Conference Program & Abstract Booklet

Rostock 1996
Cleveland 1998
Rostock 2000
Tallahassee 2002
Lyon 2004
Krems 2006
Vancouver 2008

8TH

INTERNATIONAL CONFERENCE ON THE

SCIENTIFIC AND CLINICAL APPLICATIONS OF MAGNETIC CARRIERS

Rostock, Germany | May 25-29, 2010

www.magneticmicrosphere.com

Welcome Message

It is our great pleasure to welcome you all to the now already 8th International Conference on the Scientific and Clinical Applications of Magnetic Carriers. We will again have lots of new and exciting presentations, concentrated on magnetic particles and their applications.

As in the past, we wish to cultivate discussions and a familiar atmosphere not only during the talks, but also during breaks, lunches and the boat trip. Nothing will help that more than the beautiful sand beach in front of our conference venue or the fresh salty air. And remember, the beach chair was invented here at the beach - so take everything at a leisurely pace, think about your research and that of your colleagues here, and get ready to start new collaborations. This will advance our field even more!

We wish you all a great conference, lots of new "aaah" moments, and just a wonderful time.

Your organizers,

Urs Hafeli, University of British Columbia, Vancouver, Canada
Wolfgang Schuett, IMF Krems, Austria & Rostock, Germany
Maciej Zborowski, Cleveland Clinic Foundation, Cleveland, U.S.A.

Contents

Sponsors

We are particularly thankful to our main sponsors this year. They have allowed us to again hand out many travel grants. Without their generous contributions this meeting would not be possible. Exhibits will be up during the entire meeting.

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NEW TOOLS IN BIOSCIENCES

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Warnemünde Attractions

The Lighthouse
You can see this 31-metre-high lighthouse from almost everywhere in Warnemünde. Built as a marine navigation aid in 1897, its beam points the way to the safety of the harbour. Halfway up the lighthouse and towards the very top, there are viewing galleries that run right around the tower. These offer fantastic panoramic views over the entire resort.

The "Teepott"
The "Teepott" centre at the foot of the lighthouse lies just a few metres from the promenade, the "Alter Strom" canal and the Westmole jetty. Built between 1927 and 1928, the tea pavilion soon became known as the "Teepott" (teapot). It was gutted by fire 1945, and rebuilt in 1968. Because of the extraordinary design of the roof, it is now a listed building. With its variety of cuisine and culture the "Teepott" ranks as one the resort's top attractions.

Westmole Jetty
This 530-metre-long jetty is one of Warnemünde's must-see attractions. From the Westmole jetty you can look out over the sea, watch ships pass by and breathe in the pure sea air. It's also a great base for walks along the coast with its steep cliffs and sandy beaches. If you're lucky you'll even come across one or two pieces of amber.

Maritime Simulation Centre
Warnemünde's state-of-the art Maritime Simulation Centre is the only one in the world that can simulate nautical and technical shipping operations, while at the same time taking into account shore-based support provided by the port control centres. It is used for the training and development of seagoing personnel and offers an excellent base for research. Guided group tours are possible by prior arrangement.

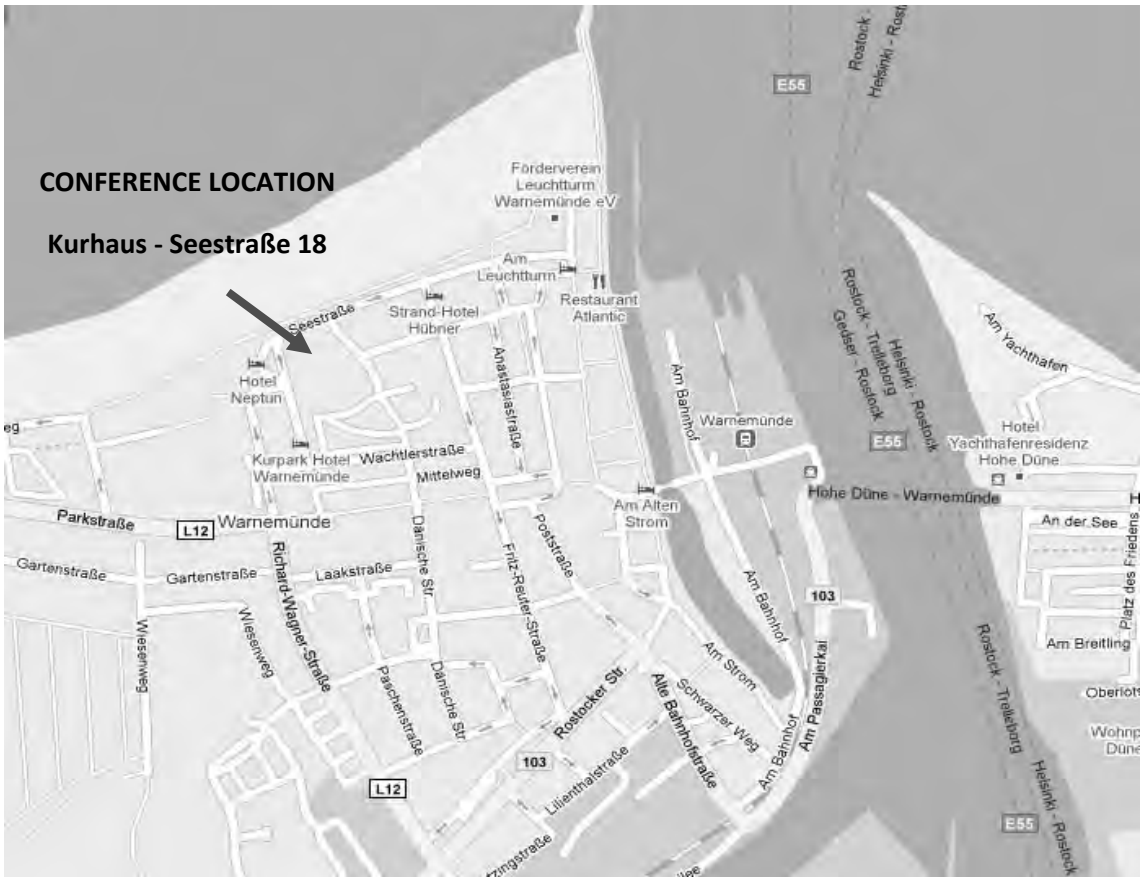
Local History Museum
The local history museum is housed in an old fisherman's cottage, which was built in 1767. Exhibitions provide information on what life was like for sailors and fishermen in the 19th century, the harbour pilot service and how Warnemünde became a seaside resort.

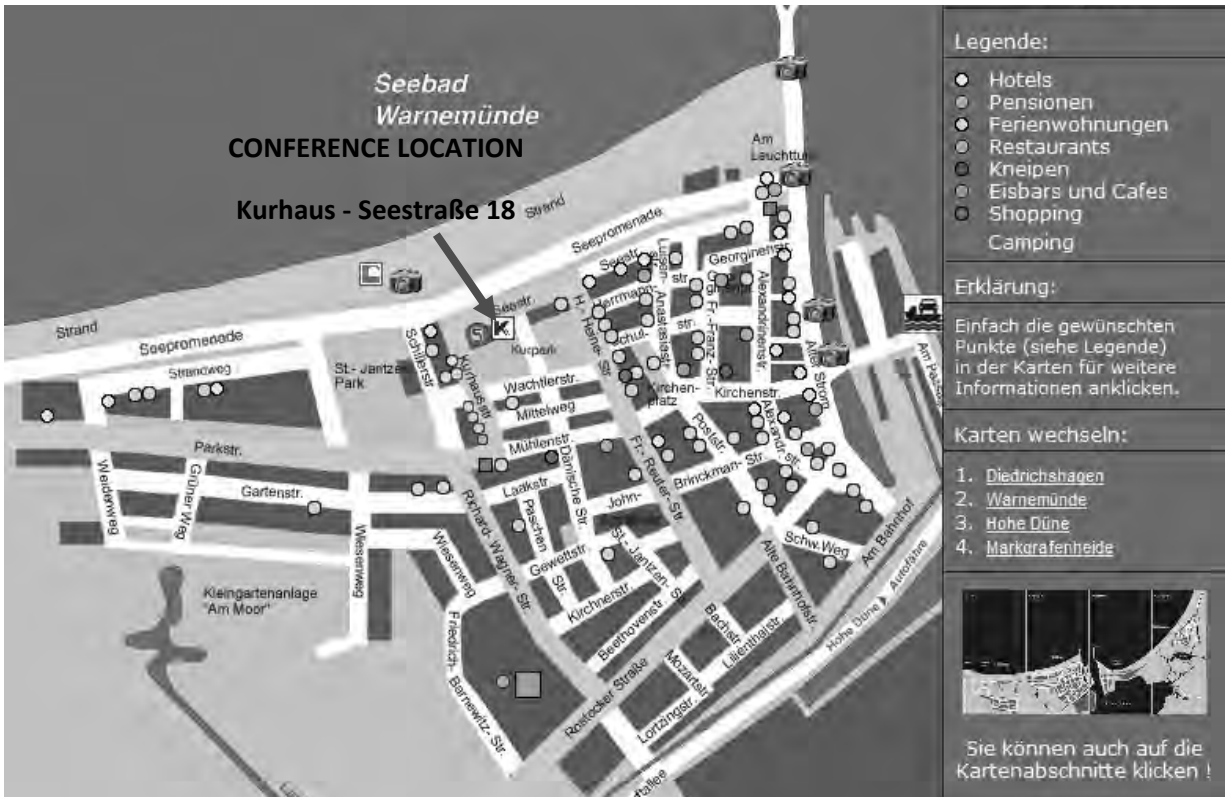
Alter Strom
A mile-long idyllic stroll past shops and restaurants in lovingly restored fishermens huts and fresh fish sales directly off the boat.

Getting Around

For information on all public transport in the Rostock area: ferry, bus, tram and train:
http://www.rsag-online.com/english/home

Maps


CONFERENCE LOCATION
Kurhaus - Seestraße 18
Förderverein Leuchtturm Warnemünde eV
Am Leuchtturm
Restaurant Atlantic
Strand-Hotel Hübner
Seestraße
Hotel Neptun
Kurpark Hotel Warnemünde
Wachtlerstraße
Mittelweg
Anastasiastraße
Parkstraße
L12
Warnemünde
Gartenstraße
Laakstraße
Richard-Wagner-Straße
Wiesenweg
Dänische Str.
Fritz-Reuter-Straße
Poststraße
Am Alten Strom
Am Bahnhof
Warnemünde
E55
Rostock - Helsinki
Rostock - Trelleborg
Gedser - Rostock
Helsinki - Rostock
Am Yachthafen
Hotel Yachthafenresidenz Hohe Düne
Hohe Düne - Warnemünde
An der See
Am Breitling
103
Rostocker Str.
Alte Bahnhofstraße
Schwarzer Weg
Am Passagierkai
Paschenstraße
Lilienthalstraße


Seebad Warnemünde
CONFERENCE LOCATION
Kurhaus - Seestraße 18
Strand
Seepromenade
Strandweg
St.-Jantzen-Park
Parkstr.
Gartenstr.
Gartenweg
Wiesenweg
Richard-Wagner-Str.
Kleingartenanlage "Am Moor"
Friedrich-Barnewitz-Str.
Rostocker Straße
Lortzingstr.
Mozartstr.
Beethovenstr.
Kirchnerstr.
Gewerbestr.
Laakstr.
Dänische Str.
Mühlenstr.
Wachtlerstr.
Mittelweg
Kurpark
Kirchenplatz
Kirchenstr.
Fr.-Franz-Str.
Brinckman-Str.
Fr.-Reuter-Str.
John-Brinckman-Str.
Poststr.
Alexandrinenstr.
Georginenstr.
Am Leuchtturm
Am Bahnhof
Alte Bahnhofstr.
Schw.Weg
Lilienthalstr.
Hohe Düne
Autobahn
Legende:
Hotels
Pensionen
Ferienwohnungen
Restaurants
Kneipen
Eisbars und Cafes
Shopping
Camping
Erklärung:
Einfach die gewünschten Punkte (siehe Legende) in der Karten für weitere Informationen anklicken.
Karten wechseln:
1. Diedrichshagen
2. Warnemünde
3. Hohe Düne
4. Markgrafenheide
Sie können auch auf die Kartenabschnitte klicken !

Social Program

And as always, we will not let science prevent us from learning new stuff, having fun together and enjoying Rostock / Warnemünde to the fullest.

Tuesday Evening, May 25, 2010
A welcome reception will be held at the Kurhaus Warnemünde, **open to all participants of the conference**. It will start at 6:30 PM and go till 11 PM. Chemicell generously sponsored this reception. Conference registration will be available beginning at 5:00 PM till closure at the Kurhaus.

Wednesday Evening, May 26, 2010
After the talks, there will be a poster session with Beer and Pretzels generously sponsored by Magnisense and Stemcell Technologies. This is followed by a banquet and social evening, all included in the registration!

Thursday, May 27, 2010
During the day, we will have a **spouse tour** starting at 9 AM. Please tell us on the previous day if your spouse would like to take part in this! This tour is complimentary.

After the talks, there will be a poster session with Beer and Pretzels generously sponsored by Diagnostic Biosensors LLC and Life Technologies Corp.

The rest of the evening is free. Go and explore Warnemünde!

Friday Evening, May 28, 2010
On this evening, we will have our traditional boat cruise. Micromod generously sponsored this highlight of our conference. We will also visit downtown Rostock during this trip.

Saturday, May 29, 2010
The meeting will end at 4 PM.

Conference Proceedings

After every "Magnetic Carrier Meeting", we publish peer reviewed articles in a special journal issue that contains the most-up-to date research in our area. **The deadline for the submission of these full papers is June 14, 2010.**

During the last few years, we published these full size articles in the Journal of Magnetism and Magnetic Materials. Please check them out here for 2009 or 2007 (by the way, you can still order a copy from these two years - all older ones are sold out).

This year, we will publish the proceedings with the American Institute of Physics in their Conference Proceeding Series. This AIP publication is well indexed and you can find your papers with search engines such as SciFinder Scholar.

Specific Details for Paper Submission:

- There are no page limits for this journal, although **we do recommend a 6-8 page size**; but if you have lots of interesting results to report, go ahead!

- Best of all, **all your figures can be in colour!**

- Submission is all electronic on our website: www.magneticmicrosphere.com. **Deadline is Monday, June 14**, and papers will be sent out for peer review the following day. Please use the same password to login that you used for the abstract submission.

- Details of formatting are given on our website (www.magneticmicrosphere.com), as well as on the AIP website (http://proceedings.aip.org/).

8th International Conference on the Scientific and Clinical Applications of Magnetic Carriers - Rostock, Germany

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17:00	Posters can be put up (pins will be provided)			
18:30	**Informal reception and welcome cocktail (Apero) in the Kurhaus Warnemünde -** *generously sponsored by* ***Chemicell***			
Wednesday, May 26, 2010				
7:30	**Registration desk opens / Posters can be put up** (pins will be provided)			
	Opening Session			
9:00	Schuett, Wolfgang	Opening of the conference / Welcome	Rostock, Germany	
9:15	Hafeli, Urs	Short review of the last 2 years of magnetic carriers research	Vancouver, Canada	Talk 0
9:35	Giaever, Ivar	From Quantum Physics to Biotechnology: Reflections and Advice from a Nobel Prize Carrier	Oslo, Norway	Invited talk 1
10:10	**Coffee break / posters / exhibitors**			
	Session 1: Magnetic Drug Delivery I - ***Chair: Christian Plank (Germany)***			
10:45	Alexiou, Christoph	Cancer Therapy with Drug Loaded Magnetic Nanoparticles-Magnetic Drug Targeting	Erlangen, Germany	Talk 1
11:00	Azevedo, Ricardo	Toxicity, biodistribution and magnetic attraction of a magnetic fluid containing maghemite nanporticle surface-coated with a double-layer lauric - in vitro and in vivo tests	Brasilia, Brazil	Talk 2
11:15	Nacev, Alek	Predicting the behavior of magnetic particles in-vivo: simulations vs experiments	College Park, U.S.A.	Talk 3
11:30	Darton, Nicholas	Manipulation and tracking of superparamagnetic nanoparticles using MRI	Cambridge, U.K.	Talk 4
11:45	Georgelin, Thomas	Coupling bleomycin to multifunctionalized magnetic nanoparticles, toward an efficient cancer treatment	Paris, France	Talk 5
12:00	Gitter, Kurt	Quantitative targeting-maps as result of experimental investigations on a branched tube model in magnetic drug targeting	Dresden, Germany	Talk 6
12:15	**Lunch**			
	Session 2: Gene Delivery - ***Chair: Gustav Steinhoff (Germany)***			
13:40	Steinhoff, Gustav	Enhanced Thoracic Gene Delivery by Magnetic Nanobead-Mediated Vector	Rostock, Germany	Invited talk 2
14:20	Ma, Yongjie	Enhancement of the efficiency of non-viral gene delivery by magnetofectins formed via electrostatic self-assembly to COS-7 cell lines in vitro	Shanghai, China	Talk 7
14:35	Mykhaylyk, Olga	Optimizing virus-magnetic nanoparticle complexes for gene transfer in cell lines and stem cells	Munich, Germany	Talk 8
14:50	Trueck, Christina	Targeting of lentiviral vectors and positioning of transduced cells by magnetic nanoparticles and magnetic field	Bonn, Germany	Talk 9
	Session 3: Magnetic Drug Delivery II - ***Chair: Dhirendra Bahadur (India)***			
15:10	Gutierrez, Lucia	Detection of magnetic nanoparticles in a tumour after cytokine delivery in the presence of a magnetic field	Madrid, Spain	Talk 10
15:25	Isa, Lucio	Nitrocatechol-dispersant stabilized superparamagnetic iron oxide nanoparticle assembly into magnetically actuated membranes	Zurich, Switzerland	Talk 11
15:40	**Coffee break / posters / exhibitors**			
16:25	Klostergaard, Jim	Magnetically-Responsive Nanoparticles for Vectored Delivery of Cancer Therapeutics	Houston, U.S.A.	Talk 12
16:40	Levasseur, Steve	Systemically Delivered Magnetic Nanoparticles for Targeted Ocular Molecular Imaging and Drug Delivery	Vancouver, Canada	Talk 13
16:55	Liu, Shao-Kai	Interaction of Low-Frequency Ultrasound and Magnetic Force on Magnetite Nanoparticle Carrying Tissue Plasminogen Activator	Tao Yuan, Taiwan	Talk 14
17:10	Louguet, Stéphanie	Engineering the surface of magnetic nanoparticles by adsorption of double hydrophilic block copolymers	Pessac, France	Talk 15
17:25	MacDonald, Cristin	Effects of magnetite loading and time-varied magnetic field on MNP transport in soft medium and cellular uptake	Philadelphia, U.S.A.	Talk 16
17:40	Newman, Brant	Magnetic Endothelialization of Vascular Stents Facilitates Healing and Improves Patency	Rochester, U.S.A.	Talk 17
17:55	Pirmoradi, Fatemeh	A Magnetically Controlled Drug Delivery Device	Vancouver, Canada	Talk 18
18:10	Russo, Alessandro	Magnetic Scaffold for Advanced Osteochondral Tissue Engineering	Bologna, Italy	Talk 19
18:25	Wu, Tony	Intra-Arterial Application of Magnetic Nanoparticles for Targeted Thrombolytic Therapy: A Rat Embolic Stroke Model	Tao Yuan, Taiwan	Talk 20
18:40	**Poster session (odd numbers) with beer and pretzels - PLEASE RATE POSTERS** *- sponsored by* ***Magnisense*** *and* ***Stemcell Technologies***			
20:10	**Banquet and social evening, included in registration fee**			

Thursday, May 27, 2010				
7:30	**Registration desk opens**			
8:30	Krishnan, Kannan	**Tutorial I** on Magnetic Things We All Should Know	Seattle, U.S.A.	Tutorial 1
	Session 4: Microsphere Synthesis - *Chair: Kathy Saatchi (Canada)*			
9:00	Ettenauer, Marion	Preparation of Magnetic Cellulose Microparticles as Markers in Extracorporeal Blood Purification	Krems, Austria	Talk 21
9:15	Furlan, Marco	Magnetic Gelation: A new Method to Produce Anisotropic Porous Polymeric Materials	Zurich, Switzerland	Talk 22
9:30	Kuznetsov, Anatoly	Magnetically guided microscopic electric batteries: manufacturing and biological applications.	Moscow, Russia	Talk 23
9:45	Xu, Hong	Development of immunomagnetic beads for rapid detection of Escherichia coli O157:H7 by immunomagnetic enrichment and real-time PCR	Shanghai, China	Talk 24
10:00	**Group photograph**			
10:15	**Coffee break / poster session / exhibitors**			
	Session 5: Nanoparticle Synthesis and Analysis - *Chair: Kannan Krishnan (U.S.A.)*			
10:45	Amstad, Esther	Adding Different Functionalities to Ultra-stable Superparamagnetic Iron Oxide Nanoparticles through Controlled Surface Modification	Zurich, Switzerland	Talk 25
11:00	Wawrzik, Thilo	Multi-Variant Magnetic Particle Spectroscopy for Magnetic Nanoparticle Characterization	Braunschweig, Germany	Talk 26
11:15	Dutz, Silvio	Physical Properties of Water Based Large Single Domain Particle Dispersions of Magnetite	Jena, Germany	Talk 27
11:30	Guerler, Celin	Magnetically Induced Hot Spots Promote a Locoregional Chemical Reaction	Düsseldorf, Germany	Talk 28
11:45	Jain, Nirmesh	Anchored Steric Stabilisation of Superparamagnetic Nanoparticles for Biomedical Applications	Sydney, Australia	Talk 29
12:00	Wang, Shan X.	Magneto-Nano Chips for Biomedical Diagnostics	Stanford, U.S.A.	Invited talk 3
12:40	**Lunch**			
	Session 6: Nanoparticle Synthesis and Analysis - *Chair: Thompson Mefford (U.S.A.)*			
14:00	Lellouche, Jean-Paul	Hydrophilic Maghemite (gamma-Fe2O3) Nanoparticles - Aggregation Control Using an Ultrasound-Assisted Doping Process of Particle Surface	Ramat Gan, Israel	Talk 30
14:15	Mahmoudi, Morteza	Synthesis of Rod-Shaped Superparamagnetic Iron Oxide Nanoparticles with Polyvinyl Alcohol	Tehran, Iran	Talk 31
14:30	Mistlberger, Guenter	Magnetically controlled, multifunctional nano devices	Graz, Austria	Talk 32
14:45	Nikitin, Maxim	Self-assembly of multifunctional nanoparticles via barnase and barstar	Moscow, Russia	Talk 33
15:00	Pellegrino, Teresa	Magnetic based nanobeads as multifunctional platforms for biomedical applications	Lecce, Italy	Talk 34
15:15	Truonc Phuoc, Lai	Magnetic nanosystems for the sentinel nodes detection	Strasbourg, France	Talk 35
15:30	Turcu, Rodica	Synthesis and characterization of biocompatible magnetically controllable nanostructures using different polymers or block copolymers	Cluj-Napoca, Romania	Talk 36
15:45	Yang, Liangrong	Fabrication of Temperature- and pH- Responsive Magnetic Nanoparticles and Their Reversible Agglomeration in Aqueous Milieu	Beijing, China	Talk 37
16:00	Yoo, Myung-Ik	Synthesis of Individually Water-Soluble, Biocompatible, and Angiogenesis-Targeting Superparamagnetic Iron Oxide Nanoparticles	Seoul, Korea	Talk 38
16:15	Zierold, Robert	Synthesis of Biocompatible Magnetic Test-Tube-Shaped Nanoparticles by Atomic Layer Deposition	Hamburg, Germany	Talk 39
16:30	**Coffee break / poster session / exhibitors**			
	Session 7: Analytical Methods - *Chair: Sara Majetich (U.S.A.)*			
17:00	Dennis, Cindi	Interactions in Magnetic Nanoparticle Systems: How to Identify Them and Their Consequences	Gaithersburg, U.S.A.	Talk 40
17:15	Lim, JitKang	Magnetophoretic motion control of individual Brownian nanoparticles	Penang, Malaysia	Talk 41
17:30	Liu, Wenzhong	Magnetic Nanoparticle Temperature Estimation Using AC Susceptibility	Wuhan, China	Talk 42
17:45	Salaklang, Jatuporn	Fixed bed magnetic reactor for surface derivatization of Superparamagnetic Iron Oxide Nanoparticles	Fribourg, Switzerland	Talk 43
18:00	Schaller, Vincent	Determination of nanocrystal size distribution in magnetic multi-core particles including interactions and magnetic anisotropy	Göteborg, Sweden	Talk 44
18:15	Tarn, Mark	Continuous magnetic microparticle-based sandwich immunoassays in a multilaminar flow microreactor	Hull, U.K.	Talk 45
18:30	Teste, Bruno	Magnetic core shell nanoparticles based homogeneous immunoassay	Paris, France	Talk 46
18:45	**Poster session (even numbers) with beer and pretzels - PLEASE RATE POSTERS** *- sponsored by* ***Diagnostic Biosensors LLC*** *and* ***Life Technologies Corp.***			
20:15	**Free evening - use it to meet old friends, discuss new collaborations, and enjoy Warnemünde on your own!**			

Friday, May 28, 2010				
7:30	**Registration desk opens**			
8:30	Krishnan, Kannan	**Tutorial II** on Magnetic Things We All Should Know	Seattle, U.S.A.	Tutorial 2
	Session 8: Magnetic Hyperthermia I - ***Chair: Carlos Rinaldi (Puerto Rico)***			
9:00	Berndl, Bettina	Antibody Targeted Magnetic Nanoparticle Hyperthermia for Cancer Therapy	London, U.K.	Talk 47
9:15	Gogoi, Manashjit	Thermosensitive magnetoliposomes containing biphasic mixture of metal oxide composite nanoparticles for combined cancer drug delivery and hyperthermia	Mumbai, India	Talk 48
9:30	Ivkov, Robert	Development of magnetic nanoparticles and devices for thermal therapy of cancer: A radiosensitization study in mice bearing human prostate cancer xenografts	Baltimore, U.S.A.	Talk 49
9:45	Kashevsky, Bronislav	Substantiation and in-vivo approbation of the low-frequency ferromagnetic hyperthermia	Minsk, Belarus	Talk 50
10:00	**Coffee break / poster session / exhibitors**			
	Session 9: Magnetic Hyperthermia II - ***Chair: Quentin Pankhurst (U.K.)***			
10:30	Martinez-Boubeta, Carlos	Self-assembled multifunctional fe/mgo nanospheres for MRI and hyperthermia	Barcelona, Spain	Talk 51
10:45	Pradhan, Pallab	Multifunctional magnetic liposomes for magnetic drug targeting and hyperthermia applications	Mumbai, India	Talk 52
11:00	Rau, Beate	Thermotherapy of oesophageal cancer with superparamagnetic iron oxide nanoparticles	Berlin, Germany	Talk 53
11:15	Southern, Paul	Real-time In Vitro Analysis of Magnetic Hyperthermia using Optical and Fluorescent Microscopy	London, U.K.	Talk 54
11:30	Plank, Christian	Magnetofection – magnetically enhanced nucleic acid delivery, from research tool towards clinical application	Munich, Germany	Invited talk 4
12:10	**Lunch**			
13:25	**POSTER PRIZE** - *Presented by Cordula Gruettner*			
	Session 10: Magnetic Imaging / MRI - ***Chair: Tim St. Pierre (Australia)***			
13:30	Jing, Ying	Dual Functional Au coated Fe70Co30 High-Magnetic-Moment Nanoparticles for MR imaging and Therapy	Minnesota, U.S.A.	Talk 55
13:45	Lee, Yi-Cheng	MnO Nanocrystals as in vivo Time-Dependent T1 MRI Contrast Agents	Seattle, U.S.A.	Talk 56
14:00	Misri, Ripen	Molecular Imaging Bioprobes (MRI/SPECT) for Mesothelin Expressing Cancers	Vancouver, Canada	Talk 57
14:15	Nikitin, Petr	Non-invasive in vivo mapping and long-term monitoring of magnetic nanoparticles in different organs of an animal	Moscow, Russia	Talk 58
14:30	Rahn, Helene	Calibration phantom for quantitative tomography analysis of biodistribution of magnetic drug carriers	Dresden, Germany	Talk 59
14:45	Rühmer, Dennis	Magnetic relaxation imaging using a fluxgate based scanner	Braunschweig, Germany	Talk 60
15:00	Soenen, Stefaan	Intracellular iron oxide nanoparticle coating stability determines nanoparticle usability and cell functionality	Leuven, Belgium	Talk 61
15:15	Tokarev, Alexander	Magnetic Nanoneedles for Optofluidic Applications	Clemson, U.S.A.	Talk 62
15:30	Trekker, Jesse	Effect of the core size of monodisperse superparamagnetic nanoparticles on their relaxometric enhancing properties for MRI	Leuven, Belgium	Talk 63
15:45	Zabow, Gary	Cylindrical Magnetic Nanoshells for Multispectral MRI	Bethesda, U.S.A.	Talk 64
16:00	**Coffee break / poster session / exhibitors**			
	Session 11: Magnetic Separation - ***Chair: Maciej Zborowski (U.S.A.)***			
16:30	AlHetlani, Entesar	On-chip generation and manipulation of magnetic w/o and o/w droplets	Hull, U.K.	Talk 65
16:45	Andreu, Jordi	Equilibrium and nonequilibrium aggregation af superparamagnetic colloids	Bellaterra, Spain	Talk 66
17:00	Balasubramanian, P.	Circulating tumor cell detection by magnetic depletion of normal bloopd cells	Columbus, U.S.A.	Talk 67
17:15	Earhart, Christopher	Improved Designs of a Microfabricated Magnetic Sifter for Biomolecule and Cell Purification	Stanford, U.S.A.	Talk 68
17:30	Fischer, Thomas	Colloidal transport and separation on magnetic garnet films	Bayreuth, Germany	Talk 69
17:45	Hoshino, Kazunori	Microfluidic Chip-Based Immunomagnetic Detection of Circulating Tumor Cell	Austin, U.S.A.	Talk 70
18:00	Schwarz, Sebastian	Nanoparticle Size and Surface Charge Determine Formation of Protein Corona, Cellular Uptake and Magnetic Resonance Imaging Properties	Aachen, Germany	Talk 71
18:30	**Traditional boat cruise / visit of downtown Rostock** - sponsored by **micromod**			

Saturday, May 29, 2010				
7:30	**Registration desk opens**			
8:30	Krishnan, Kannan	**Tutorial III** on Magnetic Things We All Should Know	Seattle, U.S.A.	Tutorial 3
	Session 12: Biological Applications I - *Chair: Shan Wang (U.S.A.)*			
9:00	Antosova, Andrea	Magnetic fluids have ability to decrease amyloid aggregation associated with amyloid-related diseases	Kosice, Slovakia	Talk 72
9:15	Ho, Vincent	Magnetic cell labelling and manipulation in 2D and 3D cell cultures	Cambridge, U.K.	Talk 73
9:30	Bakandritsos, Aristides	Development of Functional Magnetic Targeted Carriers for Cationic Drugs	Patras, Greece	Talk 74
9:45	Laurencin, Mathieu	Human Erythrocytes Covered with Magnetic Core–Shell Nanoparticles as Multifunctional Drug Carriers	Paris, France	Talk 75
10:00	Palfreyman, Justin	Magnetic Barcoded Microcarriers for Biomolecular Labelling Applications	Cambridge, U.K.	Talk 76
10:15	**Coffee break / exhibitors**			
	Session 13: Biological Applications II - *Chair: Jeff Anker (U.S.A.)*			
11:00	Riegler, Johannes	A simplified mathematical model for targeted magnetic delivery of cells using an MRI scanner	London, U.K.	Talk 77
11:15	Shoshi, Astrit	GMR-based real-time cell endocytosis monitoring of magnetic particles	Vienna, Austria	Talk 78
11:30	Sureshkumar, Manthiriyappan	Multifunctionalized magnetic bionanocomposites for biotechnology applications	Taipei, Taiwan	Talk 79
11:45	Thanh, Nguyen TK	Superparamagnetic Fluorescent Nickel-Enzyme Nanobioconjugates: Synthesis and Characterization of a Novel Multifunctional Biological Probe	London, U.K.	Talk 80
12:00	Torres-Lugo, Madeline	Examination of the Enhanced Potentiation of Combined Cisplatin Treatment with Magnetic Fluid Hyperthermia	Mayaguez, U.S.A.	Talk 81
12:15	Rozhkova, Elena	Biofunctionalized Magnetic-Vortex Microdiscs for Biomechanical Cell Actuation	Argonne, U.S.A.	Invited talk 5
12:55	**Lunch**			
	Session 14 : Biosensors - *Chair: Frank Ludwig (Germany)*			
14:00	Dempsey, Nora	Micro-structured hard magnetic films for lab-on-chip applications	Grenoble, France	Talk 82
14:15	Donolato, Marco	On chip detection and manipulation of biological entities carried by magnetic beads via domain wall conduits	Como, Italy	Talk 83
14:30	Enpuku, Keiji	AC susceptibility measurement of magnetic markers for liquid phase detection of biological targets	Fukuoka, Japan	Talk 84
14:45	Kinnunen, Paivo	Growth Measurements of Individual Bacteria with a Magnetic Bead Rotation Sensor	Ann Arbor, U.S.A.	Talk 85
15:00	Li, Yuanpeng	Biomarker quantification in unprocessed human sera by a competition-based nanomagnetic protein assay and high-magnetic-moment nanoparticles	Minneapolis, U.S.A.	Talk 86
15:15	Østerberg, Frederik	Chip-based measurements of Brownian relaxation of magnetic beads using a planar Hall effect magnetic field sensor	Lyngby, Denmark	Talk 87
15:30	Sivagnanam, Venkataragavalu	Study of spatio-temporal immunofluorescence on magnetic bead patterns in a microfluidic channel	Lausanne, Switzerland	Talk 88
15:45	**Closing Comments and Announcement of the NEXT MEETING: Wolfgang Schuett / Jian Ping Wang**			
16:00	**Meeting ends**			

Talk and Poster Abstracts

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10:10	**Coffee break / posters / exhibitors**			
	Session 1: Magnetic Drug Delivery I - *Chair: Christian Plank (Germany)*			
10:45	Alexiou, Christoph	Cancer Therapy with Drug Loaded Magnetic Nanoparticles-Magnetic Drug Targeting	Erlangen, Germany	Talk 1
11:00	Azevedo, Ricardo	Toxicity, biodistribution and magnetic attraction of a magnetic fluid containing maghemite nanporticle surface-coated with a double-layer lauric - in vitro and in vivo tests	Brasilia, Brazil	Talk 2
11:15	Nacev, Alek	Predicting the behavior of magnetic particles in-vivo: simulations vs experiments	College Park, U.S.A.	Talk 3
11:30	Darton, Nicholas	Manipulation and tracking of superparamagnetic nanoparticles using MRI	Cambridge, U.K.	Talk 4
11:45	Georgelin, Thomas	Coupling bleomycin to multifunctionalized magnetic nanoparticles, toward an efficient cancer treatment	Paris, France	Talk 5
12:00	Gitter, Kurt	Quantitative targeting-maps as result of experimental investigations on a branched tube model in magnetic drug targeting	Dresden, Germany	Talk 6
12:15	**Lunch**			
	Session 2: Gene Delivery - *Chair: Gustav Steinhoff (Germany)*			
13:40	Steinhoff, Gustav	Enhanced Thoracic Gene Delivery by Magnetic Nanobead-Mediated Vector	Rostock, Germany	Invited talk 2
14:20	Ma, Yongjie	Enhancement of the efficiency of non-viral gene delivery by magnetofectins formed via electrostatic self-assembly to COS-7 cell lines in vitro	Shanghai, China	Talk 7
14:35	Mykhaylyk, Olga	Optimizing virus-magnetic nanoparticle complexes for gene transfer in cell lines and stem cells	Munich, Germany	Talk 8
14:50	Trueck, Christina	Targeting of lentiviral vectors and positioning of transduced cells by magnetic nanoparticles and magnetic field	Bonn, Germany	Talk 9
	Session 3: Magnetic Drug Delivery II - *Chair: Dhirendra Bahadur (India)*			
15:10	Gutierrez, Lucia	Detection of magnetic nanoparticles in a tumour after cytokine delivery in the presence of a magnetic field	Madrid, Spain	Talk 10
15:25	Isa, Lucio	Nitrocatechol-dispersant stabilized superparamagnetic iron oxide nanoparticle assembly into magnetically actuated membranes	Zurich, Switzerland	Talk 11
15:40	**Coffee break / posters / exhibitors**			
16:25	Klostergaard, Jim	Magnetically-Responsive Nanoparticles for Vectored Delivery of Cancer Therapeutics	Houston, U.S.A.	Talk 12
16:40	Levasseur, Steve	Systemically Delivered Magnetic Nanoparticles for Targeted Ocular Molecular Imaging and Drug Delivery	Vancouver, Canada	Talk 13
16:55	Liu, Shao-Kai	Interaction of Low-Frequency Ultrasound and Magnetic Force on Magnetite Nanoparticle Carrying Tissue Plasminogen Activator	Tao Yuan, Taiwan	Talk 14
17:10	Louguet, Stéphanie	Engineering the surface of magnetic nanoparticles by adsorption of double hydrophilic block copolymers	Pessac, France	Talk 15
17:25	MacDonald, Cristin	Effects of magnetite loading and time-varied magnetic field on MNP transport in soft medium and cellular uptake	Philadelphia, U.S.A.	Talk 16
17:40	Newman, Brant	Magnetic Endothelialization of Vascular Stents Facilitates Healing and Improves Patency	Rochester, U.S.A.	Talk 17
17:55	Pirmoradi, Fatemeh	A Magnetically Controlled Drug Delivery Device	Vancouver, Canada	Talk 18
18:10	Russo, Alessandro	Magnetic Scaffold for Advanced Osteochondral Tissue Engineering	Bologna, Italy	Talk 19
18:25	Wu, Tony	Intra-Arterial Application of Magnetic Nanoparticles for Targeted Thrombolytic Therapy: A Rat Embolic Stroke Model	Tao Yuan, Taiwan	Talk 20
18:40	**Poster session (odd numbers) with beer and pretzels - PLEASE RATE POSTERS** - *sponsored by* ***Magnisense*** *and* ***Stemcell Technologies***			
20:10	**Banquet and social evening, included in registration fee**			

The Nobel Prize and the Future of Science (?)

Ivar Giaever
Emeritus Professor at Rensselaer Polytechnic Institute, Troy, U.S.A.
Professor at large, University of Oslo, Norway

In the year 2001 the Swedish people celebrated the 100 years anniversary of the Nobel Prize. During this period science has changed remarkably. In this talk I will explain how the Nobel Prize came about and give my personal views about where I think science is headed in the future. It is my belief that most fundamental laws of science are known, but that scientists are still needed to sort out details and to make new inventions.

Prof. Giaever is most famous for his experimental discoveries regarding tunneling phenomena in superconductors. For this work, he received in 1973 the Nobel Prize in Physics. Prof. Giaever's research later in his career was mainly in the field of biophysics. He also worked with Prof. Ugelstad, the inventor of the Dynabeads.

Cancer Therapy with Drug Loaded Magnetic Nanoparticles-Magnetic Drug Targeting

Christoph Alexiou[1)]*Rainer Tietze[1)], Stefan Lyer[1)], Eveline Schreiber[1)], Jenny Stiller[1)], Lutz Trahms [2)], Stefan Odenbach[3)]

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[3]*Chair Magnetofluiddynamics Technical University Dresden, Germany*

Introduction: Cancer is a leading cause of death worldwide: In spite of all efforts deaths from cancer are projected to continue rising, with an estimated number of 12 million in 2030. The major disadvantage of chemotherapy, a major therapeutic column in cancer therapy, is that often the dose of systemically applied agents needed to annihilate all tumor cells will cause severe negative side effects in patients. The use of magnetic nanoparticles is one attempt to overcome this dilemma. To date different application forms and methods are investigated to use these particles for treating different solid tumor entities. Superparamagnetic iron oxide nanoparticles (Fe_3O_4) are biodegradable and they are still in use as contrast agent in magnetic resonance imaging (MRI). Magnetic Drug Targeting (MDT) is a promising attempt for treating tumors. This method applies magnetic nanoparticles bound to chemotherapeutics and injected into the supplying vascular system of the respective tumor while focused by a strong external magnetic field to target selectively this region (**Figure. 1**). This leads to higher doses of the chemotherapeutic agent in the tumor region with a reduced overall dose.
Materials and Methods: Experimental VX-2 squamous cell carcinomas were implanted at the left hind limb of rabbits. After four to six weeks MDT was performed with one cycle of treatment. Mitoxantrone was bound to superparamagnetic Fe_3O_4-nanoparticles (hydrodynamic diameter: ~100 nm) in an aqueous solution (= ferrofluid). The range of the body weights of the rabbits was between 3.1 kg and 3.9 kg. The applied doses were approximately less than 10 % compared to the regular systemic dose (10mg/m^2). The drug loaded nanoparticles were given through the femoral artery close to the tumor. The magnetic nanoparticles were attracted to the tumors by a focused external magnetic field during the application. This could be proven with histological investigations, analytical methods concerning the drug concentration (HPLC) and particle enrichment (Magnetorelaxometry) and imaging techniques (X-Ray-Tomography). The magnetic field was generated by a water cooled electromagnet (Siemens Healthcare, Erlangen, Germany) with a field gradient of 72 T/m directly under the tip of the poleshoe. Tumor bearing animals were examined with a 4.7 Tesla MRI Bruker Biospec scanner.
Results and Discussion: Histological cross sections of the tumors showed, that the particles can penetrate the vascular wall and concentrate into the tumor. This could be also documented with X-Ray-Tomography. Magnetorelaxometry, a very sensitive and fast method for detection of magnetic nanoparticels show a very high concentration in the tumor region and a very low in the remaining body compartments. The development of therapeutic nanoparticles is an ongoing process. Beside progresses in colloidal stability, an increasing amount of mitoxantrone, which is the therapeutic agent, can now be adhered to the nanoparticles. Therapy control can be performed using MRI to display particle enrichment by signal extinction of iron oxide. Results from HPLC-biodistribution experiments showed that Magnetic Drug Targeting allows to enrich the therapeutic agent up to 50 times higher in the desired body compartment (i.e. the tumor region) compared to the commonly used systemic application.

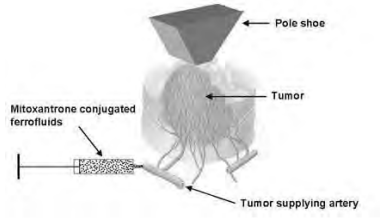

Figure 1: Schematic drawing of Magnetic Drug Targeting

Conclusion: Magnetic Drug Targeting is a promising new therapeutic model for cancer therapy. The selective concentration of the nanoparticels and the bound chemotherapeutic agent in the tumor region leads to complete tumor remissions with no negative side effects. The particles can also reach the intracellular space, which is crucial for chemotherapy. With X-Ray-Tomography the biodistribution of nanoparticles can be visualized and Magnetorelaxometry allows the possibility to quantify *in vivo* the amount of the particles *in vivo*. Further studies have to be done to transfer this innovative treatment modality into human trials.
Acknowledgement: Deutsche Forschungsgemeinschaft DFG (AL552/3-1) and Else Kröner-Fresenius Foundation (Bad Homburg v.d.H., Germany).

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Toxicity, biodistribution and magnetic attraction of a magnetic fluid containing maghemite nanoparticle surface-coated with a double-layer lauric acid – *in vitro* and *in vivo* tests

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Nanomaterials, due to its unique physical properties, are promising agents for biomedical use Toxicity and biodistribution of double-layer lauric acid-coated maghemite nanoparticles were evaluated *in vitro* in tree cell linage (mouse fibroblast, kidney epithelium and peritoneal macrophage) and *in vivo* by light microscopy and transmission electron microscopy observation of mice organs. Cell linage showed high tolerance for subjected nanoparticles; the nanomaterials were found in animals' liver, lung and spleen, without pathological tissue alteration. A magnetic field emission devise were used in brain and lung of live animals to evaluated nanoparticles accumulation in animals tissue. The results showed a light accumulation in brain and in large/medium vessels of lungs. Results suggest that lauric acid nanoparticles are promising materials for drug delivery systems and contrast agent in animal models, since it has low toxicity to cells and tissues, lack of genotoxicity, and ability to suffer magnetic attraction by an external field.

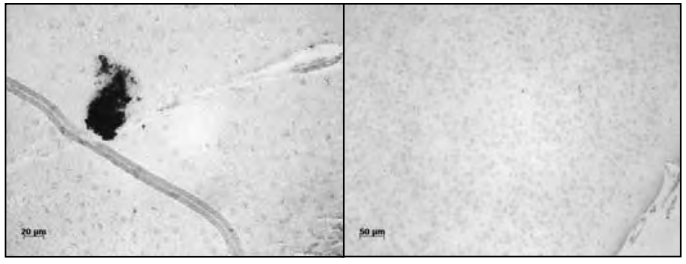

Figure . LM Photography of brain of mice treated with magnetic nanoparticles coated with lauric acid (MNP-LA) and killed after 30 minutes of magnetic field exposition (a). MNP-LA were found as blue clusters (arrows). Compare with animals treaded with MNP-LA without magnetic field (b).

Predicting the Behavior of Magnetic Particles *In-Vivo*: Simulations vs Experiments

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[b]Computational Mathematics, California Institute of Technology, Pasadena, CA.

Advanced simulations of particle motion under diffusion, blood convection, and magnetic forces were used to predict particle distribution in blood vessels and surrounding tissue. We found excellent agreement with published experimental data including an ability to explain results that were not previously understood.

In magnetic drug delivery ferro-magnetic particles are injected systemically into the blood flow and are then focused to disease locations by magnets. A critical issue is whether the applied magnetic forces can hold particles against blood flow, at which depth and in which blood vessels, and how far do particles travel from vessels into surrounding tissue.

We defined a model from physical first principles and implemented an ADI (Alternating Directions Implicit) numerical method [2] (×500 faster than COMSOL, could solve cases where COMSOL failed) to solve for ferrofluid spatial distribution. An exhaustive search of the parameter space revealed 3 distinct behavior types.

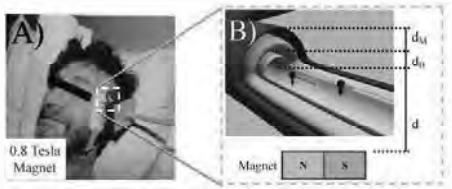

Figure 1: A) Magnetic drug focusing in clinical trials [1]. B) We consider a single blood vessel (of any size, depth, and blood velocity) and surrounding tissue for our simulations.

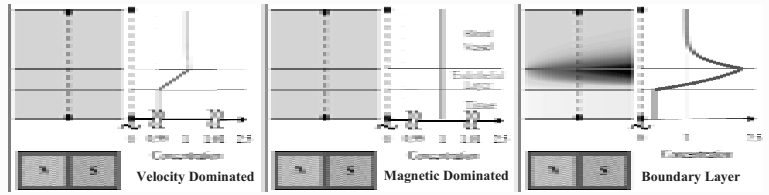

Figure 2: The three behaviors. In the velocity dominated case blood flow forces far exceed magnetic forces and particles are washed out. In magnetic dominated the magnet sweeps the particles down and out. In the last case, the two effects compete and a boundary layer forms at the vessel/tissue interface.

The type of behavior observed is uniquely set by 3 non-dimensional parameters: the magnetic-Richardson number Ψ (magnetic force vs. Stokes drag), the mass Péclet number ***Pe*** (convection vs. diffusion), and the Renkin reduced diffusion coefficient Φ (diffusion in blood vs. in tissue). The behavior in prior experiments [1, 3-6] (overlaid in Figure 3) matches our predictions. In particular, we accurately and quantitatively predict when focusing will occur even in cases where the observed behavior was previously unexplained.

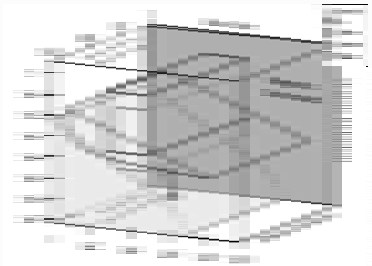

Figure 3: Prior experiments vs. our predictions in the three non-dimensional parameters space.

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Manipulation and tracking of superparamagnetic nanoparticles using MRI

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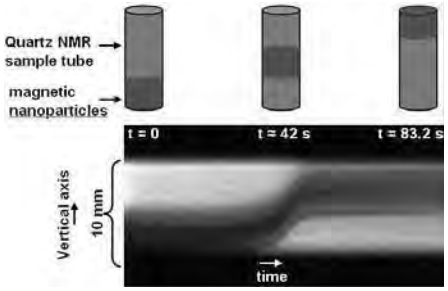
The use of magnetic fields in magnetic resonance imaging (MRI) for the tracking and delivery of chemotherapeutics bound to superparamagnetic nanoparticles offers a promising method for the non-invasive treatment of inoperable tumours. Studies of inflow magnetic targeting of superparamagnetic nanoparticles in plastic microcapillary films, representative of the human blood vessels, with different magnetic field geometries has yielded a computer model to optimise capture conditions [1]. We have demonstrated that superparamagnetic magnetite nanoparticles fabricated by an easily scalable method can be driven and tracked in real time at high velocities *in vitro* using MRI hardware [2]. Force balance calculations are consistent with the magnetic properties of individual 10 nm diameter particles that move collectively as micron sized agglomerates with hydrodynamic diameter similar to that inferred from zero-magnetic-field dynamic light scattering measurements. Magnetotactic bacteria are currently being explored as a potential future scalable source of monodisperse magnetic nanoparticles for therapeutic and biosensing applications.



2-D MRI manipulation of superparamagnetic iron oxide nanoparticles

1. Darton, N.J., B. Hallmark, T. James, P. Agrawal, V.H.B. Ho, and N.K.H. Slater, *On the magnetic field architecture required to capture superparamagnetic nanoparticles in a microcapillary flow.* Journal of Nanoparticle Research, 2010. **12**(1): p. 307-317. DOI: 10.1007/s11051-009-9615-0
2. Darton, N.J., A.J. Sederman, A. Ionescu, C. Ducati, R.C. Darton, L.F. Gladden, and N.K.H. Slater, *Manipulation and tracking of superparamagnetic nanoparticles using MRI.* Nanotechnology, 2008. **19**: p. 395102-395106. DOI: 0957-4484/19/395102

Coupling bleomycin to multifunctionalized magnetic nanoparticles, toward an efficient cancer treatment

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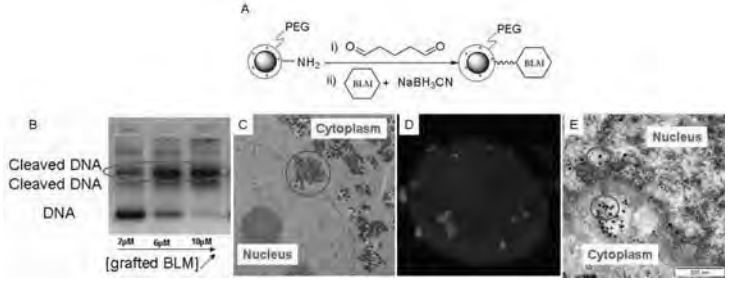
In the context of theranostic applications, we have developed a nanometric platform in order to graft an anticancer drug, the bleomycin (BLM). Bleomycin, which is an antitumor antibiotic, is currently used in some cancer treatment but suffers of weak efficiency, due to a poor biodisponibility, a weak translocation for some cancerous cells lineage and is also responsible for important side effects like pneumonia. Synthesis, functionalization and physico-chemical characterization of multifunctionalized magnetic core shell nanoparticles (γFe_2O_3@SiO_2(Fluorescent dyes)-NH_2/PEG) were performed [1, 2]. Positively charged nanoparticles revealed a hydrodynamic size of 40nm. The covalent grafting of biological entities was first optimised with an enzyme [3]. The anchoring of bleomycin was then performed (A). A suspension of bleomycin activated nanoparticles was obtained, colloidaly stable in cells culture medium, thanks to PEG chains. Biological activity of supported bleomycin was studied on DNA fragments (plasmids and oligomers). Grafted bleomycin kept its capacity to induce DNA cleavage (B). Moreover, selectivity and specificity of supported bleomycin to DNA were not altered in comparison with free bleomycin. Both bleomycin activated nanoparticles and nude nanoparticles internalization were studied at different times and translocation into cells by two different pathways were observed. Interestingly, confocal fluorescence microscopy and electronic transmission microscopy indicated strong interactions between nanoparticles and cells nuclei, with major accumulation near the nucleus (C, D). Some nanoparticles were observed too into the nucleus (E). Cells viability assays were also carried out (WST1 assays, clone efficiency). Bleomycin activated nanoparticles were able to induce a cytotoxic effect on human cancer cells (HT1080) whereas nude nanoparticles did not thus demonstrating the role played by the grafted anticancer drug.



Bleomycin grafting (A). DNA cleavage (B). Nanoparticles translocation into human fibroblastom (TEM (C, E), fluorescent confocal microscopy (D)).

The combination of bleomycin activity with properties of those multifunctionalized nanoparticles (multimodal imaging, magnetic guiding, hyperthermia) is of great interest for theranostic applications. Moreover, cells translocation and nucleus targeting allows the confinement of bleomycin close its therapeutic target and enlightens the possibility of very high efficiency bleomycin based cancer treatment with few side effects.

[1] V. Maurice, T. Georgelin, J-M Siaugue, V. Cabuil. J Magn Magn Mater. 321(10), 1408 (2009).
[2] F. d'Orlye, A. Varenne, T. Georgelin, J-M Siaugue, B. Teste, S. Descroix, P. Gareil. Electrophoresis. 30(14), 2572 (2009).
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Quantitative targeting-maps as result of experimental investigations on a branched tube model in magnetic drug targeting

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Magnetic drug targeting (MDT) is a promising approach for tumour treatment due to its high targeting efficiency, since the drug-loaded nanoparticles are concentrated within the target-region due to the influence of a magnetic-field. The understanding of transport phenomena of the particles in an artery system is still challenging. This work presents experimental results on a branched tube-model. As a result novel quantitative drug-targeting-maps are presented.

The artery-model, see Fig. 1a, is a half-Y-branched glass tube (inner diameter d_i=1.6mm), where the branching tube is thought to supply the target area. Fig. 1a shows the variation of the magnet-position and a scheme of the chosen cascade of measure-points. Fig. 1b shows additional definitions used in the map. For each measure-point the procedure of a medical application is simulated. Therefore, to obtain measure-data for one magnet-position, 1ml of ferrofluid with a volume-concentration of Φ=2.95 $\%_{vol}$ is injected during 10 minutes into the tube with the flow-velocity of distilled water of v_{max} = 12.3mm/s. Quantitative data is obtained by measurements of inductivity of calibrated coil-like containers capturing the outflow of each branch. The magnetic field is generated by an axially magnetized cylindrical permanent magnet. The ferrofluid is chosen as a water-based W12-MSG of Ferrotech® with magnetite particles with a measured average particle size d_{mean}=7.5nm.

The targeting-map Fig. 2 shows the percentage of net amount of ferrofluid targeted into the branch as (coloured) surface-plot. This map combines the magnetic volume-force (MVF) in the centre-point (lower scale), the MVF in the branch-point (left scale) and the position of the magnet (top and right scales). With the chosen concentration of ferrofluid, two basic mechanisms can be interpreted: 1. The (red) areas (see arrow) indicate a yield >88% (max 97%) with an active targeting due to attraction of the particles within the branching-zone. 2. On the contrary, the (dark blue) areas with low or no yield [<13%] (right and lower right regions) indicate constriction, where an accretion builds along the wall, leading to a plug or a positive yield in the other branch. An increase of MVF will not necessarily result in a higher yield but on the contrary to a rather abrupt drop of the targeted amount.

The quantitative targeting-maps are valuable for evaluation and comparison of setups and are also helpful for the design and optimization of a magnet-system with an appropriate strength and distribution of the field-gradient. The maps indicate the danger of accretion within the tube and also show the promising result for MDT that up to 97% of the nanoparticles were successfully targeted.

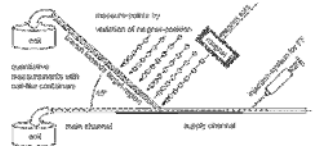

Fig. 1a: Artery model and cascade of measure-points

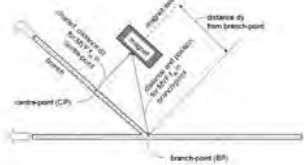

Fig. 1b: Artery-model: additional definitions

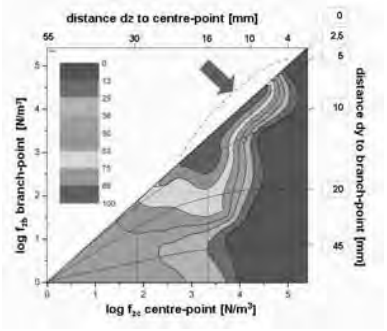

Fig. 2: Targeting map, percentage of targeted net-amount of FF

Enhanced thoracic gene delivery by magnetic nanobead mediated-vector

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Targeted gene delivery into the thoracic tissue is challenging, which needs the collection of enough gene carrier particles to the specific area. We developed a targeted gene delivery method, which employed a magnetic field to drive magnetic nanobead (MNB)/polymer/DNA complexes after systemic administration to the left thorax of mouse to induce localized gene delivery. Nonviral polymer (poly ethyleneimine, PEI) vector-gene complexes were conjugated to MNBs with the Sulfo-NHS-LC-Biotin linker. In vitro transfection efficacy of MNB/PEI/DNA was compared with PEI/DNA in three different cell lines as well as primary endothelial cells under magnetic field stimulation. In vivo, MNB/PEI/DNA complexes were injected into the tail vein of mice and an epicardial magnet was employed to attract the circulating MNB/PEI/DNA complexes. The results suggested the endocytotic uptake of MNB/PEI/DNA complexes and intracellular gene release with nuclear translocation were observed in vitro, whereas the residues of MNB/PEI complexes were localized at the perinuclear region. Compared with PEI/DNA complexes alone, MNB/PEI/DNA complexes had a 36- to 85-fold higher transfection efficiency under the magnetic field. In vivo, the epicardial magnet effectively attracted MNB/PEI/DNA complexes in the left side of the thorax, resulting in strong reporter and therapeutic gene expression in the left lung and the heart. Gene expression in the heart was mainly achieved within the endothelium. MNB-mediated gene delivery could comprise an efficient method for gene delivery to the appropriate target organ of thorax including the lung and the heart.

Enhancement of the efficiency of non-viral gene delivery by Magnetofectins Formed via Electrostatic Self-assembly to COS-7 Cell Lines *in vitro*

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Abstract: One of the major challenges for successful gene therapy is improving the transfection efficiency of non-viral vectors. Magnetic nanoparticles (MNPs) has been developed as non-viral gene delivery vehicles. Charged MNPs modified with polyethyleneimine (PEI), citric acid (CA) or carboxylmethyl-dextran (CMD) enhanced magnetofection efficiency. Both positively charged and negatively charged MNPs could spontaneously form transfection complexes (magnetofectins) with plasmid DNA (pDNA) and PEI/liposome via electrostatic self-assembly. Charged MNPs apparently enhanced PEI/liposome transfection efficiency and/or gene expression level into COS-7 cells with reduced transfection time from 4 h to 15 min under a magnetic field *in vitro*. These results suggest that charged MNPs could improve non-viral vectors transfection by simply mixing with them and by exerting a magnetic force. Such MNPs may also found applications in remotely controlled vector targeting *in vivo*.

Keywords: Gene delivery; Liposome; Magnetofection; Magnetic Nanoparticles; Non-viral vector; PEI

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OPTIMIZING VIRUS-MAGNETIC NANOPARTICLE COMPLEXES FOR GENE TRANSFER IN CELL LINES AND STEM CELLS

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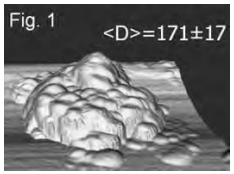
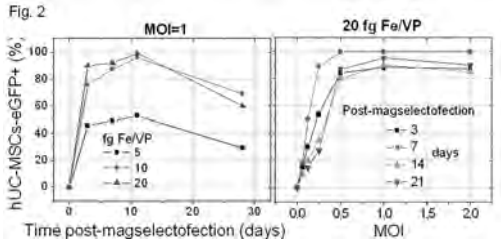
The goal of this work was providing magnetic nanoparticles (MNPs) and their formulations for efficient viral genetic modification of cell lines and primary cells *in vitro* and *ex vivo*.

MNPs of the core-shell type with magnetite cores of about 10 nm stabilized and decorated with surfactants and charged polymers were selected from our library of in-house synthesized MNPs for self-assembly with viral particles. Selected MNPs were characterized with respect to the core composition, crystallite size, magnetization, coating composition using XRD, TEM, magnetization and XPS methods. To achieve maximal vector binding and to avoid excess of MNPs, magnetic vectors were optimized with account for the vector association and magnetic sedimentation with MNPs using radioactively labelled adenovirus or ELISA for p24 for lentivirus. A simple method was used for the evaluation of the magnetophoretic mobility, measuring the time course of the turbidity of suspensions of the virus-MNP complexes in defined magnetic fields. We have learned that for magnetic viral vectors, it is reasonable to express the composition in terms of iron weight per PHYSICAL virus particle, and NOT per infectious virus particle, taking into account that both infectious and non-infectious virus particles are associated with appropriate MNPs. We suggest a "rule" to formulate virus magnetic complexes with suitable MNPs, based on the association and magnetic sedimentation of the virus with MNPs as well as with account for the functionality of gene delivery. The optimal complex composition of 2.5-20 fg iron per physical virus particle, depending on the MNPs used, is applicable to both adenoviral and lentiviral vectors. Optimized magnetic virus complexes were stable in 50% FCS. Electron and atomic force microscopy data showed structurally intact viruses surrounded by multiple MNPs as shown in Fig.1.



Genetic modification of cells on a cell separation column modified with formulated magnetic viral vectors (magselectofection) is highly efficient in hematopoietic stem cells and mesenchymal stem cells from human umbilical cord, hUC-HSCs and hUC-MSCs, respectively. Under optimized transduction conditions, viral magselectofection of hUC-MSCs with SO-Mag2 lentivirus complexes at MOI as low as 0.5 pfu/cell (determined using CMS5 cells and a standard polybrene infection) resulted in 60-100% transduced cells depending on the donor (Fig. 2). The “paradoxical” result of achieving 100 % transduced hUC-MSCs at 0.5 MOI makes it obvious that the infectivity of the virus is determined by both the fraction of infectious particles in the preparation and internalization efficiency of the particles. It suggests that virus internalization is significantly increased when using magnetic complexes and magnetic force. We speculate that magselectofection or magnetofection can be used as a tool to estimate more realistically biological virus titers. We have also found a two-fold increase in the percentage of the reporter gene expressing cells post-magselectofection of the hUC-MSCs pre-labelled with 20 pg Fe/cell of MNPs. This observation is important for the optimization of protocols for the generation of magnetically labeled and genetically-modified cells.



ACKNOWLEDGEMENTS: Financial support through the European Union grant FP6- SHB-CT-2006 019038 "Magselectofection", through the German Research Foundation through project PL 281/3-1 "Nanoguide" and through the German Excellence Cluster "Nanosystems Initiative Munich" are gratefully acknowledged.

Targeting of lentiviral vectors and positioning of transduced cells by magnetic nanoparticles and magnetic field

Christina Trueck[1] , Olga Mykhaylyk[2], Ulrich M Becher [3], Bernhard Gleich[4], Daniela Wenzel[5], Bernd Fleischmann[5], Christian Plank[2] and Alexander Pfeifer[1]

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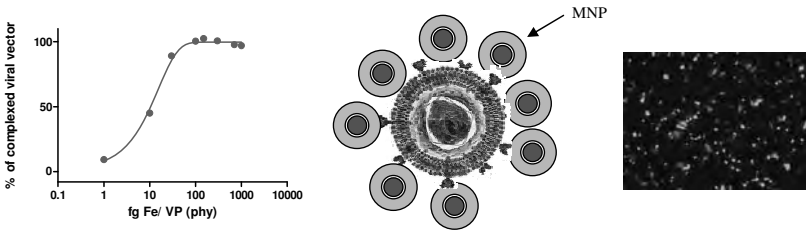
Magnetic nanoparticles (MNPs) are promising tools in regenerative medicine. In this study, we combined targeting of lentiviral vectors (LVs) and positioning of transduced cells by magnetic nanoparticles and magnetic fields. The complexation of LVs with MNPs enhanced transduction efficiency of endothelial cells and enabled direct targeting of LVs by magnetic force, even in perfused vessels. The magnetic moments of the transduced cells were high enough to enable positioning of MNP-containing endothelial cells at the intima of vessels under flow conditions *ex vivo* and *in vivo* in an injury model of the mouse carotid artery. To further optimize the transduction efficiency, we tested a range of MNPs of the core-shell type with an iron oxide core diameter of about 10 nm and different coatings in a variety of bioassays. Some of the newly analyzed MNPs improved magnetic sedimentation of lentiviral particles compared to previously tested, commercially available ones. Importantly, the new MNPs significantly increased lentiviral transduction efficiency with enhanced provirus integration per genome. Furthermore, we tested different solvents and determined an optimal ratio for the complexation of MNPs and viral particles by a p24 Elisa. Thereby we obtained an increased transduction efficiency in several cell lines including Human Umbilical Vein Endothelial Cells (HUVECs), bovine Pulmonary Arterial Endothelial Cells (bPAECs) and HL-1 (murine cardiomyocytes) as analyzed by Flow cytometry and Fluorescence microscopy. These results are the basis for further *in vivo* studies of MNP-assisted lentiviral gene and cell targeting.



(A) (B) (C)

Binding kinetic of a MNP-LV complex (A); schematic drawing of a MNP-LV complex (B); fluorescence image of CMV-GFP transduced HUVECs (C)

Detection of magnetic nanoparticles in a tumour after cytokine delivery in the presence of a magnetic field

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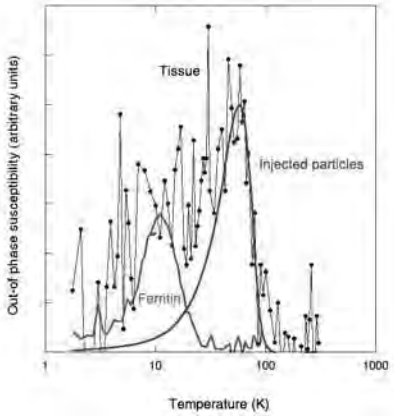
Over the last decades, a great effort is being performed in the development and optimization of magnetic nanoparticles for controlled local drug release. The necessary next step is to check whether they reach their target in a living organism and their following removal.

In this work, magnetic nanoparticles were synthesized by thermal decomposition of iron acetylacetonate in an organic medium and modified with dimercaptosuccinic acid (DMSA). Afterwards, γ-Interferon, one of the most promising cytokines, was attached to the DMSA-coated magnetic nanoparticles and the whole compound (INF-NP) was intravenously injected to mice with induced tumours in both back legs. A magnet was externally attached to one of the legs, to concentrate the particles, while the other was used as control.

The results show that the tumour size is significantly reduced only in the leg treated with the INF-NP and the external magnet. The presence of the magnetic nanoparticles in the tumorous tissues, has been studied by Transmission Electron Microscopy (TEM) and AC susceptibility measurements. Aggregates of magnetic nanoparticles have been observed in the targeted tissue (see figure). Furthermore, the magnetic characterisation, has also confirmed the presence of the injected magnetic carrier, with the ability of discerning also the endogenous ferritin, previously characterized by the same technique.



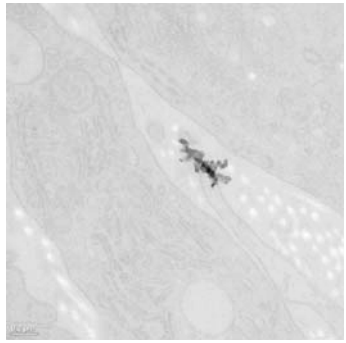


Fig. Left: Temperature dependence of the out-of-phase susceptibility of the tumorous tissue. The magnetic behaviour of the injected particles and the endogenous ferritin are also plotted for comparison. Right: Transmission electron micrograph of the studied tissue showing the presence of the injected particles.

Nitrocatechol-dispersant stabilized superparamagnetic iron oxide nanoparticle assembly into magnetically actuated membranes

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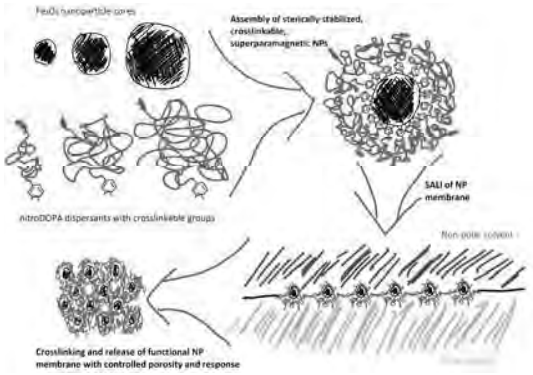
Well stabilized superparamagnetic iron oxide nanoparticles (SPIONs) are attractive building blocks for assembling smart materials which respond to external magnetic fields. Creating ferrofluids of superparamagnetic particles which can be processed at high temperatures and in aqueous conditions for applications in hydrogels or polymers for biotechnology applications are a big challenge. The most important requirement is particle stability, which requires irreversibly bound anchor groups for dispersants that control the chemical properties of the individual SPION under these conditions. A controlled chemistry of the individual SPION combined with a bottom up approach to assemble magnetic nanoparticles will make it possible to assemble new nanomaterials such as responsive membranes for in vitro and in vivo applications where externally controlled and reversibly switched permeability is of interest.



We have recently demonstrated that we can stabilize SPIONs which fulfill the stability criteria described above using nitrocatechol anchors for self-assembling dispersants [1]. This allows us free control over the dispersant type (hydrophilic/hydrophobic, molecular weight, linear/dendritic, end functionality type and density) and grafting approach, e.g., grafting to for easy assembly of mixed functionalities or grafting from for radical polymerization of responsive polymer shells. Furthermore, we have shown that the magnetite core size can be tuned by synthesis in the microwave in the range from 3-17 nm [2] and recently also shown how synthesis in the oil bath can be used in a similar way to synthesize even higher quality SPION cores in the same size range. These developments give us control over core and shell size and properties independently of each other. We will present a toolbox of mix-and-match properties of SPIONs with the themes above varied and an example of how a variation in core size and dispersant shell thickness affects the stability of SPIONs [2].

Assembly into smart membrane materials of stable and homogenous properties also requires guiding stable SPIONs into a superstructure. We will demonstrate how oil-water interfaces known to produce ordered assemblies of microparticles can be used to assemble monolayers of dispersant stabilized SPIONs with unique adsorption behavior at the interface and how particle stability at such interfaces is related to, but more demanding than, bulk particle stability [2]. The new methodology being developed to probe and control the assembly of such membranes will also be highlighted. As outlook we will show our current efforts to combine SPION building blocks with different properties with tailored self-assembly to produce nanoparticle-polymer hybrid membrane materials which can respond to actuation with externally applied direct or alternating magnetic fields.

1. Amstad, E., et al., *Ultrastable Iron Oxide Nanoparticle Colloidal Suspensions Using Dispersants with Catechol-Derived Anchor Groups*. Nano Letters, 2009. **9**(12): p. 4042-4048.
2. Isa, L., et al., *Self-assembly of iron oxide-poly(ethylene glycol) core-shell nanoparticles at liquid–liquid interfaces*. Chimia, 2010. **64**(3).

Talk 11

Magnetically-Responsive Nanoparticles for Vectored Delivery of Cancer Therapeutics

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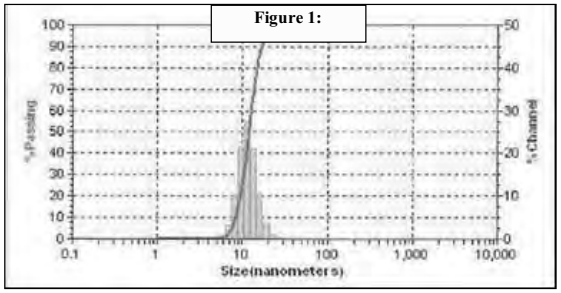
A major cause of therapeutic failure in medical oncology is the development of tumor cell resistance to chemotherapeutics, the intra-tumoral levels of which may be pharmacologically and physiologically limited. Thus, the ability to physically target therapeutics to the tumor microenvironment and overcome the limitations of both tumor interstitial pressure and inadequate drug levels, while limiting off-target toxicity, could favorably impact therapeutic indices. We propose that magnetite-based, silica-coated superparamagnetic nanoparticles (MNPs) could effectively serve this purpose for superficial and even visceral tumors using clinically-scalable, designed magnetic field shapes and gradients.



MNPs with a narrow dispersion around a prescribed average particle diameter (Figure 1: Dynamic light scattering determination of particle size and size distribution of silica-coated MNPs), are prepared using a modification of the Massart procedure [*IEEE Transactions on Magnetics,* **17,**1247,1981]. Drug constructs can then prepared via carbodiimide reactions with vinyl silane modified MNPs, with stable dispersions obtained in specific dispersants.

The ability to magnetically-enhance tumor extravasation of intravenously (iv)-administered MNPs was evaluated in nude mouse models of human ovarian and breast tumor xenografts with encouraging results. Human breast tumor cells were injected in the mammary fatpad, and when resulting tumors reached > 3-4 mm diameter, MNPs were injected iv while a focused magnetic field was juxtaposed over the tumor. Magnetic resonance imaging (MRI-Figure 2) and scanning electron microscopy (SEM-Figure 3) were used to evaluate MNP tumor localization. Evidence from both techniques supports the successful tumor localization of MNPs.

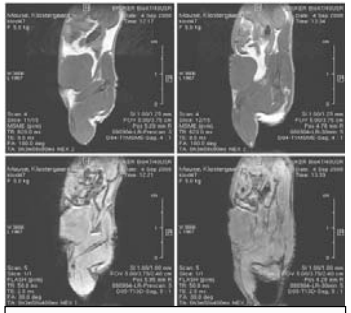


Figure 2: Pre- (left panels) and post- (right panels) MNP injection MRIs

These localization studies will be extended to toxicity and anti-tumor efficacy studies in human tumor xenograft models with drug-loaded MNPs, and with comparisons to free drugs, as well as biodistribution analyses of the fate of the MNPs themselves. We propose that this technology is scalable to many types of presentations of both superficial as well as certain viscerable tumors with existing magnetic technology.

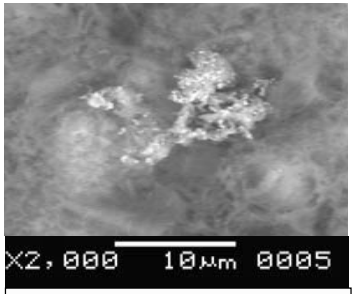


Figure 3: SEMs of tumor sections

Support: Department of Defense grants through the Alliance for Nanohealth and the Breast Cancer Research Program, and a sponsored research agreement with NanoBioMagnetics, Inc.

Talk 12

SYSTEMICALLY DELIVERED MAGNETIC NANOPARTICLES FOR TARGETED OCULAR MOLECULAR IMAGING AND DRUG DELIVERY

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The eye is an ideal target organ for novel drug delivery and *in vivo* imaging for common retinal pathology. In contrast to the rest of the body, the clear optical media of the eye offers a unique opportunity to directly visualize the progression of diseases as well as the effects of their respective treatments.

The current methods for intraocular drug delivery, topical and intravitreal injections, both have inherent shortcomings. The former is typically limited to the anterior segment of the eye while the latter comes with risks of serious complications such as endophthalmitis and retinal detachment, both of which can lead to blindness.

Systemically delivered biocompatible magnetic nanoparticles could be a viable alternative to drug delivery and molecular imaging of the eye. The unique properties of the nanoparticles highlight the value and efficacy of magnetic drug delivery. Drugs bound to magnetic particles can be injected directly into the bloodstream and concentrated in the eye with the use of a magnetic field. This enables us to direct medications and biological imaging markers to the eye.

We attempted to direct magnetic particles in the bloodstream to the eye. Cobalt nanoparticles sized 50 nm in diameter and magnetite microspheres sized 1 µm were radiolabeled with ^{99m}Tc for biodistribution study purposes. These particles were injected by tail vein into C57Bl6 mice. A targeted magnetic field was applied to the mice during a 30 minute circulation period. Subsequently, the mice were sacrificed and organs removed for radioactive biodistribution and histological studies. Confocal microscopy was used to visualize particles in cross-sections of the eye, retinal whole mounts and the liver.

Radioactive biodistribution studies indicated slightly increased particle activity in the eyes of mice treated with a magnetic field compared to non-magnet treated mice. These results were confirmed by confocal images (**Figure 1**).

These preliminary results show the potential of discovering a novel method of ocular drug delivery and targeted molecular imaging using systemically delivered magnetic nanoparticles.

Figure 1. Left - flat mount image without magnet use. Middle – flat mount image with magnet use. Right – cross section view of retina (red) showing nanoparticle incorporation (white/blue).

Talk 13

Interaction of Low-Frequency Ultrasound and Magnetic Force on Magnetite Nanoparticle Carrying Tissue Plasminogen Activator

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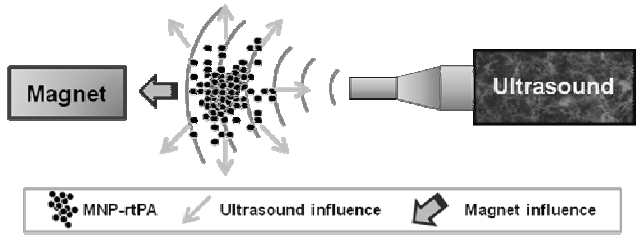
We recently demonstrated feasibility of target thrombolysis with recombinant tissue plasminogen activator (rtPA) covalently bonded to magnetic nanoparticles (MNP) under magnetic guidance in a rat embolic model (Biomaterials 30:3343, 2009). Since ultrasound may facilitate thrombolysis in thromboembolic diseases, we tested the feasibility of combination of ultrasound and the magnet/MNP-rtPA system in vitro and determined whether ultrasound may enhance dispersion of MNP-rtPA under magnetic influence without jeopardizing its enzyme activity. Burst-mode low-frequency (28 kHz) ultrasonic waves were delivered concurrently with magnet guidance procedure on the silastic tube mimicking isolated artery. The ultrasound energy penetrated through the wall of the silastic tube appears to be similar to that through the wall of aortic artery from the rat, as measured by a needle hydrophone. A bolus of MNP (0.2 mg) with magnetite core and polyacrylic acid coating was delivered and guided by a permanent magnet (maximal magnetic strength of 4000 gauss) to the middle of the silastic tube filled with phosphate-buffered saline; the pellet of MNP thus formed under the influence of the magnet was subjected to 28 kHz ultrasound and recorded by video microscopy. By analysis of the dispersion area in response to ultrasound, it was found that when peak pressure and burst length were fixed to be 3.7 MPa and 100 ms/pulse, pulse repetition frequency equal or larger than 0.2 Hz may cause consistent dispersion of MNP. A single pulse with a burst length of up to 1.5 sec did not alter the enzyme activity of MNP-rtPA; however, ultrasound with higher energy (7.1 MPa, 500 ms/pulse) attenuated enzyme activity of MNP-rtPA by ~40%. Nevertheless, repeated exposure to acoustic field (3.7 MPa, 500 ms/ pulse) for up to 20 pulses did not alter the enzyme activity. In conclusion, integrating ultrasound and magnetic targeting to enhance thrombolytic efficacy appears to be feasible, where ultrasonic sonication exists conditions to concurrently facilitate MNP-rtPA dispersion and at the same time minimize the detrimental effect on rtPA activity.



Talk 14

Engineering the surface of magnetic nanoparticles by adsorption of double hydrophilic block copolymers

Stéphanie Louguet [a], Christophe Schatz [a], Romain Epherre [b], Stéphane Mornet [b], Etienne Duguet [b], Sébastien Lecommandoux [a]

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Magnetic nanoparticles are currently widely investigated and used in life sciences as contrast agents for biomedical imaging applications as well as heating sources for hyperthermia. However, their lack of stability in *in vivo* conditions, their potential toxicity due to ion leakage and their poor stealthiness and targeting abilities must be improved through chemical modifications of their surface. Polymers and copolymers have been widely used for nanoparticle coating either by covalent chemistry or through physical adsorption involving weak interactions.

This project deals with the development of stimuli-responsive hybrid nanoparticles that can offer new therapeutic strategies for pathologies involving the crossing through the blood brain barrier. These systems are built around a magnetic core based on perovskite, with a Curie temperature tunable in the body temperature range and are able to act as contrast agents in magnetic resonance imaging (MRI) as well as heat inductors in AC magnetic fields. This inorganic core is surrounded by a thermo-responsive polymeric corona that controls the loading and the release of drugs, and can be functionalized by specific ligands ensuring the targeting specificity.

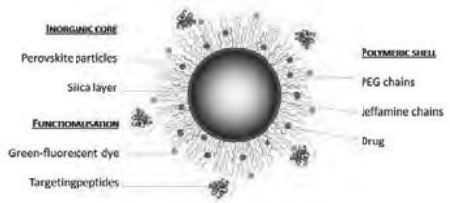

Figure 1: Schematic representation of bio-functionalized hybrid magnetic particles.

We report here on a new method to functionalize the surface of perovskite-based nanoparticles by adsorption of a double hydrophilic poly(ethylene oxyde)-*block*-polylysine (PEO-*b*-PLys) copolymer. A careful investigation of the adsorption conditions by dynamic light scattering, adsorption isotherms, isothermal titration calorimetry and neutron scattering allowed us to fully understand the behaviour of this copolymer at the interface and to determine an experimental window where polysine blocks are completely adsorbed and poly(ethylene oxide) PEO chains freely protruding in solution. In addition, the poly(ethylene oxide) chains were end-functionalized by either a targeting peptide or a fluorescent probe. The biodistribution, the efficiency of the targeting and the potential of the modified magnetic nanoparticles as contrast agents were assessed by *in vitro* MRI experiments and *in vivo* evaluation.

Another interesting aspect of this study lies in the thermo-sensitivity of the corona which enables the release of previously entrapped bioactive molecules thanks to the increase in temperature induced by the magnetic cores. Co-adsorption of polymer chains with different thermo-sensitivities allows controlling the transition temperature in a biologically relevant range (about 42°C). The physical entrapment of hydrophobic drugs within the adsorbed copolymer layer was investigated as it may be of interest to have a colloidal system with both imaging and therapeutic capabilities. Common techniques such as fluorescence spectroscopy were carried out to evaluate the drug loading in the copolymer layer. Lastly the co-adsorption of PEO-*b*-PLys and thermoresponsive copolymers was explored from a fundamental point of view at first and then as a mean to trigger the drug release when particles are heated by application of an AC magnetic field.

Talk 15

Effects of magnetite loading and time-varied magnetic field on MNP transport in soft medium and cellular uptake

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Many magnetic nanoparticle (MNP) studies have shown that they are promising vectors for targeted drug-delivery. However, clinically, their full potential has not been realized due in part to the difficulties in transporting the carriers through soft tissues to targeted sites within the body. In order for MNPs to move within a tissue, the MNP must overcome an initial yield stress, present in the tissue. Both, the yield stress and viscous drag may depend strongly on the rate of variation of the magnetic forces, on MNP size, shape, degree of loading with magnetic material and on MNP surface properties. Our preliminary studies in viscous gel have shown that the addition of an AC field perpendicular to the desired direction of motion and the static magnetic field gradient can enhance transport of MNPs, by decreasing the effective local viscosity of the gel resulting in lower magnetic force needed to pull the particles. Similarly, it is hypothesized that the "oscillating" effect caused by an AC field will also increase the cellular uptake of MNPs into cells in vitro and in vivo. Polylactide-based magnetic particles (~300nm) were prepared by a modified emulsification-solvent evaporation method incorporating 10-70% (w/w) magnetite. MNP velocity experiments were conducted using a viscous (15,000 cp), uniform, translucent gel with MNPs varying in magnetite loading. It was found that the "oscillating" effect of an AC magnetic field greatly improves the ability of MNP transport within viscous medium. Combined application of an AC magnetic field with the static field gradient resulted in a nearly 30-fold increase in MNP transport efficiency in viscous gel for 30%(w/w) magnetite loaded particles as compared to static field conditions. It is expected that this result is occurring as a consequence of a reduction of viscous drag within the medium. The effects of the combined application of the AC field with the static gradient were then examined for cellular uptake experiments. Static field only experiments showed that there was a significant difference ($p<0.01$) between 10% (w/w) magnetite loaded MNPs and 70% (w/w) magnetite loaded MNPs at all time points. However, a significant difference ($p<0.05$) was only present for 30% (w/w) magnetite loaded MNPs and 70% (w/w) magnetite loaded MNPs for the first three time points (totaling 6 hours), Figure 1. Our data suggests a relationship between the force applied by the MNPs and the uptake of these particles into cells. It is evident that the particles seem to be driven into the cells. At this point it is unknown whether the mechanism is through a passive transport or via an active cellular response triggered by the forces exerted on the cell by the particle. MNP uptake experiments incorporating the effect of an AC field are currently in progress in order to determine whether the "oscillating" effect will increase the cellular uptake of varying magnetite incorporated MNPs.

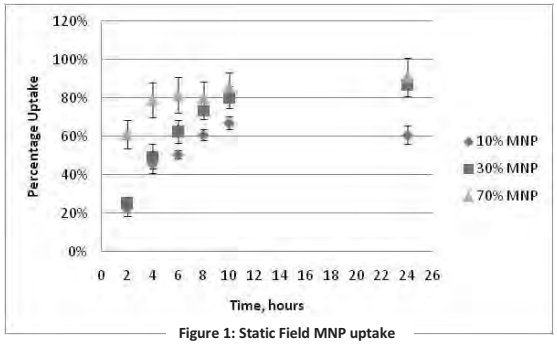

Figure 1: Static Field MNP uptake

*Research funded by NIH Ruth Kirschstein National Research Service Award for Pre-Doctoral Fellows

Talk 16

Magnetic Endothelialization of Vascular Stents Facilitates Healing and Improves Patency

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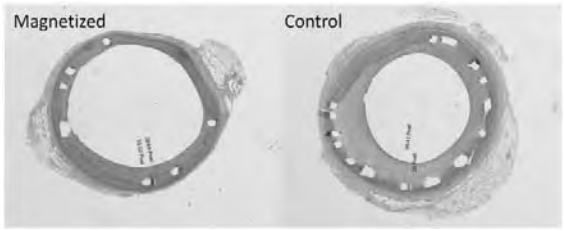
Treatment of symptomatic atherosclerosis often involves percutaneous transluminal (balloon) angioplasty with subsequent stent deployment to prevent restenosis of the diseased vessel. These procedures cause varying degrees of injury to the vascular endothelium, which can cause neointimal hyperplasia and result in restenosis[1]. Recently, drug-eluting stents (DES) have become the preferred option to using bare metal stents (BMS) for placement in coronary arteries. Paclitaxel and Sirolimus - eluting stents show improved patency, but are susceptible to late stent thrombosis[2]. The current work indicates that magnetic vascular stents endothelialized using superparamagnetic nanoparticles exhibit improved patency without the need for prolonged anticoagulative therapy.

Bare metal stents were milled and laser cut from Carpenter 2205, a high chromium content (~21%) austenitic-ferritic stainless steel. Electrochemical polishing and acid pickling were used to improve surface smoothness. Stents were magnetized via exposure to a 3T MRI field, and residual magnetism was verified using iron filings. Autologous endothelial cells (EC) were isolated from pigs and endocytosed with 200 nm PLGA-coated magnetite particles (PMP). Pilot studies confirmed cell viability and specific attraction to magnetized stents. Seven magnetized, and as many control stents were implanted into pigs, and SPM-labeled EC were delivered *in vivo*. After one month, stents were removed, sectioned, stained and micrographed. Image analysis software was used to measure media penetration depth and neointima thickness corresponding to each stent strut.



All 14 stents remained fully patent, and subjective examination revealed no traces of thrombosis, inflammation, or corrosion in any of the 14 stents. Quantitative histomorphometric analyses indicated that, for similar penetration depths into the *tunica media* (sub-endothelial layer of the native vessel), magnetically-seeded stents had less prominent neointima. The current study suggests that magnetic nanoparticles can be used to rapidly endothelialize magnetic grafts, thereby facilitating healing and improving stent patency in pigs within one month.

[1] R. Kornowski et al., "In-Stent Restenosis: Contributions of Inflammatory Responses and Arterial Injury to Neointimal Hyperplasia," *Journal of the American College of Cardiology* 31, no. 1 (1998): 224–230.
[2] Aloke V. Finn et al., "Vascular Responses to Drug Eluting Stents: Importance of Delayed Healing," *Arterioscler Thromb Vasc Biol* 27, no. 7 (July 1, 2007): 1500-1510.

A Magnetically Controlled Drug Delivery Device

F. N. Pirmoradi[1], L. Cheng[1], J. Jackson[3], H. Burt[3], M. Chiao[1,*]
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We report a magnetically controlled, on-demand drug delivery device that does not need an on-board power source. Drug-loaded micro reservoirs (∅6 mm × 480 μm) are sealed by a magnetic PDMS (polydimethylsiloxane) membrane (∅6 mm × 35 μm) containing a via (∅200 μm). Controlled drug release is demonstrated by modulating the magnetic actuation frequency of the magnetic PDMS membrane.

Polymeric and thin-film drug delivery systems that are responsive to external stimuli such as electrical [1] or magnetic fields [2] have the advantage of on-demand drug release compared to diffusion or degradation-release based polymer delivery systems. However, the drug delivery rate is often diffcult to control from such systems. In the past decade, Microelectromechanical System (MEMS)-based controlled drug delivery devices have provided precise dose control but all of them require an on-chip battery for operation [3,4]. As a result, the overall device volume may be dominated by the size of the battery. This paper demonstrates a MEMS device for on-demand and controlled drug release that uses direct magnetic to mechanical energy conversion to drive a magnetic diaphragm – drug pumping system. Although pumping drugs is not a new concept, previous MEMS approaches use channels in their systems which result in requiring higher magnetic fields and larger membranes due to pressure loss.

As shown in Fig. 1, a 35 μm-thick magnetic PDMS membrane seals a drug-loaded PDMS reservoir (∅6 mm × 480 μm). The PDMS membrane has a via (∅200 μm) that is fabricated by a laser ablation process. When a magnetic force is applied, the membrane deforms down and pushes the drug out in an analogous fashion to squeezing water out from a flexible water bottle. The amount of drug delivered and the timing of the dose may be precisely controlled by adjusting the external magnetic field. Figure 2 demonstrates a pilot study on drug release as a function of time. The slope of the drug release curve is a quantitative measure of the drug release rate. It is shown that when the device is actuated using a periodic magnetic field (~0.2 T, 25 actuations/minute), the release rate of a mock up drug, methylene blue, is on average 81% higher than that released when the device is in a diffusion state (i.e. no applied magnetic field). The device demonstrates controlled drug release rates using different magnetic actuation frequencies. When the frequency of the magnetic actuation increases from 25 to 90 actuations/minute, the release rate increases from 56.6 ng/minute to 98.9 ng/minute. This paper will detail the fabrication process and in vitro drug release experiments.

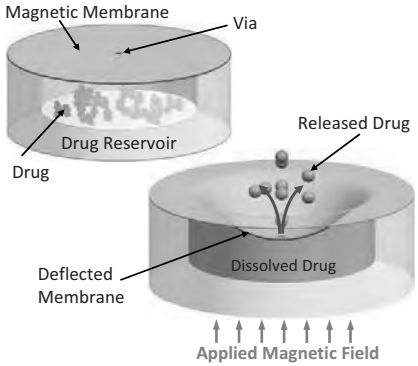

Figure 1. Principle of operation.

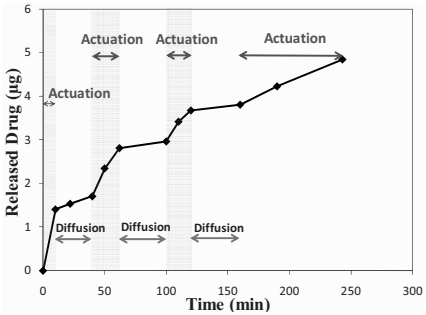

Figure 2. Drug release based on the sequences of excitation of the device in a periodic magnetic field (25 actuations per minute) and diffusion (without actuation).

References

[1] K. C. Wood, N. S. Zacharia, et al. *PNAS*, vol. 105, pp. 2280, 2008.
[2] T.-Y. Liu, S.-H. Hu, et al. *J. of Controlled Release*, vol. 126, pp. 228, 2008.
[3] J. T. Santini, M. J. Cima, and R. Langer, *Nature*, vol. 397, pp. 335, 1999.
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Magnetic Scaffold for Advanced Osteochondral Tissue Engineering

Alessandro Russo,[1,2] Daniela Casino,[1,2] Tatiana Shelyakova,[1,2] Silvia Panseri,[1,2] Valentin Dediu,[3] Manuel Bañobre,[4] José Rivas,[4] Maurilio Marcacci,[1,2]
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Introduction

Osteochondral region has a very low capacity to regenerate, therefore, biodegradable polymer and hybrid materials have been widely investigated as scaffolds for osteochondral regeneration. Although, they have not yet been incorporated into daily clinical practice. In fact, the overall success of osteochondral tissue engineering strongly depends on two fundamental aspects: stability of bone/scaffold interface and nutrients, oxygen and angiogenic factors transfer into the scaffold. Our project challenges further development in osteochondral tissue engineering using magnetism. A magnetizable scaffold has been developed to improve angiogenesis by growth factors delivery *via* magnetic guiding. Magnetic forces evaluations for scaffold design and in vitro tests to evaluate cellular activity on magnetic scaffold are presented.

Materials and methods

A commercial biomimetic porous scaffold of hydroxyapatite and collagen for osteochondral defects (Finceramica S.r.l.) has been magnetized by consecutive impregnation with a magnetite based ferrofluid [1]. A scaffold prototype with a saturation magnetization of 25 emu/g has been considered. Nanoparticles adopted in our simulations are $Fe_3O_4@SiO_2$ with about 15 emu/g saturation magnetization at 10 kOe at room temperature (nanoparticles characteristics in Table 1).

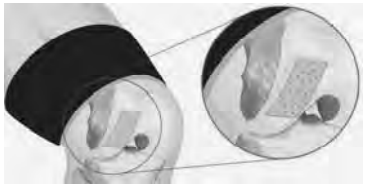
Table 1. *$Fe_3O_4@SiO_2$ Magnetic nanoparticle characteristics*

Particle size	20 nm (diameter)
Size distribution	20±4 (total particle size) 9±2 (magnetite core)
Mass fraction	20-30% iron oxide, 80-70% silica

Computer modelling (COMSOL Multiphysics) has been adopted to evaluate optimal strategies for magnetic forces generation inside a magnetic scaffold for a severe osteochondral defect (3x3x1cm^3). Two strategies have been hypothesized for clinical application and simulated by software: first, implanting small magnets into the bone under the scaffold; second, placing an external ring magnet around the knee. In vitro test with mesenchymal stem cells from human bone marrow have been performed in order to evaluate cellular activity on magnetic scaffold.

Results

Magnetic forces evaluations suggest that implanting four small biocompatible magnets (Ø 2, h 8 mm, Br=1.4 T) into the bone under the scaffold, magnetic attraction force on a nanoparticle inside the scaffold is 0.2-1.5*10^{-21} N. Differently, adopting an external ring magnet ($Ø_{ext}$ 21,$Ø_{int}$ 15, h 10 cm, Br=1.4 T) around the knee, magnetic attraction force is 3-4*10^{-19} N, two orders of magnitude greater compared to the first strategy. Such force overcomes nanoparticle gravity (0.57*10^{-19} N) and in a condition of very low flux as in osteochondral region, this should be suitable for nanoparticles guiding. The *in vitro* preliminary results demonstrate the ability of these new magnetic scaffolds to support cell adhesion and proliferation. The cells exhibited a high rate of proliferation, covered the whole surface and filled most of the macropores (diameter from 100 to 550 µm) of the scaffold after 10 days with 10 fold increase in the cell amount compared to day 0.



Conclusions

Multiphysics modelling was useful to design magnetic configuration for a novel magnetic scaffold. According to preliminary results, this scaffold allows cellular proliferation and can be suitable for magnetic guiding of nanoparticles inside the scaffold, opening the way to a new material for large joint defects treatment combining magnetic properties with tissue engineering strategies.

References

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Acknowledgments

The authors thanks EU for the financial support ("MAGISTER" project NMP3-LA-2008-21468).

Talk 19

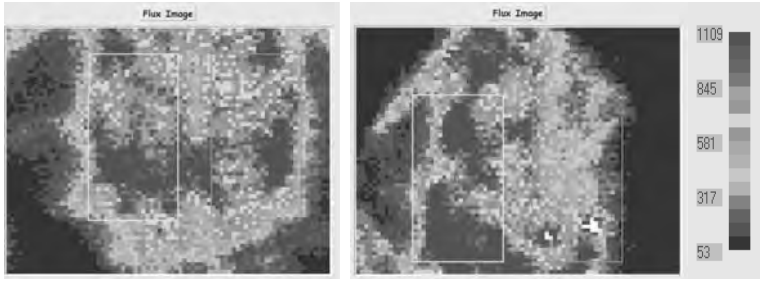
Intra-Arterial Application of Magnetic Nanoparticles for Targeted Thrombolytic Therapy: A Rat Embolic Stroke Model

Tony Wu[1]; Hsiao-Pin Lin[2]; Chia-Ning Hu[1]; Jyh-Ping Chen[3]; Yeu-Jhy Chang[1] Shih-Tseng Lee[4]; Yunn-Hwa Ma[2*]

[1]Department of Neurology, Chang Gung Memorial Hospital; [2]Department of Physiology and Pharmacology, College of Medicine, Chang Gung University; [3]Department of Chemical and Materials Engineering, College of Engineering, Chang Gung University; [4]Department of Neurosurgery, Chang Gung Memorial Hospital, Tao-Yuan, Taiwan.

Recombinant tissue plasminogen activator (rtPA) is used for acute ischemic stroke therapy with high incidence of hemorrhagic side effect. We recently demonstrated feasibility of magnetic target delivery of rtPA using magnetic nanoparticle as the drug carrier in a rat embolic model with the emboli in the iliac artery. To further determine the feasibility of this approach in the treatment of ischemic stroke, a rat embolic stroke model with an easy access of a magnet appears to be imperative. In anesthetized rats, the right internal carotid artery was occluded by injection of a whole blood clot (1×1 mm) containing indocyanine green, allowing visualization of the clot lodging in the artery. Introduction of a clot to the right internal carotid artery reduced the right, but not left, cerebral blood flow measured by a laser Doppler imager (see the Figure below). Intra-arterial infusion of rt-PA at 0.5 and 1 mg/kg significantly reversed the brain perfusion within 30-40 min (n=5-6). Placement of an NdFeB magnet above the internal carotid artery caused retention of polyacrylic acid-coated magnetic nanoparticle (100 nm) against hemodynamic dragging force. Our results demonstrated feasibility of this rat embolic stroke model for the study of magnetic targeted delivery of thrombolytic drugs for treatment of cerebral thromboembolic diseases.

(-) clot **(+) clot**



Talk 20

Thursday, May 27, 2010				
7:30	**Registration desk opens**			
8:30	Krishnan, Kannan	**Tutorial I** on Magnetic Things We All Should Know	Seattle, U.S.A.	Tutorial 1
	Session 4: Microsphere Synthesis - *Chair: Kathy Saatchi (Canada)*			
9:00	Ettenauer, Marion	Preparation of Magnetic Cellulose Microparticles as Markers in Extracorporeal Blood Purification	Krems, Austria	Talk 21
9:15	Furlan, Marco	Magnetic Gelation: A new Method to Produce Anisotropic Porous Polymeric Materials	Zurich, Switzerland	Talk 22
9:30	Kuznetsov, Anatoly	Magnetically guided microscopic electric batteries: manufacturing and biological applications.	Moscow, Russia	Talk 23
9:45	Xu, Hong	Development of immunomagnetic beads for rapid detection of Escherichia coli O157:H7 by immunomagnetic enrichment and real-time PCR	Shanghai, China	Talk 24
10:00	**Group photograph**			
10:15	**Coffee break / poster session / exhibitors**			
	Session 5: Nanoparticle Synthesis and Analysis - *Chair: Kannan Krishnan (U.S.A.)*			
10:45	Amstad, Esther	Adding Different Functionalities to Ultra-stable Superparamagnetic Iron Oxide Nanoparticles through Controlled Surface Modification	Zurich, Switzerland	Talk 25
11:00	Wawrzik, Thilo	Multi-Variant Magnetic Particle Spectroscopy for Magnetic Nanoparticle Characterization	Braunschweig, Germany	Talk 26
11:15	Dutz, Silvio	Physical Properties of Water Based Large Single Domain Particle Dispersions of Magnetite	Jena, Germany	Talk 27
11:30	Guerler, Celin	Magnetically Induced Hot Spots Promote a Locoregional Chemical Reaction	Düsseldorf, Germany	Talk 28
11:45	Jain, Nirmesh	Anchored Steric Stabilisation of Superparamagnetic Nanoparticles for Biomedical Applications	Sydney, Australia	Talk 29
12:00	Wang, Shan X.	Magneto-Nano Chips for Biomedical Diagnostics	Stanford, U.S.A.	Invited talk 3
12:40	**Lunch**			
	Session 6: Nanoparticle Synthesis and Analysis - *Chair: Thompson Mefford (U.S.A.)*			
14:00	Lellouche, Jean-Paul	Hydrophilic Maghemite (gamma-Fe2O3) Nanoparticles - Aggregation Control Using an Ultrasound-Assisted Doping Process of Particle Surface	Ramat Gan, Israel	Talk 30
14:15	Mahmoudi, Morteza	Synthesis of Rod-Shaped Superparamagnetic Iron Oxide Nanoparticles with Polyvinyl Alcohol	Tehran, Iran	Talk 31
14:30	Mistlberger, Guenter	Magnetically controlled, multifunctional nano devices	Graz, Austria	Talk 32
14:45	Nikitin, Maxim	Self-assembly of multifunctional nanoparticles via barnase and barstar	Moscow, Russia	Talk 33
15:00	Pellegrino, Teresa	Magnetic based nanobeads as multifunctional platforms for biomedical applications	Lecce, Italy	Talk 34
15:15	Truonc Phuoc, Lai	Magnetic nanosystems for the sentinel nodes detection	Strasbourg, France	Talk 35
15:30	Turcu, Rodica	Synthesis and characterization of biocompatible magnetically controllable nanostructures using different polymers or block copolymers	Cluj-Napoca, Romania	Talk 36
15:45	Yang, Liangrong	Fabrication of Temperature- and pH- Responsive Magnetic Nanoparticles and Their Reversible Agglomeration in Aqueous Milieu	Beijing, China	Talk 37
16:00	Yoo, Myung-Ik	Synthesis of Individually Water-Soluble, Biocompatible, and Angiogenesis-Targeting Superparamagnetic Iron Oxide Nanoparticles	Seoul, Korea	Talk 38
16:15	Zierold, Robert	Synthesis of Biocompatible Magnetic Test-Tube-Shaped Nanoparticles by Atomic Layer Deposition	Hamburg, Germany	Talk 39
16:30	**Coffee break / poster session / exhibitors**			
	Session 7: Analytical Methods - *Chair: Sara Majetich (U.S.A.)*			
17:00	Dennis, Cindi	Interactions in Magnetic Nanoparticle Systems: How to Identify Them and Their Consequences	Gaithersburg, U.S.A.	Talk 40
17:15	Lim, JitKang	Magnetophoretic motion control of individual Brownian nanoparticles	Penang, Malaysia	Talk 41
17:30	Liu, Wenzhong	Magnetic Nanoparticle Temperature Estimation Using AC Susceptibility	Wuhan, China	Talk 42
17:45	Salaklang, Jatuporn	Fixed bed magnetic reactor for surface derivatization of Superparamagnetic Iron Oxide Nanoparticles	Fribourg, Switzerland	Talk 43
18:00	Schaller, Vincent	Determination of nanocrystal size distribution in magnetic multi-core particles including interactions and magnetic anisotropy	Göteborg, Sweden	Talk 44
18:15	Tarn, Mark	Continuous magnetic microparticle-based sandwich immunoassays in a multilaminar flow microreactor	Hull, U.K.	Talk 45
18:30	Teste, Bruno	Magnetic core shell nanoparticles based homogeneous immunoassay	Paris, France	Talk 46
18:45	**Poster session (even numbers) with beer and pretzels - PLEASE RATE POSTERS** *- sponsored by* ***Diagnostic Biosensors LLC*** *and* ***Life Technologies Corp.***			
20:15	**Free evening - use it to meet old friends, discuss new collaborations, and enjoy Warnemünde on your own!**			

Preparation of Magnetic Cellulose Microparticles as Markers in Extracorporeal Blood Purification

Marion Ettenauer,[a] Steffen Fischer,[b] Katrin Thümmler,[b]
Viktoria Weber,[a,*] Dieter Falkenhagen[a]

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[b]*Institute of Plant- and Wood Chemistry, Technische Universität Dresden, D- 01737 Tharandt, Germany*
*email: viktoria.weber@donau-uni.ac.at

Magnetic micro- and nanoparticles have found widespread application in biomedicine, medical diagnosis and therapy.[1] For these applications, iron oxide particles such as magnetite (Fe_3O_4) or its oxidized form maghemite (γ-Fe_2O_3) are commonly used because of their favourable chemical and physical properties, e.g. superparamagnetic behaviour at room temperature.
For special biomedical *in vivo* applications, a spherical morphology with a defined particle size, the possibility of surface functionalization, as well as biocompatibility are prerequisite.[2] A new extracorporeal blood purification technique, the Microspheres-Based Detoxification System (MDS), utilizes combined membrane, separation and adsorption based on microparticles (particle size <5 µm). The adsorbent microparticles are separated from the patient`s blood only by one membrane, which implies the potential risk of particle entrance into the patient in case of a membrane rupture. To guarantee first fault safety of the system in case of a membrane rupture, as required for medical devices, a particle detector based on a magnetic trap combined with an optical detection system was developed. Magnetic fluorescent particles are added to the adsorbent circuit. Detection of these particles in the venous blood line leads to immediate shut-down of the pumps.
The work presented here includes the synthesis of magnetic spherical microparticles based on cellulose with a particle size of less than 5 µm. The synthesis of such magnetic microparticles was performed in two ways: (a) porous cellulose microparticles were synthesized by the acetate process[3] and magnetite was incorporated into the particles by co-precipitation of Fe(II)/Fe(III) in alkaline media or (b) commercially available magnetite nanoparticles were incorporated into the polymer microparticles during the polymer synthesis process. In both cases, various parameters, such as iron salt or magnetite concentration, incubation time, dispersion parameters, as well as the influence of protective colloids were investigated. The resulting magnetic microparticles were characterised by electron microscopy, measurement of the particle size distribution as well as by determination of their magnetic properties. As a result, spherical magnetic cellulose microspheres could be synthesized with a narrow particle size distribution ranging from 1 to 10 µm, respectively. In summary, we are establishing spherical magnetic cellulose microparticles as matrix for the use of marker particles in the Microspheres-based Detoxification System.

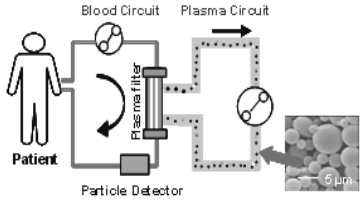

Figure 1: Schematic drawing of the Microspheres-Based Detoxification System (MDS). The blood circuit is separated from the plasma circuit by a plasma filter. In the plasma circuit, plasma is recirculated and kept in suspension together with the adsorbent particles. The purified plasma is filtered back to the blood circuit, and whole blood is reinfused into the patient.

[1]Gupta A.K.; Gupta M. Synthesis and surface engineering of iron oxide nanoparticles for biomedical applications. Biomaterials 2005 (26), 3995-4021.
[2]Ettenauer M., Posnicek T., Brandl M., Weber V., Falkenhagen D. Magnetic fluorescent microparticles as markers for particle transfer in extracorporeal blood purification. Biomacromolecules 2007, 8(12), 3693-6.
[3]Wagenknecht W.; Fanter C; Loth F. Process for producing spherical microparticles on the basis of cellulose acetate. European Patent EP0750007.

MAGNETIC GELATION: A NEW METHOD TO PRODUCE ANISOTROPIC POROUS POLYMERIC MATERIALS

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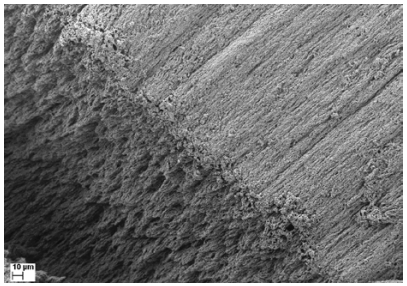
In this work, we introduce a novel procedure to create porous polymeric materials using a process that we named magnetic gelation. We start by preparing magnetic polymer nanoparticles, composed of magnetite nanocrystals dispersed into a polymer matrix, via miniemulsion polymerization. Due to the superparamagnetic behavior of the nanocrystals embedded in the polymer matrix, the nanoparticles prepared in this manner develop strong and reversible dipolar interactions only in the presence of an external magnetic field. Both the size of the nanoparticles and the amount of magnetite nanocrystals encapsulated inside them has been varied, so as to tune the strength of the magnetic interactions. Dispersions containing a few volume percentages of these magnetic nanoparticles, which are stabilized by means of electrostatic interactions, have been partially destabilized through the addition of controlled amounts of electrolytes and their self-assembly and gelation behavior in the presence and in the absence of an external magnetic field have been investigated. In the absence of magnetic fields, the particles self-assemble into random fractal clusters, which eventually percolate to form a colloidal gel. Instead, when an external magnetic field is applied, the particles align themselves in columnar structures in the direction of the field, as shown in the figure. By tuning the duration of magnetic field application, as well as the intensity of magnetic interactions, different extent of anisotropy and characteristic pore sizes in the final material are obtained. The materials obtained through this magnetic gelation process have been hardened though partial fusion of the nanoparticles and characterized by means of electron microscopy and magnetic torque measurements. The magnetic torque measurements allow one to determine the magnetic anisotropy of the materials, and confirm that various degrees of anisotropy can be reached by tuning duration of magnetic field application, as well as the intensity of magnetic interactions.



In order to gain a better insight into the physics of the magnetic gelation process, and have some guidance in the choice of the optimal experimental conditions, Brownian Dynamic Simulations have been performed. A qualitative and semi-quantitative comparison between the simulation results and the experimental findings is presented.

Magnetically guided microscopic electric batteries: manufacturing & biological applications.

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[b]*Institute of Nuclear Physics, Moscow University, Moscow, Russia*
[c]*NanoSynthesis, Columbus, OH, USA*

Our group developed a technology of producing a new class of magnetic nano- and microparticles (10-1000 nm in diameter): the particles which also produce electric field and current in small vicinity around them due to electrochemical processes [1]. The particles can be introduced into biological tissues and cells, and potentially can have interesting research and clinical applications.

The surface of each particle is covered with at least two distinctly different substances on two different sides. In [2] such "two faced" particles were ingeniously called "Janus particles" in honor of the double-faced Roman god. Such gradient of surface composition creates interesting properties of the particles, depending on each of the substances on the surface and their interaction with the environment the particle is in. For example, with proper design, microparticles coated with n- and p-type semiconductors on different sides could serve as nano- or microscopic photovoltaic elements and generate photocurrent in the surrounding medium when illuminated [1].

Another example of electric current generating particles is a miniature galvanic element. If a particle's surface consists of one metal on one side, and another metal with different electrochemical potential on the other side, such particle will generate electric field and electric current in the medium when placed into an electrolyte (e.g., cytoplasm or cell culture medium). For example, an iron (standard electrode potential E^0_{Fe} = -0.441V) particle coated with copper (E^0_{Cu} = 0.52V) on one side should remain stable while dry, but will generate electric field when submerged into an electrolyte (e.g., placed into a solution, or phagocyted by a cell, Fig. 1). The electric potential difference for this pair is 0.961V. The electric current density will depend on the conductivity of the electrolyte and the particle size. The more active electrode (in this case iron) will dissolve over time. Biological activity of such particles could involve both biochemical activity of the ions of the dissolving electrode and the influence of the electric current on cell processes. Previous studies suggest that in some cases this could suppress cell proliferation and/or stimulate cellular immune activity.

The iron particles (ca. 1 μm diameter), produced by the plasmachemical technique, were partially imbedded onto a thin layer of glue, and submerged into a 5% aqueous solution of Cu_2SO_4 for 5 min. The hydrophobic glue partially protected the particles, while in the exposed parts of the particles the iron was replaced with copper: $Cu_2SO_4 + Fe = Fe_2SO_4 + Cu$. After washing of the Cu_2SO_4 off, the particles were dried and removed using non-polar organic solvents. The particle composition was studied using Hitachi S-3000H SEM with EDAX EDS detector. Placing the particles into an electrolyte connects the electric circuit and starts the current flow. Experiments with phagocytosis of the particles (both current generating and non-generating) by mice macrophages are under way.

The particles contain ferromagnetic (or superpara-magnetic) core, therefore they can be controlled by the magnetic field and standard methods of magnetically controlled drug delivery can be applied to them. But these new particles also generate electric currents around them, which can also interact with the magnetic field, offering new ways of controlling them. We believe that this new class of the magnetic nano- and micro-particles can have exciting new biological applications.

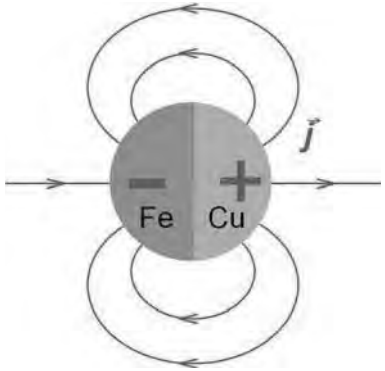

Figure 1. Microscopic galvanic element in an electrolyte and the current around it

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2. A. Walther & A. Muller, "Janus particles" *Soft Matter*, 2008, 4, 663–668

Development of immunomagnetic beads for rapid detection of Escherichia coli O157:H7 by immunomagnetic enrichment and real-time PCR

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In this paper, we developed rapid quantitative method for detection of E. coil O157:H7 via combining immunomagnetic enrichment and real time PCR technique[1]. Firstly, magnetite nanoparticles were assembled into sphere-like aggregates by membrane emulsification equipment (SPG). Secondly, the obtained magnetite aggregates were coated by silica with amino groups using modified stöber method[2]. Carboxyl groups were then grafted onto the surface of silica via succinylation and the carboxyl magnetic microspheres diameter of 200nm and 1000nm were obtained respectively (Fig 1). Thirdly, As synthesized carboxyl magnetic microspheres activated by EDC/NHS were covalently bound with protein A to prepare immunomagnetic microspheres, protein A density onto the surface of magnetic microspheres could be adjusted from 7.4μg /mg beads to 35μg/mg beads by changing conjugation protocol such as protein A doses, EDC/NHS concentration and pH. Fourthly, the above obtained protein A magnetic beads were added into 1ml artificially contaminated milk sample mixed with a certain amount rabbit-anti-O157 polyclonal antibody and E. coil O157:H7 with number of 0, 4, 9,18,36,72 and 144 copies respectively. The mixture was incubated one or two hours at 28℃ to perform enrich Bacteria procedure, and then the magnetic beads were collected, washed and re-dispersed by PBS buffer with the help of magnetic fields to obtain magnetic suspension. Several factors, such as the amount ratio of protein A beads and antibody and incubation times were optimized. Finally, the half part of magnetic suspension was carried out real time PCR analysis process using ABI commercial real time PCR kit, the detection number of E. coil O157:H7 in milk was calculated from the Ct of PCR amplification standard curves, which came from the corresponding number of bacterial enrichment through culturing the other part suspension 18hours at 37℃using E.Coli Broth. The results showed that the enrichment efficiency based on our homemade immunomagnetic beads was larger than 80% when there existed lower than 150 copies O157:H7 per ml milk sample, when combined with real time PCR, O157:H7 DNA was detected with 2 copies /ml milk only, the whole detection time was less than 3hours. In conclusion, we developed rapid quantitative detection method combining immunomagnetic enrichment and real time PCR technique to pave a new way of opening many possibilities in food safety field.

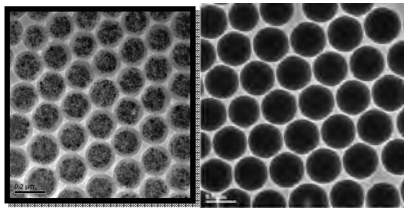

Figure 1 TEM image of carboxyl silica magnetic microspheres

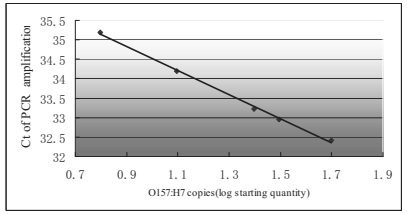

Figure 2 Real time PCR standard curve

Keywords: Rapid detection, Escherichia coli (E. coli) O157:H7; Immunomagnetic enrichment, PCR
Acknowledgements: This work was supported by NSFC (20874061) and shanghai nano project (0852nm04100)
Reference:
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Adding Different Functionalities to Ultra-stable Superparamagnetic Iron Oxide Nanoparticles through Controlled Surface Modification

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The biocompatibility and magnetic properties of iron oxide nanoparticles (NPs) render them attractive for a variety of biomedical applications such as magnetic resonance (MR) contrast agents, for triggering drug release and cell separation purposes. Good NP stability under physiologic conditions and controlled surface chemistry are key to successful application. Consequently, poor NP stability limits many applications of iron oxide NPs especially in the biomedical field.

Due to the high importance of dispersants for NP stability and functionality, we tested different low molecular weight dispersants consisting of catechol derived anchor groups covalently linked poly(ethylene glycol) (PEG). A stable, dense, brush-like PEG dispersant layer will provide a biocompatible stealth coating for the NPs *in vivo*. Catechols such as dopamine and DOPA are considered to be well-suited for anchoring low molecular weight dispersants to iron oxide NPs [1]. However, we found catechol derivatives, namely nitrocatechols, which have a considerably higher affinity to iron oxide, leading to vastly higher NP stability, compared to catechols [2]. Electron transfer reactions taking place between nitrocatechols and iron ions located at the surface of iron oxide NPs lead to a strong, and for practical purposes irreversible, bond of nitrocatechols to iron oxide. This strong bond allowed us to not only synthesize ultra-stable iron oxide NPs by using PEG-nitrocatechols as dispersants but it additionally enabled us to tune the dispersant shell thickness, surface functionality and density of functional units by changing the PEG molecular weight and end groups of said dispersants[2,3]. Thus, thanks to the well-defined adsorption of nitrocatechols, we can independently tune the core size, important for tailoring the magnetic properties, the dispersant layer thickness which determines NP stability and NP functionality by co-adsorbing differently end-functionalized and unfunctionalized PEG-nitrocatechol dispersants to the iron oxide NP surface (Figure 1)[4].

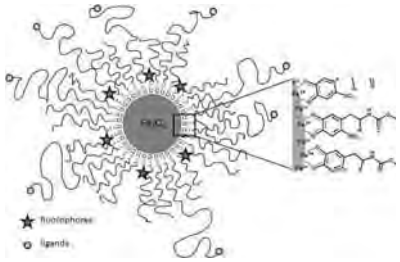

Figure 1: Ultrastable multifunctional superparamagnetic iron oxide NPs.

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Multi-Variant Magnetic Particle Spectroscopy for Magnetic Nanoparticle Characterization

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Magnetic Nanoparticles (MNP), especially superparamagnetic iron oxide nanoparticles, are commonly used as a contrast agent for MRI. Recently, a new imaging modality, capable of taking realtime snapshots of spatial magnetic nanoparticle distributions, has been introduced [1]. The method, called Magnetic Particle Imaging (MPI), relies on the nonlinear magnetization curve of the tracer to generate higher harmonics of a sinusoidal excitation field. A Magnetic Particle Spectrometer (MPS) takes the same approach of analyzing the harmonics originating from the particle sample for characterization purposes. It has been demonstrated [2] that such a device can be used to measure the harmonics generation. By applying a mathematical model, that describes the signal chain and the magnetization behavior of the particles, it is possible to estimate the particle size distribution of the sample by means of curve fitting the model to the measured signal spectrum.

MPS is used to analyze the core properties of the magnetic sample, including its size distribution [2]. However, MPS is also applicable for characterization of the hydrodynamic properties of the MNPs. For this purpose we have developed an extended MPS setup that enables parameter-measurements of the harmonics (Fig. 1), depending on the amplitude and frequency of the excitation signal and the amplitude of a static offset field.

So far, a magnetization model based on the Langevin function has been used to fit the measurement results. This basic model includes the amplitude of the excitation field and the static offset field parameters. However, for the extended MPS setup we also developed an extended model comprising the magnetization dynamics of the MNP sample, which allows to take the frequency dependence of the harmonics generation as an additional parameter. Based on that model we can utilize a multi-variant fitting routine, derived from Levenberg-Marquardt, to describe the sample's core size distribution and hydrodynamic properties. The additional parameters in the measurements and the associated model provide a higher stability of the fitting results, yielding even more properties of the MNPs compared to a simple MPS experiment.

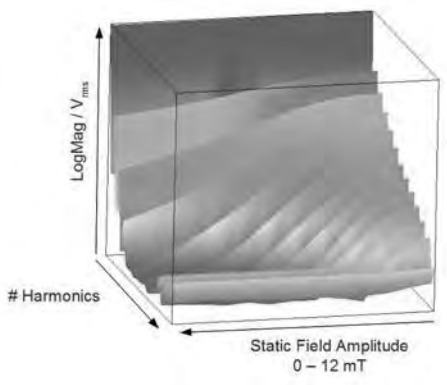

Figure 1: Measured harmonics of the excitation frequency in dependence of a static offset field

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Physical Properties of Water Based Large Single Domain Particle Dispersions of Magnetite

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Definition and motivation: Dispersions with Large Single Domain Particles (LSDP) of 16 to 80 nm magnetite core sizes with narrow size distribution have extraordinary physical properties which can be advantageously used for magnetorelaxometry (MRX), magnetic resonance imaging (MRI), magnetic particle imaging (MPI), hyperthermia, drug targeting, and magnetfection.
For fundamental research and application two important problems are to solve: the preparation of these LSDP and the sufficiently colloidal stabilization of the corresponding LSDP-dispersions.
Materials and methods: First, LSDP of magnetite of a different mean core size were prepared modifying the NISHIO method [1]. We focused our investigation on using magnetite core sizes below 35 nm, because bigger particles were too big for stabilization. After this, these particles were covered with various stabilizers, all well known for the particles stabilization of ferrofluids as citrate, oleoylsarcoside and carboxylated dextran (mw 10000 to 15000).

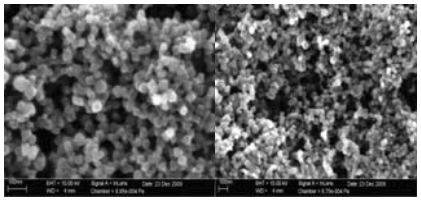

Fig.1 Typical SEM images of LSDP of magnetite.

For physical characterization the following equipments were used: VSM, TEM, SEM, XRD, SAR, and a Zetasizer for hydrodynamic size and zeta-potential.
Results: LSDP of magnetite of average core sizes between 20 and 60 nm were prepared by changing the reaction temperature between 25 and 50 °C.
The carboxylated dextran is biocompatible as well as showed good stabilization effects and therefore was preferentially used. It was found that the average hydrodynamic particles size Z(ave) depends on the particle concentration: Z(ave) of diluted LSDP-dispersions are in range of 150 nm while concentrated dispersions (1 to 5 w%) are in range of 500 nm. Of course, the particles slowly sediment under gravitation but the particles can be redispersed for several hours by shaking or at sensible ultrasound conditions.

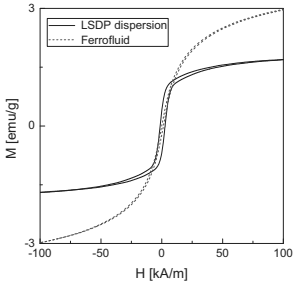

Fig. 2. Typical hysteresis curves of ferrofluids and LSDP dispersions

Summary: Water based dispersions with LSDP of magnetite of narrow size distribution were prepared using the favored carboxylated dextran as the particles shell. The colloidal stability depends on the core size, the particles concentration, and the composition of the stabilizer.
To become a candidate for the mentioned applications further work is necessary in preparing tailored LSDP-dispersions.

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Magnetically Induced *Hot Spots* Promote a Locoregional Chemical Reaction

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Dipolar nanoparticles are of great interest in medical research and application because of their ability to transform electromagnetic energy into thermal energy. This opens the opportunity to activate thermal, physical or chemical processes in a specifically confined environment by selective heating. Such particles find their application in hyperthermia, a mild and effective therapeutic method in cancer treatment.[1] Another important aspect of application for nanoparticle is in bioanalytics, which allows a fast analysis of DNA, and DNA defects, within microseconds instead of hours.[2] However, single particle heating remains a controversy in actual research, and the basic mechanisms are not yet fully understood.

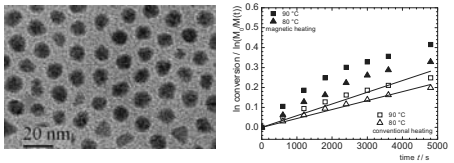

Figure 1: a) TEM of cobalt nanoparticles, b) plot of logarithmic conversion vs. time for conventional and magnetically heated samples.

The concept of our work is to compare the growth step kinetics of a surface-initiated polymerization process performed under different (conventional and magnetically induced) heating methods in order to obtain insight into the effective heat transfer mechanisms. The particles employed are magnetically blocked cobalt nanoparticles (d_n= 12 nm) that undergo fast heat dissipation by Brownian motion in an alternating field in the kHz regime. By using cobalt nanoparticles decorated with hydroxyl functional groups on the particle surface, initiation sites are provided for the ring opening polymerization of ε-caprolactone, and a brush-like core-shell architecture is obtained (Figure 2).[3] The magneticall induced local heat of particles (*hot spot approach*) is used to activate the polymerization process predominantly on the particle/medium-interface. The growing polymer chains possess an increasing distance of the activated reaction center from the cobalt core in the course of reaction. Monitoring the reaction kinetics therefore delivers information on the efficiency of the different heating methods. For all investigated reaction temperatures, we observe a faster conversion in the magnetically heated samples compared to conventionally heated ones. In particular, the reaction rate is enhanced for short reaction times, while for longer reaction times, the rate is similar to the conventionally heated samples.

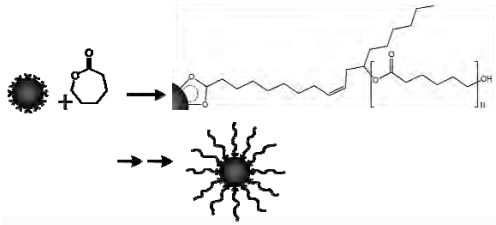

Figure 2: Surface-initiated ring opening polymerization of ε-caprolactone

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Anchored Steric Stabilisation of Superparamagnetic Nanoparticles for Biomedical Applications

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In more than 90% of cancer patients with solid tumors, an initial positive response to chemotherapy is followed by relapse with the tumors then being resistant to further chemotherapy. This is a consequence of incomplete eradication of the tumors in the first treatment due to incomplete or partial penetration of the drug into the tumor and/or the use of doses that are insufficient to kill all cancer cells. Thus, treatments that achieve better targeting and penetration of tumor cells are urgently required. Functionalized magnetic nanoparticles have been recognized as a promising vehicle for the targeted delivery of therapeutic moieties[1]. For most biomedical applications the proper functionalization of nanoparticles is vital to avoid the particles being immediately coated by plasma protein and taken up by the macrophages of the reticuloendothelial system. Another challenge is achieving high concentrations of unaggregated, very high specific surface area particles within polymer matrix particles. In this work, we have developed an approach for the steric stabilization of magnetic nanoparticles using short chain block copolymers prepared by reversible addition fragmentation chain transfer (RAFT) to largely overcome these problems.

We have devised dispersions of sterically stabilized nanoparticles that are stable indefinitely at high ionic strengths and over a broad pH range. The block polymers used to stabilize these particles form the thinnest possible steric stabilizing layer while remaining strongly attached to the nanoparticle surface over a wide range of nanoparticle concentrations. The anchored stabilizer can be readily modified to carry targeting groups, anticancer agents, fluorescent visualization aids, and groups that confer stealth properties. Figure 1 shows the result of the in vitro uptake of fluorophore functionalized magnetic nanoparticles by ovarian cancer cells. These cells can potentially be viewed using MRI as magnetic nanoparticles are shown to behave as negative contrast agents[3].

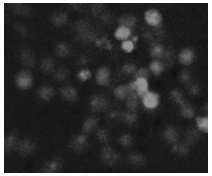

Figure 1 A2780 cells containing magnetic nanoparticles with fluorophore attached

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Magneto-Nano Chips for Biomedical Diagnostics

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Reproducible and multiplex protein assays are greatly desired by cancer biologists as well as clinical oncologists to rapidly follow numerous proteins in clinical samples. By simply applying patient serum or tissue samples to the magneto-nano sensor chip developed in our group, one can readily and quantitatively ascertain the presence or absence of a large number of tumor markers, such as those involved in HER-kinase axis pathway, in a multiplex format. This will allow physicians to determine the efficacy of relevant chemotherapy in real time. Combined with a different set of tumor markers, the new protein assays will also allow physicians to detect cancer early, e.g., stage 1 ovarian cancer, so that cancer survival rates can be improved greatly with early intervention. Combined with yet another set of protein markers such as CDKN1A and H2AX, this new tool may permit the rapid stratification of absorbed radiation exposure doses from a radiological incident. We have now successfully applied magneto-nano biochips based on giant magnetoresistance (GMR) spin valve sensor arrays and magnetic nanoparticle labels (nanotags) to the detection of biological events in the form of multiplex protein assays (4- to 64-plex) with great speed (30 min. – 2 hours), sensitivity (1 picogram/milliliter concentration levels or below, Figure 1), selectivity, and economy [1-3]. The technology is highly scalable to deep multiplex detection of biomarkers in a complex disease, and amenable to integration of microfluidics and CMOS electronics for portable applications. This platform technology is ideal for measuring multiple protein levels in a volume of only 10-50 uL of blood sample from a human patient with minimal invasiveness, either in a laboratory setting or at point of care. Successful applications in pilot studies of animal tumor models will be presented and discussed. In addition, a microfabricated magnetic sifter device [4] with excellent characteristics for capturing and enriching low abundance biomarkers and rare cells will be presented briefly.

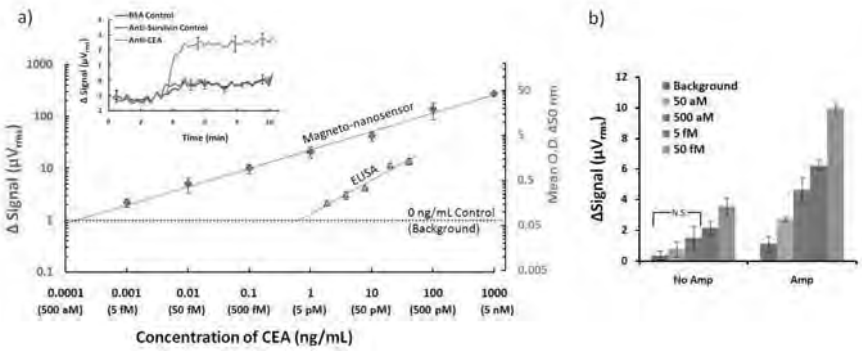

Figure 1 | Sensitivity and linear dynamic range (on a log-log plot) of magneto-nanosensors and ELISA. (a) Superimposed serial dilution curves of CEA detection on the magnetic nanosensor (blue) and ELISA (green) comparing the linear dynamic range and the lower limit of detection in 0.1% BSA in PBS (the same antibody pairs were used for both assays). (b) Demonstration of protein detection using amplification to quantifiably distinguish ($P < 0.05$) protein concentrations in the attomolar concentrations.

References:

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Hydrophilic Maghemite (γ-Fe_2O_3) Nanoparticles - Aggregation Control Using an Ultrasound-Assisted Doping Process of Particle Surface

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Iron oxide-based nanoparticles (NPs) and/or nanocomposites found quite diverse and numerous applications in magnetism-driven separation of cells, magnetic field-guided drug- and gene-delivery (magnetic drug targeting/gene therapy), detoxification of undesirable toxic species, relaxation and contrast enhancement in non-invasive magnetic resonance imaging (MRI) of tissues, piezoelectric immunosensors, and magnetic fluid hyperthermia for cancer therapy. Amongst various well-known technological bottlenecks of existing nanopar-ticle/nanocomposite fabrication methods, the *detrimental aggregation phenomenon* is one of the most difficult to address. Our recent work in the field led to the discovery and successful implementation of a novel method/concept for the aggregation control of **hydrophilic magnetically responsive maghemite (γ-Fe_2O_3) nanoparticles** (Fig. 1).

Quite remarkably and in contrast to any process described till now, *this novel method does not make use of any passivating organic species such as surface-interacting polymers or ligands*. Indeed, we demonstrated that the ultrasound-assisted doping of the surface of 45/50 nm-sized maghemite nanoparticles using **positively charged metallic cations such as Ce^{3+} cations** arising from the monoelectronic oxidant CAN (Ceric Ammonium Nitrate, $Ce(IV)(NH_4)_2(NO_3)_6$) strongly modified their surface charge to highly positive values (ζ-potential = +40.8 mV, Fig. 2). This doping process enabled (i) a full charge-control of particle aggregation due to charge repulsive effects (as checked by ζ-potential measurements, Fig. 2), as well as (ii) their water-compatibility for biological applications.

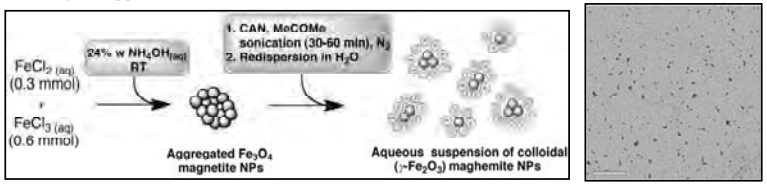

Figure 1. Fabrication process (*left*) & illustrative TEM microphotograph (*right*) of CAN-stabilized maghemite NPs

Deep characterization investigations (FT-IR, high resolution TEM with compositional EDAX analysis, DLS/ζ-potential, Mössbauer spectroscopy) have been conducted in order to identify the influential parameters of this new fabrication/"*inorganic*" stabilization method of maghemite nanoparticles. A plausible mechanism that will explain this innovative key Ce^{3+}-based doping will be proposed and discussed.

In addition, this new nanosized magnetic support has been tested for cell toxicity (HeLa, HEK 293, and MEF 3T3 cell lines), and for DNA covalent attachment/hybridization, including DNA detection using a blue-colored HRP-based enzymatic amplifying system. Preliminary results that investigated the formation of Ce^{3+}-doped maghemite nanoparticle/RNA polycatio-nic/polyanionic complexes for gene silencing will be also presented as a first step towards siRNA/microRNA delivery.

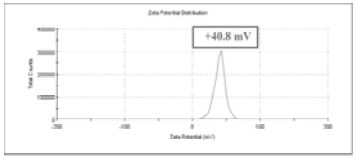

Figure 2. ζ-potential measurement of CAN-stabilized maghemite NPs (doubly distilled H_2O, 25°C)

Synthesis of Rod-Shaped Superparamagnetic Iron Oxide Nanoparticles with Polyvinyl Alcohol

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Fabrication of nanomaterials with controlled size and shape is of great scientific and technological interest.[1] The saturation of magnetized superparamagnetic iron oxide nanoparticles (SPIONs), which play a key role in biomedical applications, can be significantly increased by their arrangement in one direction (i.e. formation of nanorods). A previous report showed that small amounts of PVA act as a template in hot water (70 °C), leading to the oriented growth of Fe_3O_4 nanorods, which was confirmed by selected area electron diffraction (SAED). Synthesis temperature and polymer concentration have significant influence on the morphology and size of SPIONs. Although the precise configuration of the polymer chains within the crystalline regions is poorly understood, the fiber periodicities suggested a twisted or helical structure at low temperatures and a fixed relaxed-shape at high temperatures.[2] The aim of this work is to synthesize rod-shaped magnetite nanoparticles by a co-precipitation route. Polyvinyl alcohol (PVA)/water mixtures were used as a primary aqueous medium. During the hot-water processing, the magnetic beads were forced to grow unidirectionally along the interface by the fixed polymer molecules. The freeze/thaw technique was applied to increase the PVA crystallinity. The nanorod-shaped materials show a higher stability upon exposure to electron beam due to the PVA stability in the cluster walls. The X-ray diffraction patterns indicate the formation ($10\bar{1}$) of oriented PVA planes. This observation confirms the crystallinity-enhancing effect of the freeze-thaw process for PVA.

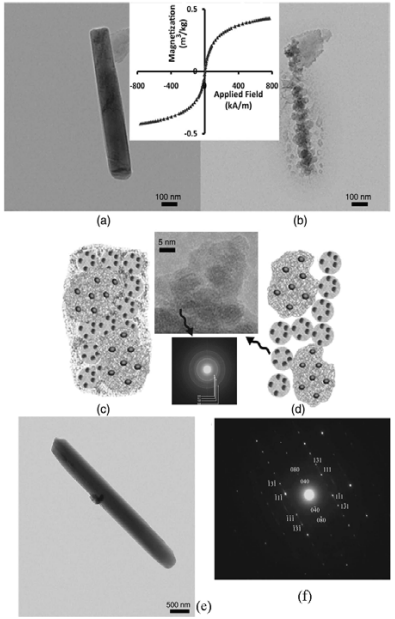

Figure 1: TEM micrographs of nanorods: (a) and (c) upon exposure to electron beam; (b) and (d) after destruction of PVA by electron beam exposure. VSM image of the nanorods shows the superparamagnetic properties (e) TEM micrograph of nanorods with crystalline PVA coating which is stable upon exposure to electron beams and (f) the SAED pattern of nanorods showing that the rods are single crystalline with the preferred growth direction imposed by PVA.

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Magnetically controlled, multifunctional nano devices.

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Polymeric magnetic nanoparticles as carriers for luminescent dyes are commonly used for applications such as multi-modal imaging, magnetically assisted fluoro-immunoassays and magnetic cell-labelling, -sorting and -tracking. Recently, the incorporation of chemical indicator dyes into polymeric particles resulted in magnetically remote-controlled optical sensor particles (MOSePs) [1–3]. Such magnetic nano sensor particles are useful for imaging changes of biologically important parameters, such as oxygen and pH in biological samples.

Here, we employed a nanoprecipitation method for the production of magnetic polymer particles with tunable sizes in the range of 50-180 nm (Fig. 1). The hydrodynamic diameter was adjustable by varying precipitation parameters such as the polymer concentration and type (see Fig. 2). Surface modifications of such magnetic particles with enzymes, polyelectrolytes or stimuli-responsive polymers resulted in multifunctional MOSePs [4]. As an example, the coupling of glucose oxidase to the particle surface resulted in magnetic nanosensors capable of monitoring changes in glucose concentration with the incorporated oxygen sensor. Finally, the incorporation of a singlet oxygen producing dye inside the particle core resulted in MOSePs for simultaneous photodynamic therapy and trace oxygen sensing. Such particles represent an excellent basis for various biomedical applications and combine diagnostics and therapy ("theranostics").

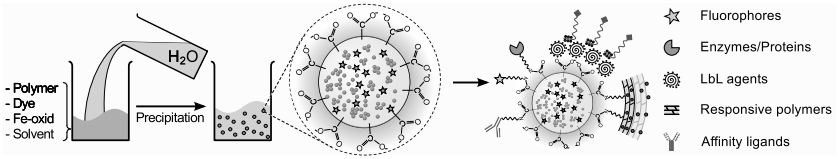

Figure 1: Synthetic route towards multifunctional magnetic nanosensors. After mixing the cocktail containing a polymer, magnetic nanoparticles and indicator dyes with water, spontaneous particle formation occurs. During this process, carboxyl groups are formed and orient themeselves to the particles' surface. This enables the introduction of additional functionalities *via* covalent or polymeric surface modifications.

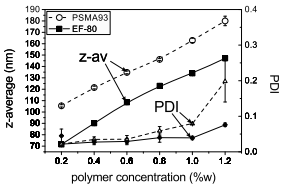

Figure 2: The particle sizes depend on precipitation parameters, such as the polymer concentration in the cocktail and the polymerization degree of the matrix polymer. The low average PDI values indicate narrow size distributions.

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Self-assembly of multifunctional nanoparticles via barnase and barstar

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Nanomedicine, being the frontier of biomedical research, incorporates several diagnostic and therapeutic approaches, using a large number of nanoparticles and biomolecules, each possessing unique properties and functions. In particular, magnetic particles (MP) stand out because of the possibility to be manipulated by external magnetic field and to be delivered to the point of interest. That, however, is not enough for most medical applications, because it is usually necessary to combine MP with functions intrinsic for toxins, antibodies, etc. Synergistic combination of several highly specialized particles may lead to significant advances in each particular application. Therefore, we believe, that a generic approach for assembly of absolutely different by nature particles would be beneficial for development of next-generation nanomedicine. For that goal, in this work an attempt to create a universal technique for assembly of multifunctional structures for various life science applications has been made [1].

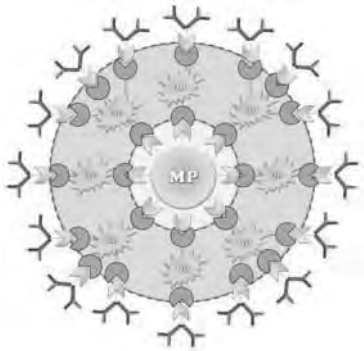

Fig. 1. Assembled trifunctional structures based on MP, quantum dots (QD) and anti-HER2/neu antibodies linked by barnase (green) and barstar (pink).

A bioengineering method for self-assembly of multifunctional superstructures has been proposed and realized. The method employs two unique strongly-interacting proteins, barnase and barstar, to rapidly join the structural components together directly in water solutions. The properties of the superstructures are designed on demand by linking different agents of various sizes and chemical nature, designated for specific goals. As a proof of concept, colloidally stable trifunctional structures have been assembled by binding together magnetic particles, quantum dots and antibodies using barnase and barstar (Fig.1). The assembly has demonstrated that the bonds between these proteins are strong enough to hold macroscopic (5 nm – 3 μm) particles together. Specific interaction of such superstructures with cancer cells resulted in fluorescent labeling of the cells and their responsiveness to magnetic field. A remarkable feature of the strategy is the opportunity to attach any protein in its functional form to the key components, barnase and barstar, by gene engineering methods to construct recognition, visualization or cytotoxic modules. Their assembly with nanoparticles of different biochemical and physical nature using the same approach could be an easy and convenient way to create desirable multifunctional superstructures.

The research was supported by grants of Russian Foundation of Fundamental Research.

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Magnetic Based Nanobeads as Multifunctional Platforms for Biomedical Applications

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The development of nanostructures at the nano/mesoscale which display at the same time fluorescence and magnetic properties are appealing biomedical platforms. Such nanostructures can be exploited for biomedical applications, as dual modality imaging probes based on optical and magnetic resonance imaging (MRI), or for bio-separation and bio-sensing, as for example the simultaneous magnetic separation and multiplexing optical detection of different tumour cell populations. Interesting combinations of such nanocomposites can be for instance nanostructures based on oligothiophenes (OTFs) as fluorescent portion and iron oxide nanoparticles as magnetic domain which display at the same time fluorescent and magnetic features.[1]

Different strategies to obtain such nanocomposites will be discussed in this talk.[2, 3] The first one is based on the functionalization, through spacer molecules of OTF fluorophores at the surface of individual magnetic nanoparticles.[3] The second strategy proposed is based on the controlled aggregation of iron oxide nanoparticles of 7 nm within an amphiphilic polymer in nanobeads with a size ranging from few tens of nanometers to 400 nm.[2] (Figure 1) Such control can be achieved by choosing properly the solvent added to the initial system and thus by changing the solubility of the polymer and of the magnetic nanoparticles in the environment in which they are initially present. Also in this case, functionalization of the polymer chains with OTF molecules allows us to obtain magnetic-fluorescent beads. Cell separation experiments and fluorescent detection on cell tagged with both types of nanostructures will be also discussed.

Finally, recent developments on surface functionalization with an additional layer of thermo-responsive polymers, doxorubicin encapsulation, and related viability test to verify the release of doxorubicin on tumour cells will be also presented.

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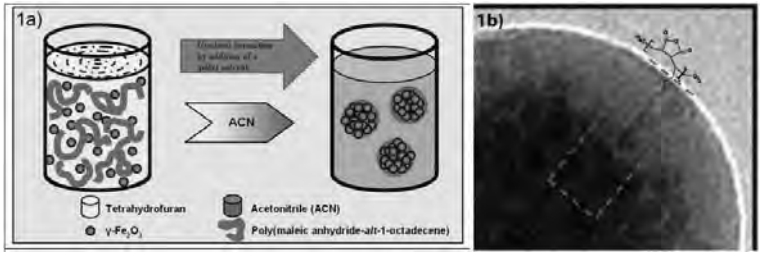

Figure 1. a) Scheme of the procedure used to obtain magnetic nanobeads. The addition of acetonitrile solvent to a solution of iron oxide nanoparticles and poly (maleic anhydride-alt-1-octadecene) polymer molecules in THF, induces a change in polarity of the system and thus promotes the bead formation. **b)** Low resolution TEM image of a typical magnetic nanobead obtained by the procedure reported in 1a).

Magnetic nanosystems for the sentinel nodes detection

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The treatment of cancer is a wide health and economical problem since over millions of citizens are concerned around the world. The success of therapy in terms of increasing life expectancy depends on the earliness of the diagnosis and of the grading of the tumour. The detection of the sentinel node (SN), which is any node receiving lymph drainage from the tumour site and containing most likely malignancy if the tumour has metastasized, has recently improved surgery strategies. We develop a novel clinical methodology based on tailored and versatile magnetic nanosystems able to target the sentinel nodes when injected in cancer tumours. The tailored biocompatible magnetic nanosystems are based on magnetic iron oxide nanoparticles on which an organic layer will be grafted through a dendron molecule and a phosphate entity. The arborescent structure of dendrons allows considering a number of functional groups in the periphery of the nanosystem such as groups ensuring the stability of the suspensions, hydrophobic groups allowing endothelial barrier -crossing or specific antibodies.

First, we have developed the synthesis of magnetite nanopaticles with sizes between 10 and 40 nm. Batches of iron oxide nanoparticles were obtained by a co-precipitation route followed (or not) by a hydrothermal treatment at 250°C with $N(CH_3)_4OH$ (T-Methyl), $N(C_2H_5)_4OH$ (T-ethyl) or $N(C_3H_7)_4OH$ (T-Propyl) bases[1]. The synthesis parameters and conditions such as pH, Base/(Ferrous+Ferric), rotation and injection speed were controlled in order to improve monodispersity. The particles mean sizes decrease when going from T-methyl to T-Propyl-substituted base. Comparison with the lattice parameters of magnetite Fe_3O_4 and maghemite γ-Fe_2O_3, equals 0.8396 nm (JCPDS file 19-629) and 0.8346 nm (JCPDS file 39-1346) respectively. The samples are mainly made of magnetite. A careful study of the structure and the composition has shown that the nanoparticles are made of a magnetite core surrounded by an oxidized layer close to maghemite. The distribution in size has been estimated by specific surface measurements, X-ray diffraction and electron microscopy techniques. Magnetization at H= 18 kOe decreases from 84 emu/g up to 52 emu/g as the size decreases from 40 nm to 10 nm.

First generation pegylated dendrons have been grafted on the nanoparticles through a phosphonate coupling agent[2]. The grafting mechanism has been investigated in view to produce biocompatible magnetic nano-objects for biomedical applications[3]. Grafting has been demonstrated to occur by interaction of negatively charged phosphonate groups with positively charged ones and hydroxyl at the iron oxide surface. The optimisation of grafting conditions has conducted to very stable water suspensions of iron oxide nanoparticles at pH=6.8. The isoelectric point of the suspension of dendronized iron oxide nanoparticles is shifted towards lower pH as the amount of dendron increases. Furthermore, it has been shown that grafting through a phosphonate improves the magnetization of the iron oxide due to super–super exchange interactions through the phosphonate group. Finally, a study of the suspensions stability will be presented.

* This work was supported by the European community (7thPCRD) through the NANOMAGDYE project, CP-FP 214032-2

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Synthesis and characterization of biocompatible magnetically controllable nanostructures using different polymers or block copolymers

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Biomedical applications of magnetic nanoparticles and various magnetically controllable nanostructures [1] require that these nanomaterials to be non-toxic and chemically stable, as well as to be quite uniform in size. For most of the applications in biotechnology, it is necessary to disperse the magnetic nanoparticles in a nonmagnetic media that can be easily processed as stable suspensions or gels, being suitable for appropriate functionalization and having biocompatible properties.

Magnetite nanoparticles and magnetic fluids prepared by chemical co-precipitation procedure and applying biocompatible sterical stabilization of nanoparticles in aqueous carrier [2] were used for the synthesis and characterization of smart magnetic hydrogels responsive to external stimuli (like pH, temperature) and conjugated with a fluorescence marker. Clusters of magnetite nanoparticles were encapsulated into spheres of thermoresponsive polymer PNIPA, figure 1. The polymerization of the monomer N-isopropylacril amide was carried out in aqueous solution in the presence of a cross-linking agent N,N`-methylenbisacrylamide, ammonium peroxydisulfate and magnetite nanoparticles (10 nm medium size). Magnetite nanoparticles were also surface-functionalized *in situ* by the addition of different molecules such as: glycolic acid, acrylic acid. For the attachment of the polymer chain on the preformed functionalized magnetite we applied the "grafting-from" strategy, scheme 1, where the polymerization is initiated directly from the particles surface to give a high number of end attached polymer chains [3]. Core-shell nanostructures were obtained by coating the magnetite core with different polymers and copolymers shells, like polycaprolactone (PCL), polylactic acid (PLA) and copolymers polycaprolactone-*co*-polylactic acid, poly(N-isopropylacril amide)-*co*-poly(N-acryloyloxysuccinimide. Physical-chemical characteristics of hybrid magnetic nanostructures were investigated by FTIR, XRD, TEM, HRTEM, DLS and magnetization measurements. The properties of smart magnetic materials nanostructured as core-shell particles or as ferrogels can be tailored for specific application such as: magnetic separation, molecular recognition, drug delivery systems.

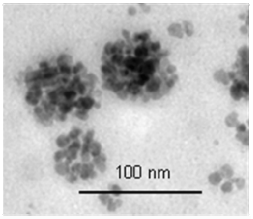
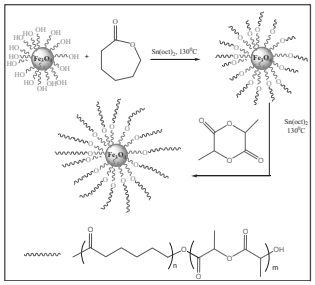


Figure.1. TEM image of magnetic PNIPA hydrogel.


Scheme 1. Copolymer (PCL-PLA) coating of magnetite functionalized with glycolic acid.

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Fabrication of Temperature- and pH- Responsive Magnetic Nanoparticles and Their Reversible Agglomeration in Aqueous Milieu

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A novel kind of pH- and temperature- responsive magnetic nanoparticle consisting of iron oxide nanoparticles coated with pH responsive chitosan oligosaccharide and temperature responsive poly(ethylene oxide)-poly-(propylene oxide)-poly(ethylene oxide) block copolymer was developed. The particles were characterized by TEM, DLS, VSM, FTIR, and TGA. The results represented that the self-aggregation of the prepared nanoparticles was not only caused by the thermo-induced self-assembly of the immobilized block copolymers, but also affected by the pH-induced charge property change of particles surface. The relative peak intensities of the dehydrated PO (propylene oxide) groups in the FTIR spectra of the aqueous nanoparticles solution with temperature-dependent were investigated. The results confirmed that the immobilized block copolymers were accompanied with a conformational change from fully extended state to highly coiled state of the Pluronic copolymer. The self-assembled behaviors can be readily reversed by adjusting back the pH or temperature value. Thus, the attractive properties of reversible and controllable dual-responsive self-assembly might endow the biocompatible magnetic nanoparticles with potential applications in biomedical fields.

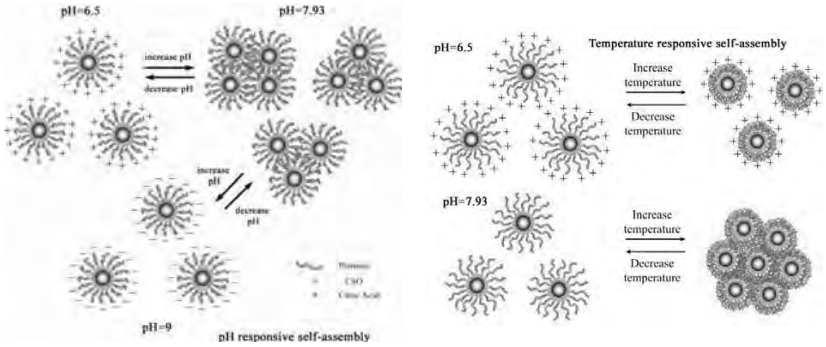

Figure 1. Mechanism for pH- and temperature-responsive self-assembly of MCP nanoparticles.

Synthesis of Individually Water-Soluble, Biocompatible, and Angiogenesis-Targeting Superparamagnetic Iron Oxide Nanoparticles

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The hydrodynamic size of water-soluble nanoparticles below 10 nm is getting more important since it greatly affects their physicochemical properties.[1] Nevertheless, the synthesis of nanoparticles have been quite challenging which are individually water-soluble, biocompatible, and targeting, all in one.

In this study, we prepared water-soluble superparamagnetic iron oxide nanoparticles with mixed ligands and PEG-cRGD conjugate, SPION(-MPA)$_{ex}$(-MHA-PEG-cRGD)$_{10}$ (MPA = mercaptopropionic acid, MHA = mercaptohexadecanoic acid, PEG = polyethylene glycol, and cRGD = cyclic RGD), and investigated their physicochemical properties by magnetic hysterisis, transmission electron microscopy (TEM), dynamic light scattering (DLS), and FT-IR. The PEG and MPA moiety were chosen to harness SPION biocompatibility and water-solubility. The cRGD-conjugation to imaging probes has been known to target and image angiogenesis, which is common to most of the tumor tissues.

Our SPION(-MPA)$_{ex}$(-MHA-PEG-cRGD)$_{10}$ was soluble in water, individually monodispersed with a diameter of 9.9 nm by DLS measurement, and showed superparamagnetic behavior. The TEM image analysis implied that each SPION(-MPA)$_{ex}$(-MHA-PEG-cRGD)$_{10}$ was aggregation-free without its size change (8.6 nm by TEM). The FT-IR spectra displayed conjugated PEG/PEG-cRGD to SPION-MHA moiety through amide bond and anchored MPA directly onto the surface of SPION. The current report provides individually water-soluble, biocompatible, and angiogenesis-targeting superparamagnetic iron oxide nanoparticles.

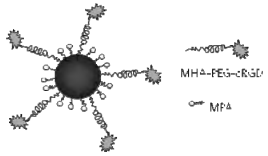

Figure 1. Individually water-soluble, biocompatible, and angiogenesis-targeting SPION.

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Synthesis of Biocompatible Magnetic Test-Tube-Shaped Nanoparticles by Atomic Layer Deposition

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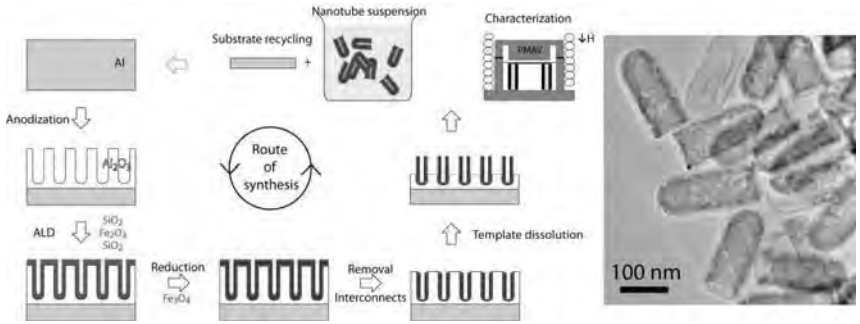
A novel, fully tunable approach to the preparation of short magnetic nanotubes is presented. Combining porous alumina as a template with atomic layer deposition (ALD) offers a new synthesis route for tubular nanostructures. Due to well-defined surface reactions of gaseous precursors not limited by the mass transport, ALD is uniquely suited to coating nanostructured substrates conformally and allows for the control of all system parameters (e.g. tube length, diameter, wall thickness, material).

By reducing the time of the electrochemical oxidation of aluminium, it is possible to form a hexagonally self-ordered thin porous structure having a small aspect ratio (<5). Silicon dioxide is first deposited into the thin porous alumina membrane [1]. Following the subsequent deposition of iron(III)oxide [2] and a second SiO_2 deposition, a three-layer wall structure of nanotubes embedded in the alumina matrix is achieved. A reduction by argon-hydrogen then transforms the iron oxide to the ferrimagnetic magnetite phase. Removing the interconnects by reactive ion etching and releasing the tubes from the porous alumina membrane by chemical etching of the matrix finally results in the formation of a ferrofluid-like suspension, whereby the nanotubes exhibit the shape of a test tube, with one closed end.

Magnetic characterization and magnetic-field-dependent viscosity measurements of nanotube ferrofluids reveal a non-linearity that contrasts with the behavior of the spherical nanoparticles in conventional ferrofluids.

In the future, applications of the novel test-tube-shaped nanoparticles have to be explored. The well-understood, biocompatible silica surface eases the inner and outer functionalization of the nanotubes before and after the releasing step, respectively. This possibility paves the way to their utilization as magnetic carriers able to transport a payload while protecting it from the surrounding environment.



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Interactions in Magnetic Nanoparticle Systems: How to Identify Them and Their Consequences

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The use of functionalized magnetic nanostructures in data storage, ferrofluids, biology, and medicine has increased dramatically during the past decade. Many applications, including viscosity, self-assembly, cell sorting, magnetic resonance imaging (MRI), hyperthermia, or drug delivery, exploit the unique physical and magnetic properties inherent in magnetic materials that possess characteristic dimensions smaller than 150 nm. Unfortunately, all models of these colloidal systems assume that (1) there are no interactions amongst the magnetic nanoparticles and (2) the magnetic field applied to the colloid has no affect on the internal structure of the colloid. Here, we present results of research on the interactions amongst magnetic nanoparticles in colloidal solution at biologically relevant concentrations.

First, we show that the existence of interactions amongst magnetic nanoparticles can be demonstrated by comparing the virgin curve from magnetic hysteresis loop with the major loop. Particle interactions are negligible if the two curves are identical; if they are not, then any model of the system must consider interactions. We also show with several different nanoparticle systems (i.e. core-shell and "plum-pudding" nanoparticles) that these interactions are always present for biologically relevant particle concentrations.

The effect of a static DC magnetic field on the internal structure of a magnetic colloid as a function of concentration and surfactant (and therefore interaction) between the magnetic nanoparticles is also explored. We present results obtained from SANS measurements of nanoparticle suspensions in which structural scattering from the 50 nm Fe_3O_4 cores is dominant. These data provide direct information about the structural correlations among the nanoparticles induced by interparticle magnetic interactions. We show that ordering in a colloid parallel to an external magnetic field occurs simultaneously with the onset of a uniaxial anisotropy in the system. Furthermore, with further increases in applied magnetic field, the particles begin to order in the direction perpendicular to the field, resulting in the formation of a unidirectional anisotropy.

Finally, we correlate the onset of these anisotropies with the onset of measurable heating (SAR) for a series of different iron oxide nanoparticle systems. The correlation between SAR and anisotropy continues beyond the onset, and remains linear over the field regime measured. The unexpected interplay between the anisotropy and SAR has the potential to have significant implications for biomedical and ferrofluid applications, where the structure of the colloid affects both its viscosity as well as its magnetic properties.

Magnetophoretic motion control of individual Brownian nanoparticles

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Abstract

To investigate within living cells, it is desirable to use a movable magnetic probe that approaches the size of the molecules of interest, normally, within the range of few to tens of nanometers. However, micromanipulation of magnetic nanoparticles is extremely challenging, due to random Brownian motion and viscous drag forces, in addition to the desired magnetophoresis. All three of these types of motion have difference size dependences.

Here we describe the combined influences of Brownian, viscous and magnetic effects on the motion of individual magnetic nanoparticles. To track their positions as a function of time, the iron oxide cores are coated with gold nanoparticles so that they can be imaged by their plasmonic response using darkfield optical microscopy. To generate a large and controllable magnetic field gradient, a thin tip of mu-metal, a soft magnetic material, was attached to the core of a solenoid. When current was passed through the solenoid coil, the tip became magnetically saturated, and rapid magnetic collection of nearby particles was achieved in a few seconds. However strong Brownian displacement was still observed, even with a magnetic field gradient as high as 3000 T/m. More deterministic magnetophoresis was seen as the particles got closer to the magnetic field source. The trajectories of the particles were analyzed to distinguish the contributions of Brownian, viscous drag, and magnetic forces. We also studied the collective motion of particles as they retreated from the magnetic tip after the magnetic field was removed. The experimental data fit with predictions made by Fick's Law for diffusion.

This technique could be an important new tool for detecting binding events and local viscosity variations within cells. Our results show the ability to detect magnetic forces as small as 1 fN. Though all of the measurements were made with aqueous dispersions, we have modeled the effect of more realistic local viscosities within cell vesicles, and found that real-time magnetic manipulation is still feasible.

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Magnetic Nanoparticle Temperature Estimation Using AC Susceptibility

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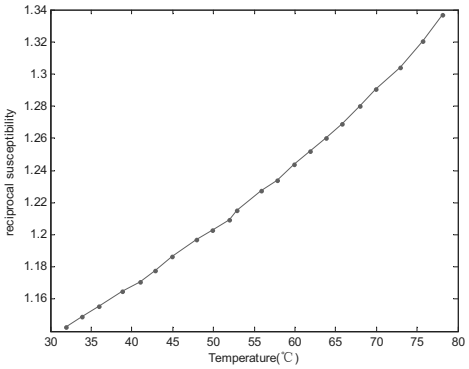
This paper presents a novel method of temperature estimation using the ac susceptibility of magnetic nanoparticles. In thermotherapy, the tumor must be heated to very specific temperatures for effective treatment. Measurements are essential because the temperature is difficult to predict due to physiological cooling, primarily blood flow. Several methods can be used for temperature measurement including thermal couple, optical fiber sensor and MRI (magnetic resonance imaging). It is also reported recently that the temperature can be estimated through the harmonics of the magnetization generated by magnetic nanoparticles in a sinusoidal field.

In our experiments, the susceptibility of the magnetic nanoparticles is found to be inversely proportional to its temperature in a small field, which can be used to generate a calibration curve and to subsequently estimate the temperature. The oil-based magnetic nanoparticles were used in the *in tube* experiments. The calibration curve was obtained by measuring the susceptibility of the oil-based magnetic nanoparticles at different temperatures in a small sinusoidal field (the maximum of which is below 5 Gauss) produced by Helmholtz coils. The relative temperature can then be estimated from any two subsequent measurements of the ac susceptibilities. The results show that the accuracy of the temperature measurement was below 1℃ between 33℃ to 70℃ using the current apparatus. The proposed method of temperature measurement under a small field using ac susceptibility enable one to non-invasive and harmless temperature estimation in the tumor thermotherapy.



Talk 42

Fixed bed magnetic reactor for surface derivatization of Superparamagnetic Iron Oxide nanoparticles

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The surface of nanoparticles plays a major role in cellular interaction/uptake, biodistribution, clearance and cytotoxicity. Surface functionalization of nanoparticles still remains a difficult task and represents not only a chemical challenge but constitutes a basic requirement for future scientific investigations. Alteration of surface charges and/or stabilization by the addition of bi- / multi-functional molecules, such as differently charged proteins or plasmids, frequently leads to particle flocculation and rapid sedimentation. The biological functionality, in such cases, is achieved by covalent binding of bio-active molecules on a preexisting single surface coating.

The yield and quality of derivatized superparamagnetic iron oxide nanoparticles (SPIONs) can be significantly improved reaction times can be reduced by using solid phase synthesis strategies. We have developed a fixed bed micro-reactor with a quadrupole repulsive arrangement of permanent magnets to allow for magnetic immobilization of the particles in order to perform the derivatization step(s) on the immobilized magnetic particles. In this way, pH changes across the isoelectric point, washing steps or even solvent exchanges are easily tolerated and the problems of colloidal instability during the derivatization steps can be overcome for SPION surface derivatization.

Several surface derivatizations were carried out exemplarily and compared to conventional liquid phase coupling chemistries. It could be shown that the surface functionalization of SPIONs using a magnetic fixed bed reactor was superior to the liquid phase reaction in terms of reaction yield, particle size distribution, colloidal stability and scalability.

In particular we show the synthesis of organelle targeting peptide derivatized SPIONs. The combination of functionalized SPIONs and their ability to be recovered using a magnetic column coupled with biomolecular mass spectrometry has allowed us to explore a complex intracellular pathway using a peptide that is known to target mitochondria. Here we demonstrated the concept of biomolecular interaction network elucidation with an organelle-targeting peptide, but the concept would also be applicable with more specific biomolecular, rather than organelle, targeting. Other applications of this technology include organelle-specific drug delivery, study of complex cellular signaling pathways and metabolism, and imaging specific pathologies such as malignant neoplasia.

Talk 43

Determination of nanocrystal size distribution in magnetic multi-core particles including interactions and magnetic anisotropy

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Magnetic multi-core particles containing from a few up to thousands of magnetic nanocrystals (MNCs) are increasingly used in biomedical applications, such as magnetic separation and biosensing, due for instance to their higher magnetic moment compared to single-core particles. A correct estimate of the size distribution (*i.e.* median diameter D and standard deviation σ) of the MNCs embedded in the particle is a necessity in most applications. These parameters are typically obtained by fitting a sum of Langevin functions to the magnetization data [1]. Since this method relies on a superparamagnetic (SPM) approximation of the MNC cluster, the intrinsic magnetic properties of the cluster (*e.g.* magnetic anisotropy, and magnetic dipolar interactions between MNCs) are neglected, which leads to an underestimate of the size parameters [2]. This finding agrees well with experimental work by others where the *physical* size D_p of the MNCs (typically calculated from TEM images) is almost systematically smaller than the *magnetic* size D_m extracted from the fitting procedure to the magnetization curve. This difference is in some cases attributed only to the presence of a magnetic dead layer around the MNCs, whereas magnetic anisotropy and dipolar interactions are not considered. To clarify whether this conjecture is justified in some cases or not, we have used a Monte Carlo method to perform an exhaustive comparison of the *apparent* size parameters D and σ for different morphologies of multi-core particles commonly synthesized. In addition to a realistic microstructure, our model also includes all the intrinsic magnetic properties mentioned above. Figure 1(a) shows the simulated values of the reduced magnetization versus applied magnetic field for the SPM model (filled symbols) and when including magnetic anisotropy and dipolar interactions between MNCs (open symbols). The deviation of the magnetization curve yields a significantly different estimate of the size parameters as illustrated in Figure 1(b) where the *apparent* magnetic diameter D_m is plotted versus the *real* physical diameter D_p for two of the simulated particle configurations: (squares) many MNCs fused in a random compact cluster (*e.g.* nanomag® particles from Micromod), and (triangles) many MNCs distributed on the surface of a large carrier sphere [3]. Based on these findings, we develop a versatile simulation platform integrating the Monte Carlo method to a least-squared optimization algorithm to fit experimental magnetization data with simulated values including dipolar interactions and magnetic anisotropy, which will yield a more reliable estimate of the size distribution parameters of magnetic nanocrystals in multi-core particles.

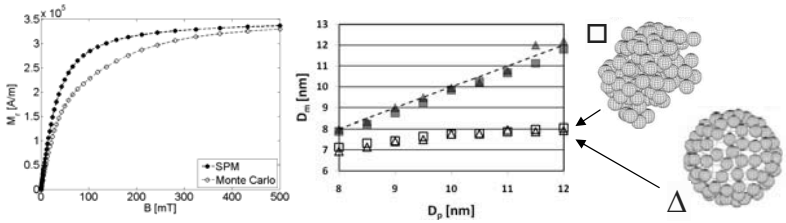

Fig. 1. (a) Reduced magnetization; (b) Magnetic diameter D_m vs. physical diameter D_p. The dashed line shows $D_m = D_p$.

[1] Chantrell RW, Popplewell, J, Charles, SW, IEEE Trans. on Magn. MAG-14 (1978) 975.
[2] Schaller V, Wahnström G, Sanz-Velasco A, Enoksson P, Johansson C, J. Magn. Magn. Mater. **321** (2009) 1400.
[3] Claesson EM and Philipse AP, Langmuir **21** (2005) 9412.

Continuous magnetic microparticle-based sandwich immunoassays in a multilaminar flow microreactor

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Magnetic microparticles are commonly used in bioanalysis as a highly efficient means of performing step-by-step reactions and assays, yet are typically employed in batch procedures that can be laborious and time consuming due to the multiple reaction and washing steps required. At the previous meeting, we presented a method for rapid magnetic particle based assays in a continuous flow lab-on-a-chip device for streptavidin-biotin assays.[1] Here, we explore the potential for performing two-reaction step sandwich assays for IgG as well as the inflammatory biomarker C-reactive protein (CRP).

A series of five laminar flow streams was generated across a microfluidic chamber in the x-direction (Fig. 1a). A magnetic field was applied in the y-direction that deflected particles through reagent and washing streams, allowing consecutive reactions to occur on the particle surface as they passed through each reagent stream. Firstly, a mouse IgG immunoassay was performed to test the capability of the system for use in biological assays. Streptavidin coated particles were deflected through a stream of biotinylated mouse IgG to allow the antibody to bind to the particle surface, before passing through a stream of fluorescently labelled goat anti-mouse IgG which bound to the captured IgG, resulting in a fluorescent particle. A concentration range of 0.1 - 10 μg mL^{-1} mouse IgG was investigated (Fig. 1b). Subsequently, we have begun studies into the analysis of CRP, usually detected in the 0.1 - 1 μg mL^{-1} range, by introducing particles coated with capture antibody into the device and deflecting them through a stream of CRP sample solution, followed by a stream of fluorescently labelled secondary antibody that binds to CRP as a fluorescent tag. First results with 10 μg mL^{-1} CRP (Fig. 1c) show an increase in fluorescence signal, indicating the potential of the platform for CRP detection.

The introduced microfluidic platform enables detection of particle fluorescence after two reaction and two washing steps in continuous flow with processing times of less than 90 seconds per particle, rather than the > 1 hour required by conventional methods. This is encouraging for the future development of the system towards real sample analysis, and opens the door for a number of particle surface based reactions and processes to be performed in continuous flow.

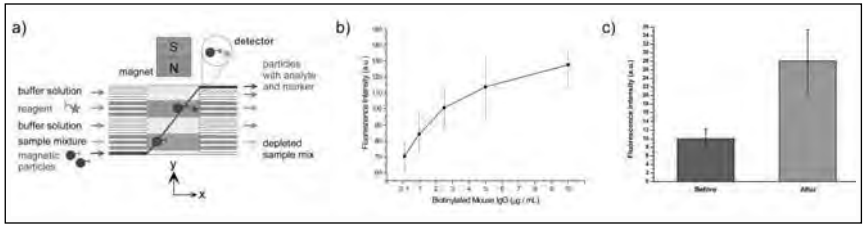

Figure 1(a). Principle of the microfluidic platform. (b) Calibration curve of particle fluorescence over a range of mouse IgG concentrations. (c) Fluorescence intensity of particles before and after crossing the CRP and labelling streams. Typically, 16 particles were analysed for the mouse IgG immunoassay, and 10 particles for the CRP assay.

Reference:

[1] S.A. Peyman, A. Iles, N. Pamme, *Chem. Commun.*, **2008**, *10*, 1220.

Magnetic core shell nanoparticles based homogeneous immunoassay

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Jean-Michel Siaugue[b*], Jan Petr[c], Anne Varenne[c], Valérie Cabuil[b]

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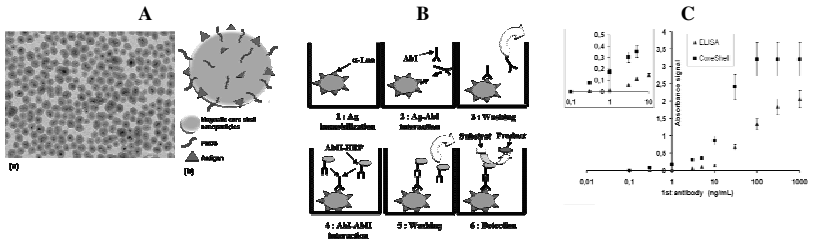
The development of original bioanalytical methods providing high specificity and sensitivity with short analysis time remains a challenge for numerous applications. In this context, an alternative immunoassay which responds to the criteria previously mentioned has been developed. To our knowledge, we report here the first homogeneous sandwich immunoassay using colloidal magnetic core shell nanoparticles [1, 2] (MCSNP) as immunosupport (**A**). The surface of these nanoparticles was functionalized with PEG chains to prevent non-specific adsorption and amino groups which allowed biomolecules covalent grafting. The charge density due to amino groups could be modulated varying the PEOS/APTS ratio [3]. The sandwich was designed for further allergy diagnostic: MCSNP grafted with α-Lactalbumin (α-Lac) antigen (**A**) was able to capture the target model analyte (goat anti α-lac immunoglobulin G (IgG)). Detection was further performed using a 2nd antibody: horseradish peroxydase conjugated rabbit anti goat IgG (**B**). Immune complex and free forms could be easily separated thanks to MCSNP magnetic properties and their colloidal behavior ensured an immunoassay in homogeneous phase. First the influence of nanosupport properties (charge and α-Lac density) and medium effect (ionic strength, buffer nature) on immunocapture efficiency and specificity was investigated. Then the performances of the original MCSNP-based immunoassay were compared with the ones of conventional enzyme-linked immunosorbent assay (ELISA). The incubation time for target analyte capture was accelerated 200 fold and we showed that 0.3 ng/mL of target antibody could be detected, this limit of detection was 20 times lower than the one evaluated with ELISA (**C**). The MCSNP system was successfully applied for IgG determination in real matrix like serum and results showed good agreement ($r^2 = 0.915$) with ELISA.



A.TEM image of MCSNP (a). Sketch of the MCSNP functionalized with α-Lac antigen (b). **B**. Schematic of the MCSNP-based homogeneous immunoassay procedure. **C.** Calibration curve obtain in ELISA and MCSNP context.

[1] V. Maurice, T. Georgelin, J.M. Siaugue, V. Cabuil, *J. Magn. Magn. Mater*. **2009**, *321*, 1408.
[2] Georgelin, T. ; Maurice, V. ; Malezieux, B. ; Siaugue, J.-M. ; Cabuil, V. ; *J. Nanopart. Res.* **2010**, *12(2)*, 675.
[3] F. D'Orlye, A. Varenne, T. Georgelin, J-M. Siaugue, B. Teste, S. Descroix, P. Gareil, *Electrophoresis* **2009**, *30*, 257.

Friday, May 28, 2010				
7:30	**Registration desk opens**			
8:30	Krishnan, Kannan	**Tutorial II** on Magnetic Things We All Should Know	Seattle, U.S.A.	Tutorial 2
	Session 8: Magnetic Hyperthermia I - *Chair: Carlos Rinaldi (Puerto Rico)*			
9:00	Berndl, Bettina	Antibody Targeted Magnetic Nanoparticle Hyperthermia for Cancer Therapy	London, U.K.	Talk 47
9:15	Gogoi, Manashjit	Thermosensitive magnetoliposomes containing biphasic mixture of metal oxide composite nanoparticles for combined cancer drug delivery and hyperthermia	Mumbai, India	Talk 48
9:30	Ivkov, Robert	Development of magnetic nanoparticles and devices for thermal therapy of cancer: A radiosensitization study in mice bearing human prostate cancer xenografts	Baltimore, U.S.A.	Talk 49
9:45	Kashevsky, Bronislav	Substantiation and in-vivo approbation of the low-frequency ferromagnetic hyperthermia	Minsk, Belarus	Talk 50
10:00	**Coffee break / poster session / exhibitors**			
	Session 9: Magnetic Hyperthermia II - *Chair: Quentin Pankhurst (U.K.)*			
10:30	Martinez-Boubeta, Carlos	Self-assembled multifunctional fe/mgo nanospheres for mri and hyperthermia	Barcelona, Spain	Talk 51
10:45	Pradhan, Pallab	Multifunctional magnetic liposomes for magnetic drug targeting and hyperthermia applications	Mumbai, India	Talk 52
11:00	Rau, Beate	Thermotherapy of oesophageal cancer with superparamagnetic iron oxide nanoparticles	Berlin, Germany	Talk 53
11:15	Southern, Paul	Real-time In Vitro Analysis of Magnetic Hyperthermia using Optical and Fluorescent Microscopy	London, U.K.	Talk 54
11:30	Plank, Christian	Magnetofection – magnetically enhanced nucleic acid delivery, from research tool towards clinical application	Munich, Germany	Invited talk 4
12:10	**Lunch**			
13:25	**POSTER PRIZE** - *Presented by Cordula Gruettner*			
	Session 10: Magnetic Imaging / MRI - *Chair: Tim St. Pierre (Australia)*			
13:30	Jing, Ying	Dual Functional Au coated Fe70Co30 High-Magnetic-Moment Nanoparticles for MR imaging and Therapy	Minnesota, U.S.A.	Talk 55
13:45	Lee, Yi-Cheng	MnO Nanocrystals as in vivo Time-Dependent T1 MRI Contrast Agents	Seattle, U.S.A.	Talk 56
14:00	Misri, Ripen	Molecular Imaging Bioprobes (MRI/SPECT) for Mesothelin Expressing Cancers	Vancouver, Canada	Talk 57
14:15	Nikitin, Petr	Non-invasive in vivo mapping and long-term monitoring of magnetic nanoparticles in different organs of an animal	Moscow, Russia	Talk 58
14:30	Rahn, Helene	Calibration phantom for quantitative tomography analysis of biodistribution of magnetic drug carriers	Dresden, Germany	Talk 59
14:45	Rühmer, Dennis	Magnetic relaxation imaging using a fluxgate based scanner	Braunschweig, Germany	Talk 60
15:00	Soenen, Stefaan	Intracellular iron oxide nanoparticle coating stability determines nanoparticle usability and cell functionality	Leuven, Belgium	Talk 61
15:15	Tokarev, Alexander	Magnetic Nanoneedles for Optofluidic Applications	Clemson, U.S.A.	Talk 62
15:30	Trekker, Jesse	Effect of the core size of monodisperse superparamagnetic nanoparticles on their relaxometric enhancing properties for MRI	Leuven, Belgium	Talk 63
15:45	Zabow, Gary	Cylindrical Magnetic Nanoshells for Multispectral MRI	Bethesda, U.S.A.	Talk 64
16:00	**Coffee break / poster session / exhibitors**			
	Session 11: Magnetic Separation - *Chair: Maciej Zborowski (U.S.A.)*			
16:30	AlHetlani, Entesar	On-chip generation and manipulation of magnetic w/o and o/w droplets	Hull, U.K.	Talk 65
16:45	Andreu, Jordi	Equilibrium and nonequilibrium aggregation af superparamagnetic colloids	Bellaterra, Spain	Talk 66
17:00	Balasubramanian, P.	Circulating tumor cell detection by magnetic depletion of normal bloopd cells	Columbus, U.S.A.	Talk 67
17:15	Earhart, Christopher	Improved Designs of a Microfabricated Magnetic Sifter for Biomolecule and Cell Purification	Stanford, U.S.A.	Talk 68
17:30	Fischer, Thomas	Colloidal transport and separation on magnetic garnet films	Bayreuth, Germany	Talk 69
17:45	Hoshino, Kazunori	Microfluidic Chip-Based Immunomagnetic Detection of Circulating Tumor Cell	Austin, U.S.A.	Talk 70
18:00	Schwarz, Sebastian	Nanoparticle Size and Surface Charge Determine Formation of Protein Corona, Cellular Uptake and Magnetic Resonance Imaging Properties	Aachen, Germany	Talk 71
18:30	**Traditional boat cruise / visit of downtown Rostock** - sponsored by **micromod**			

Antibody Targeted Magnetic Nanoparticle Hyperthermia for Cancer Therapy

B. Berndl[1,2], K. L. Vigor[1], P. Southern[2], M. Kallumadil[2], K. Page[2], H. Kogelberg[1], B. Tolner[1], M. B. Fernandez[3], K. T. Al-Jamal[3], W. Al-Jamal[3], K. Kostarelos[3], Q. A. Pankhurst[2] and K. A. Chester[1]

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Superparamagnetic iron oxide nanoparticles (SPION) have exciting potential for cancer therapy using magnetic alternating current hyperthermia (MACH). The current challenge is to achieve MACH *in vivo* and to target SPION specifically to cancer cells. We have addressed this in two parts (i) by developing the *in vitro* and *in vivo* application of MACH and (ii) by functionalizing SPION with single chain Fv antibody (scFv) fragments to confer tumour selectivity.

(i) After demonstrating the ability of the SPION-MACH system to kill tumour cells *in vitro*, an *in vivo* study was performed. Temperature increase *in vivo* was measured by real-time thermo-imaging as shown in Fig.1. Hyperthermic effects were also assessed at a cellular level using immunohistochemistry to detect the presence of heat shock protein 70 (HSP70) and cleaved caspase 3, a marker of apoptosis, in relation to the SPION localization detected by Prussian blue. The results showed that SPION effectively generate heat *in vitro* and *in vivo* when subjected to the MACH system. Furthermore, MACH enhanced *in vivo* retention of SPION and caused cellular stress in the tumour.

(ii) The possibility of specifically targeting SPION to cancer cells was explored by functionalizing with a recombinant scFv specific for the carcinoembryonic antigen (CEA). The scFv were attached to SPION of different hydrodynamic size and surface chemistry by sodium periodate oxidation or carbodiimide conjugation. In addition we investigated maleimide coupling to establish site-specific conjugation. Functionality of the scFv-SPION was confirmed by ELISA. Targeting efficacy was evaluated by cellular SPION uptake and Confocal Laser Scanning Microscopy (CLSM). The results demonstrated that scFv-functionalized SPION bound specifically to CEA-expressing human tumour cells.

In conclusion, our results showed that MACH therapy is feasible *in vivo*, and that scFv-coated SPION are functionally active and capable of specifically targeting cancer cells. This work will form a basis for addressing major challenges in the development of advanced therapeutic nanomedicines.

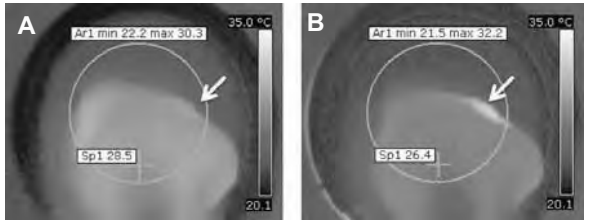

Figure 0 – Thermo-images of sub-cut human tumour xenografts (white arrows) in mice. Tumours were injected with 0.5 mg SPION and subjected to MACH for 1 min (A) or 20 mins (B). ΔT=5.8 °C

Talk 47

Thermosensitive magnetoliposomes containing biphasic mixture of metal oxide composite nanoparticles for combined cancer drug delivery and hyperthermia

Manashjit Gogoi*, Haladhar Dev Sarma[+], Dhirendra Bahadur and Rinti Banerjee***

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Thermosensitive liposomes were prepared with distearoylphosphatidylcholine (DSPC) and Cholesterol for combined cancer hypertherthermia and drug delivery. The size of the liposomes was around 225nm and the zeta potential was -41mV. In order to evaluate thermosensitivity, calcein release study was done with different composition of lipids i.e. DSPC/Cholesterol 7:3, 8:2 and 9:1, at 37^{o} and 43^{o}C. Out of these compositions, DSPC/Cholesterol 8:2 (w/w) was found to be most thermosensitive and release study was done with paclitaxel-a potent anticancer drug. The cumulative drug release at 37^{o} and 43^{o}C were 3.9 and 5.2% respectively. In order to evaluate the cellular uptake of this liposome, cellular internalization study was done with MCF-7, human breast cancer cell line. Then a biocompatible biphasic mixture containing $La_{0.75}Sr_{0.25}MnO_3$ (LSMO) and iron oxide nanoparticles were prepared and loaded in the liposomes. *In-vivo* biocompatibility and biodistribution study of magnetoliposomes done in swiss mice, suggested that these magnetoliposomes are biocompatible in nature. Finally cytotoxicity of paclitaxel loaded magnetoliposomes were evaluated in MCF-7 cell line under normothermic and hyperthermic conditions. The IC50 value of these magnetoliposomes at normothermic condition is 100nM, which reduced significantly under hyperthermia condition. Hyperthrmia experiments were done in alternating magnetic field with 425 kHz and variable field strength.

Keywords: Thermosensitive liposomes, hyperthermia, biphasic mixture, magnetic nanoparticles

Talk 48

Development of magnetic nanoparticles and devices for thermal therapy of cancer: A radiosensitization study in mice bearing human prostate cancer xenografts

Robert Ivkov, Michael Armour, Christine Cornejo, Haoming Zhou, Mohammad Hedayati, Yonggang Zhang, Theodore DeWeese

Department of Radiation Oncology and Molecular Radiation Sciences, Johns Hopkins University School of Medicine, Baltimore, MD

Heat has long been recognized as a potent sensitizer of radiation for cancer therapy. Technical hurdles associated with the selective and tumor-specific delivery of cytotoxic heat have precluded the widespread clinical adoption of heat as a therapeutic agent. Iron oxide magnetic nanoparticles, suitably formulated for in vivo applications, have recently emerged as a promising therapeutic alternative. This is particularly true when considering this approach as a delivery vehicle for mild heating as an adjuvant to radiation therapy. The potentially significant advantages offered by magnetic nanoparticle heating relate to the precision of heat delivery that can potentially improve therapeutic response while minimizing morbidity.

Challenges associated with magnetic nanoparticle heat delivery have arisen from two main sources – 1) particles delivering too little heat, either because they possess insufficient power loss factors or because of insufficient uptake by tumor tissue; and, 2) non-specific heating from excessive eddy current production in non-targeted tissues that result from the magnetic field interactions with tissue. Significant materials and engineering efforts can yield benefits demonstrating the potential for this approach.

We present results that these challenges can be overcome, at least in small animal models of human cancer. Iron oxide/starch core/shell nanoparticles that produce >400 W/g Fe @ 95 kA/m and 150 kHz were used ablate tumors and achieve complete regression. Mice tolerated field amplitudes >90 kA/m and 150 kHz for extended therapy sessions (20 minutes) due to electric shielding devices developed in our laboratory. Thermal measurements and tumor growth data are presented demonstrating the potential for delivery of therapeutic heating. Lower heat doses were combined with low-dose radiation to demonstrate radio-sensitization in mice bearing human prostate tumor xenografts.

Thermal measurements demonstrate that the combination of magnetic nanoparticles, particularly with higher dose combinations, particle and magnetic field, can produce substantial local and ablative heating of tumors. Mouse rectal temperatures, a measure of core body temperature, remained within safe levels (<40 °C) for the 20-minute therapy session with no mortality for all cohorts. Complete tumor regression was observed for the 75 kA/m therapy (and higher) and 5 mg Fe/g tumor loading of nanoparticles. Radiosensitization was achieved with lower thermal doses. For these cohorts, the heat alone was not effective.

Magnetic nanoparticle-based heat delivery has the potential to sensitize tumors to radiation even with low thermal doses (CEM <100). This offers the potential to improve therapeutic response while simultaneously minimizing therapy-related morbidity.

Substantiation and *in-vivo* approbation of the low-frequency ferromagnetic hyperthermia

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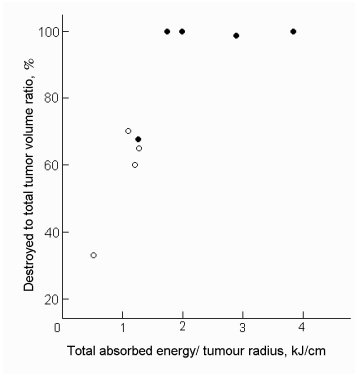
We present studies of the problem of magnetic hyperthermia with high-coercive ferromagnetic particles (FMP) and settle down the doubts [1] as to feasibility of this approach. Our previous studies included investigations of the energy absorption in liquid and solid dispersions of FMP [2-5], producing FMP with suitable sizes and coercivity [5]. Now, we summarize these results, develop a rational model of tumor heating, represent an automated experimental setup for controlled hyperthermia on small animals, describe the properties of used FM particles, present the results of experiments on rats with the RS-1 model of liver cancer.



An important finding, which ensures the possibility to strictly control the heat production inside a tumor, is that the specific energy absorption rate (SAR) of the produced FMP does not depend on the liquid matrix viscosity and is a well defined function of the AC field amplitude. The developed experimental setup generates the field with amplitude of up to 900 Oe and the frequency of 3.70 kHz. Its feedback system provides strict control of the temperature elevation at the site of a thermocouple (TC) which we place into healthy tissue, right under a heated tumor. Another TC is placed at the tumor center. Each TC is mounted in a thin hypodermic needle. During treatment, the system automatically storages the two temperatures and the energy absorption rate. 9 tumors have been treated. The first group (4 tumors) was heated at 43 °C during 30 min, the second (5 tumors), at 44 °C during 20 min. The tumors were of different shape, with volumes between 1.2 and 3.6 ml. The amount of particles syringed into each tumor was predetermined from the developed model of heating, with the average content equal to 120 mg per 1ml of tumor. The maximum SAR of used FMP was equal to 37 Wt/g. The effect of tumor destruction was estimated in 24 hours after the session using a vital staining. The figure presents the percentage of tumors destruction vs a treatment parameter representing the ratio of the total absorbed energy to the characteristic tumor radius. Empty circles stand for the first group, and filled, for the second. In the latter case, 4 tumors of 5 were destroyed almost completely. Note, that at the stationary regime of heating, the rate of energy absorption dropped down to ~0.3-0.2 of its starting (maximal) level (~ 6-10 Wt).

Our studies demonstrate, that FMP can be dispersed within tumors, provide the possibility of strictly controlled heat generation, and allow easy control of temperature level. We suggest that ferromagnetic hyperthermia is quite competitive as compared with the superparamagnetic one.

The authors appreciate financial support of the Belarusian Republican Foundation of Fundamental Research (Project X08-257)

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SELF-ASSEMBLED MULTIFUNCTIONAL Fe/MgO NANOSPHERES FOR MRI AND HYPERTHERMIA

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We are introducing (Nanomedicine: NBM, DOI: 10.1016/j.nano.2009.09.003) an alternative way for synthesizing high quality metallic, soft ferromagnetic Fe nanoparticles (NPs) with an outer protective MgO sheath, by using one-step vapour-phase condensation. Research on MgO was originally motivated because Mg^{2+} ions exhibit a biological activity for bone regeneration and it is ubiquitous and essential to all living organism. Accordingly, it was shown that as-prepared Fe/MgO particles display no toxic response over *in-vitro* 3T3 mice and glial cell cultures assessment.

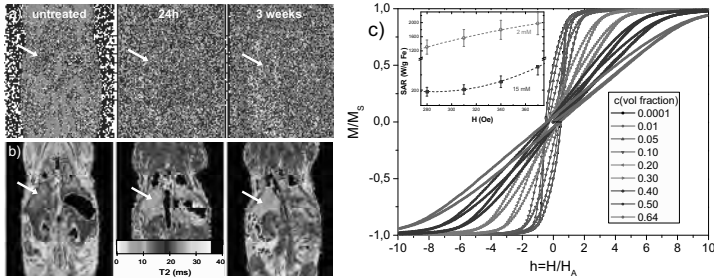

Figure. **a.** T2-weigthed MR images of mouse body before and after injection of nanoparticles. The liver appears hypo-intense in images after contrast injection (arrow). **b.** Colour-coded T2 maps showing a decrease in T2 values in the liver after contrast administration. **c.** Hysterical behavior of a system of monodisperse interacting magnetic particles with increasing concentration at a temperature well below the blocking temperature. Inset shows the SAR estimation as extracted from experimental curves.

In this report we first discuss results on particles biodistribution. In preliminary *in vivo* animal experiments, we intravenously injected 10 µl g^{-1} of saline solution at 1 mM Fe^0 molar concentration into mice (total iron dose about 0.6 mg kg^{-1}). No apparent acute toxicity or side-effects health problems were observed over a monitoring period of 3 weeks. Animals were imaged using MRI (Figure). The distribution of NPs in the mouse 24 h post injection is similar to that reported previously for iron oxides NPs with similar physical characteristics.[1]

On the other hand, *in vitro* hyperthermia experiments were performed in three different (MDA, SkBr3 and MCF7) human breast cancer cell lines in order to assess cytotoxicity, iron uptake and heating effectiveness.The losses due to magnetization reorientation in ferromagnetic particles depend on the type of remagnetization process which is determined besides by the intrinsic magnetic properties -like magnetocrystalline anisotropy- in complicated ways by particle size, shape and microstructure. It occurred to us that it might be profitable to study by Monte Carlo methods the hyperthermia performance of ferromagnetic particles. The physical model employed for our numerical simulations is the same as in Ref. [2], where the particle sample is a ferrofluid without aggregations, the positions of the particles are kept fixed and the easy axes are chosen randomly. The particle dipole interaction was simulated by varying the volume concentration of the NPs in the range from 0.0001 to 0.64. Experimental and modelling results indicate that an increase in dipolar interactions produces a decrease in the magnetic susceptibility and hysteresis losses (Figure), thus diminishing the hyperthermia output. These findings may have important clinical implications for cancer treatment.

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Multifunctional magnetic liposomes for magnetic drug targeting and hyperthermia applications

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A multifunctional magnetic liposome formulation, which is designed to combine features of biological (folate receptor mediated) and physical (magnetic field assisted) drug targeting for use in magnetic hyperthermia triggered drug release for cancer therapy (Fig. 1) has been developed.. The magnetic liposomes (MagFolDox) composed of DPPC/Cholesterol/DSPE-PEG_{2000}/DSPE-PEG_{2000}-Folate at 80:20:4.5:0.5 molar ratio released 52% doxorubicin at 43°C after 60 minutes incubation in 50% fetal bovine serum. The MagFolDox, when physically targeted to tumor cells in culture by a permanent magnetic field yielded a several fold increase in cellular uptake of doxorubicin as compared to Caelyx® (a stealth liposomal formulation of doxorubicin), nonmagnetic folate-targeted liposomes and free doxorubicin in folate receptor expressing tumor cell lines. Consequently, the MagFolDox liposomes enabled improved tumor cell killing. Moreover, MagFolDox and magnetic hyperthermia (at 42.5-43.5°C) showed synergistic cytotoxic effect in KB cells. Also, the distribution of magnetic liposomes *in vivo* can be monitored by MRI due to their contrast enhancement properties. *In vivo* real time MRI monitoring in mice confirmed significantly higher blood circulation time for MagFolDox compared with free magnetic particles. This also confirmed that the biodistribution of the MagFolDox can be monitored *in vivo* by MRI. Thus, the multifunctionality of the magnetic liposomes has been successfully demonstrated.

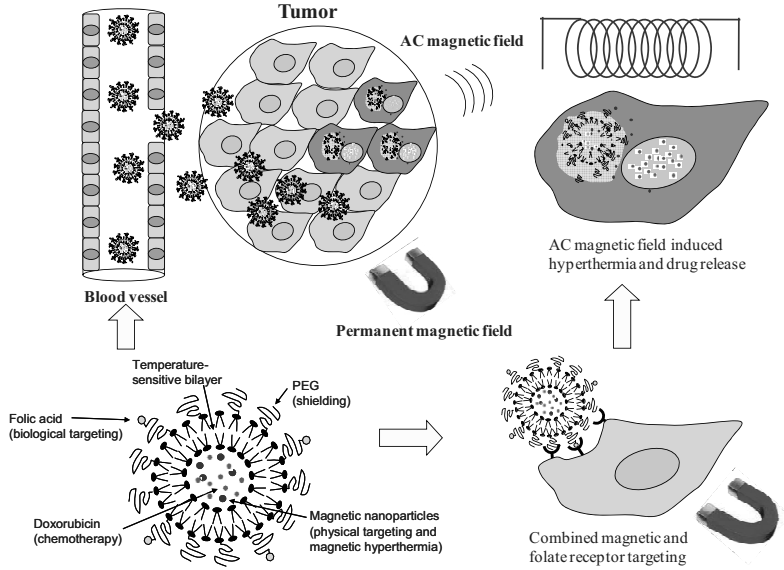

Fig. 1 Cartoon diagram showing the integrated concept of magnetic drug targeting and hyperthermia for cancer therapy using multifunctional magnetic liposomes (J Contr Rel, 2010;142: 108-121)

Thermotherapy of oesophageal cancer with superparamagnetic iron oxide nanoparticles

Beate Rau, Marcus Steinbach, Jens-Thorsten Ollek, Michael Hünerbein, Andreas Jordan

Background: Oesophageal cancer has a poor outcome according to overall and disease free survival. It is known, that the combination of hyperthermia in addition to radio- and /or chemotherapy is able to enhance the effect of the treatment. Superparamagnetic nanoparticles induced thermotherapy is an therapeutic option to further improve treatment. The objective of this study is to show that superparamagnetic nanoparticles could provide interstitial hyperthermia and can be applied safely and effectively in case of incurable oesophagus carcinoma. The method comprises direct injection of a magnetic fluid into the tumor and its subsequent heating in an alternating magnetic field.

Methods: Altogether 9 patients with an oesophageal cancer were involved in the study. Two patients received thermotherapy as monotherapy due to previous chemoradiotherapy. 7 patients received combined therapy with chemoradiotherapy (GHD 45 Gy; 5-FU 225 mg/m^2/d continuously and Cisplatin 30 mg/m^2 as bolus). Hyperthermia was applied synchronous to Cisplatin infusion once a week. The nanoparticles were placed pretherapeutically via endoscope and controlled by CT scan. Temperature was measured by a probe placed inside the oesophagus. Therapy associated toxicities were recorded according to the WHO-guidelines.

Results: Succesful endoscopic placement of the nanoparticles was performed. Measured treatment temperature inside the oesophagus was in average 40°C. Calculated temperatures according to the distribution of the nanoparticles measured by post instillation CT were 41.5°C on average. Tumour shrinkage was observed in every tumour and leads to better life quality.

Conclusion: With magnetic nanoparticles induced thermotherapy in advanced oesophageal cancer both, monotherapy or combination with chemo- and/or radiotherapy is feasible and regression of tumour size was observed with reasonable toxicity. To improve quality of temperature measurement and to proof validity of treatment further investigations are necessary.

Real-time In Vitro Analysis of Magnetic Hyperthermia using Optical and Fluorescent Microscopy.

Paul Southern[1*], Mathew Kallumadil[1], Bettina Berndl[2], Steve Nesbitt[1], Quentin Pankhurst[1]
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There is an obvious and widespread need for an alternative treatment of metastatic cancer that does not rely upon systemic therapies such as chemotherapy or radiotherapy, which can result in adverse side effects. In treating cancer cells, there is often damage sustained to healthy cells inducing increased strain on an already weakened body.

One viable solution would be to develop a specific localised treatment that would deliver biomolecular therapeutic agents, preferentially targeting cancer cells, that could then be activated in a controlled manner thus increasing the efficiency of the therapy where needed. One particular method relies upon heat generated from a magnetic nanoparticle under the influence of an externally applied alternating magnetic field (AMF). This method, known as magnetic hyperthermia, is ideally suited for treating metastatic cancers which are highly susceptible to cell death as a response to a small increase in local temperature, typically between 3-5ºC [1].

We will be presenting a novel approach using real-time live cell fluorescent and optical microscopy to study the cellular effects of magnetic hyperthermia on in vitro cell cultures. We will demonstrate using HT29 melanoma and LS174T colorectal cancer cell lines the effects of particle concentration and local AMF strengths on the cell viability.

To complement these studies a small in-vivo murine study (n=11) was performed using commercial nanoparticles, previously shown demonstrating a high specific loss power [2]. The effectiveness of magnetic hyperthermia on subcutaneuos tumours (Breast cancer cell line MDA-MB231) is discussed with particular emphasis on cell viability as a function of local heating rates as shown in Figure 1.

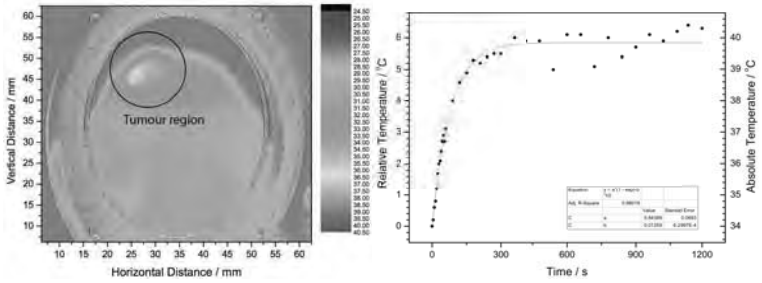

Figure 1 – Surface heat map across tumour region (left) and associated temperature profile during exposure to an alternating magnetic field (right).

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Magnetofection – magnetically enhanced nucleic acid delivery, from research tool towards clinical application

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Christian Plank's group (www.plank-lab.net) is developing systems for localized nucleic acid delivery. Equipping these systems with magnetic nanoparticles allows localizing delivery and boosting its efficiency by magnetic force. This form of magnetic drug targeting, also known in the literature as "Magnetofection", is used by researchers worldwide for nonviral and viral nucleic acid delivery.

An extension of magnetic targeting concepts is their combination with modules for triggered drug release. In this context, the Plank group develops thermosensitive magnetic liposomes and magnetic microbubbles. With the latter, localized nucleic acid delivery can be triggered by ultrasound. This has been used advantageously for tissue regeneration in a rat skin-flap model which is relevant in reconstructive surgery.

Furthest advanced towards clinical application is a nanomagnetic genetic tumor vaccine consisting of plasmid DNA, bound to magnetic nanoparticles, encoding a cytokine. When applied intratumorally in animal patients (cats) suffering from fibrosarcoma, the most frequent skin tumor in cats, this vaccine prolongs the recurrence-free survival time from 270 days to more than 1000 days.

Dual Functional Au coated $Fe_{70}Co_{30}$ High-Magnetic-Moment Nanoparticles for MR imaging and Therapy

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Nanoparticles have become a potential platform for diagnosis, therapy and many biomedical applications. Novel nanoparticles possessing both diagnostic and therapeutic function are desirable for future clinical usage. This could be achieved by designing the structure of the nanoparticle and materials specifically. Here, Au coated $Fe_{70}Co_{30}$ high-magnetic-moment core-shell nanoparticles were developed as a promising MRI (magnetic resonance imaging) contrast agent with anti-angiogenic property [1]. The high local spin density of $Fe_{70}Co_{30}$ can modify the local magnetic field distribution effectively even without the demand of a large external field. Low dosage and less side-effect are hence expected for this kind of contrast agents. The outer Au shell has anti-angiogenesis property that can be used in the therapy for angiogenesis dependent disorder.
$Fe_{70}Co_{30}$-Au core-shell nanoparticles were fabricated directly out of gas phase by a unique physical gas condensation technique [2] (Fig. 1a). Formation of Au shell in terms of diffusion was controlled in the gas phase depending on the thermal history [3]. The anti-angiogenic effect of high-magnetic-moment $Fe_{70}Co_{30}$-Au core-shell nanoparticles was determined through their inhibition of VEGF165 (vascular endothelial growth factor 165) induced proliferation of HUVECs (human umbilical vein endothelial cells) (Fig. 1b). The induced proliferation of HUVECs was greatly reduced (nearly 50%) in the presence of $Fe_{70}Co_{30}$-Au nanoparticles. Western Blot analysis of VEGFR2 phosphorylation was tested to confirm that $Fe_{70}Co_{30}$-Au inhibited function of VEGF165. It was found that VEGF165 induced phosphorylation of VEGFR2 was inhibited, which indicates that $Fe_{70}Co_{30}$-Au binds to VEGF165 and leads to abrogation of VEGFR2 phosphorylation. MR imaging property was tested on HUVECs treated with $Fe_{70}Co_{30}$-Au. $R2^*$ and $T2^*$ plotted as a function of sample dose exhibit significant relaxivity of $Fe_{70}Co_{30}$-Au(Fig. 1c). A saturation value of relaxivity was reached due to maximum intracellular uptake. These studies prove that $Fe_{70}Co_{30}$-Au nanoparticles can function as a MRI diagnostic probe and an anti-angiogenic therapeutic moiety at the same time.

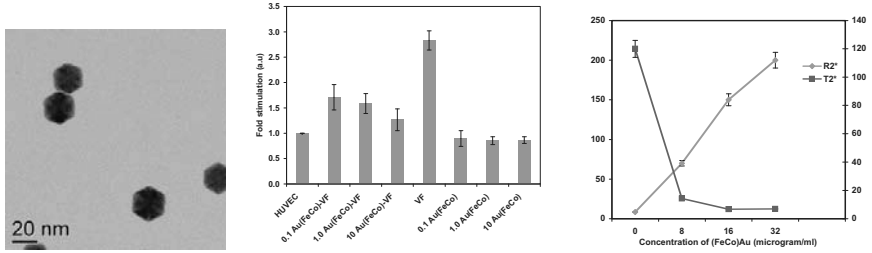

Fig.1a TEM image of $Fe_{70}Co_{30}$-Au nanoparticles; 1b Effect of $Fe_{70}Co_{30}$-Au on VEGF165 induced proliferation of HUVECs; 1c MRI experiments with $Fe_{70}Co_{30}$-Au treated HUVECs

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MnO Nanocrystals as *in vivo* Time-Dependent T_1 MRI Contrast Agents

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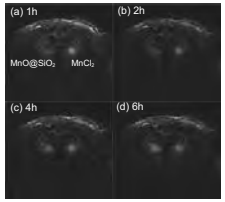
Nanostructured inorganic nanoparticles and core-shell structures can be used as MRI contrast agents with the advantages of flexible surface modification characteristics.[1] Manganese based nanoparticles have potential as agents that can be "activated" when taken into cells. For example, manganese oxides or manganese carbonates are insoluble at pH 7 but dissolve to release Mn^{2+} at the lower pH found in the endosome-lysosome pathway. The dissolution of manganese based particles in an acidic environment leads to large enhancement of the T_1 relaxation rate.[2] In addition, Mn^{2+} can leave the endosome-lysosome pathway to fill the entire cell leading to a much larger volume distribution of the contrast agent.[2] It would be advantageous to be able to control the rate of dissolution of Mn based nanoparticles to control T_1 contrast signals, *in vivo* with time. To this end, five different coatings (MSA, PMAO, PF127, PMAO-PEG and SiO_2) on MnO nanocrystals have been tested to study the release rate of the Mn^{2+} ions and change in relaxivity at pH 7 compared to pH 5.



Highly monodisperse, single-phase, MnO nanocrystals, ~10 nm in diameter, were prepared by chemical routes and their magnetic properties were extensively characterized.[3] Mercaptosuccinic acid (MSA), poly(maleic anhydride-alt-1-octadecene) (PMAO), Pluronic F-127 (PF127), PMAO-PEG and SiO_2 were then used, respectively, to transfer native hydrophobic particles to aqueous solutions for biocompatible applications. MSA-, PMAO-, PF127- , and PMAO-PEG-coated nanoparticles had relatively high relaxivities in PBS buffer solutions at pH 7 (1.8-2.5 $s^{-1}mM^{-1}$) and dissolved very quickly at pH 5. For example, it took ~20 min to reach relaxivity of 6.94 for MSA coated particles at pH 5. SiO_2 coated particles showed the smallest relaxivity (0.29 $s^{-1}mM^{-1}$) at neutral pH, which was stable over time. The MnO@SiO_2 nanoparticles had the best dynamic range for contrast change when the pH was lowered. Time dependent relaxivity measurements, at pH ~5.0 in acetic acetate buffer solution of MnO@SiO_2 nanoparticles showed values increasing to 2.44 $s^{-1}mM^{-1}$ by 53 min to 6.1 $s^{-1}mM^{-1}$ after 75 hours. This final relaxivity is equivalent to $MnCl_2$ indicating that the particles had completely dissolved. The release rate of Mn^{2+} ions was faster for the first 5 hrs, subsequently slowing down after 10 hrs. MP-RAGE images of the rat brain (Fig) showed that the signal intensity at the injection site of MnO@SiO_2 particles (left sides in images) increased with time consistent with the slow dissolution rate measured in vitro. The signal at the site of $MnCl_2$ injection (right sides of images) was elevated at the first image after injection and began to decrease slightly due to tracing of the Mn^{2+} ions to different parts of the brain.

Different coating for MnO nanoparticles can affect the T_1 relaxivity at pH 7 and the rate of dissolution at pH 5. MnO@SiO_2 particles had the lowest pH 7 relaxivity and the slowest dissolution rate at pH 5 in vitro. *In vivo* MRI of MnO@SiO_2 particles injected into the brain showed time-dependent signal changes consistent with the in vitro rates. The MnO@SiO_2 particles show the best potential for delaying the release of MRI contrast until specific biological processes have occurred, such as endocytosis.

This work was supported by NSF/DMR #0501421 and Intramural Research Program of the NIH, NINDS.

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Molecular Imaging Bioprobes (MRI/SPECT) for Mesothelin Expressing Cancers

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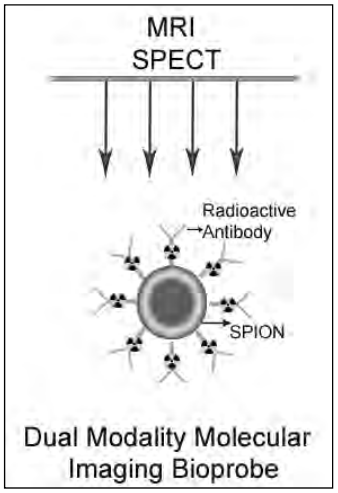
Specific molecular MRI bioprobes have enormous potential of being used in clinical settings for MR imaging of tumours as well as MRI guided laser tumour ablation for treatment of tumours [1]. Studies have shown that a highly specific MRI contrast agent capable of producing high resolution images can be prepared by coating magnetite (Fe_3O_4) particles with monoclonal antibodies directed against a tumour antigen. Such preparations maintain both the immunoreactivity of the monoclonal antibody and the full relaxing capability of the magnetite particle [2]. Our goal is to develop dual modality molecular imaging bioprobes in the form of magnetic nanoparticles conjugated to an antibody for the early single photon emission computed tomography (SPECT) and MR imaging of mesothelin antigen expressing tumours (mesothelioma, pancreatic cancer, ovarian cancer). To add anatomic imaging information, magnetic resonance imaging (MRI) of the magnetic bioprobes will be used to provide high signal sensitivity and good contrast. It is anticipated that combining both imaging modalities will provide a powerful diagnostic tool for early diagnosis and monitoring of these cancers. Antimesothelin antibody mAbMB was radiolabelled with Indium-111 (^{111}In) using a p-SCN-Bn-DTPA chelator. The radiolabelled antibody (^{111}In-mAbMB) was then characterised *in vitro* for stability, immunoreactivity, binding affinity and internalization using A431K5 cells (mesothelin expressing cells). The biodistribution of ^{111}In-mAbMB was evaluated in tumour-bearing mice to confirm its localization in the mesothelin expressing tumours. Further, tumour uptake of ^{111}In-mAbMB was also visualized using MicroSPECT/CT imaging. SPIO nanoparticles (SPION <100 nm) bearing –COOH groups were conjugated with ^{111}In-mAbMB using the carbodiimide coupling reaction. Antibody conjugation efficiencies of more than 75% were achieved. The particles (^{111}In-mAbMB-SPION) were characterized for their magnetic properties and *in vitro* cell binding properties. *In vitro* MR imaging studies were carried out using the 7 Tesla Bruker Biospec 70/30 USR (from Bruker Biospin: Ettlingen, Germany) MRI instrument. These studies showed that the contrast gradient at varying concentrations of SPION and ^{111}In-mAbMB-SPION was similar, indicating that the conjugation did not significantly affect the relaxation properties of the particles. The relaxivity values obtained were as follows**:** SPION: $r_1 = 0.0006\ \mu g^{-1}s^{-1}ml$; $r_2 = 4.371\ \mu g^{-1}s^{-1}ml$ and ^{111}In-mAbMB-SPION: $r_1 = 0.00066\ \mu g^{-1}s^{-1}ml$; $r_2 = 5.1661\ \mu g^{-1}s^{-1}ml$. The cell uptake was evaluated after 3, 6 and 24 hours using A431K5 (mesothelin positive) and A431 (mesothelin negative) cells after incubation with ^{111}In-mAbMB-SPION at 37˚C. The cell uptake of ^{111}In-mAbMB-SPION by A431K5 cells was significantly higher than A431 cells at all the time points, indicating its ability to bind specifically to mesothelin expressing cells. The above studies provide evidence that SPIO nanoparticles conjugated with ^{111}In labelled antimesothelin antibody mAbMB retain their magnetic properties and hold great promise as specific MR imaging bioprobes for mesothelin expressing tumours. Biodistribution and MR imaging properties of this dual modality imaging bioprobe are currently being studied in an *in vivo* animal model.



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Non-invasive *in vivo* mapping and long-term monitoring of magnetic nanoparticles in different organs of an animal

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Quantitative detection of magnetic particles (MP) is very important for various bioanalytical and medical applications. A highly sensitive room-temperature method is known for MP detection based on non-linear MP magnetization at two frequencies f_1 and f_2 and recording the response at combinatorial frequencies $f = mf_1 + nf_2$, where m and n are integers (or $m = 0$ or under exposure to a magnetic field of one frequency [1]; or both may be varied to get the best signal-to-noise ratio), e.g. at $f = f_1 \pm 2f_2$ [1-4]. The sensitivity of the method of few ng of MP is on the level of radioactive technique for MP based on ^{59}Fe isotope [3].

In the work discussed in this paper, the method was extended for non-invasive *in vivo* topography mapping of the MP distribution among different organs of a rat with employment of new highly sensitive room temperature devices equipped by an external inductive probe (Fig.1). The external probe quantifies MP within 20-mm depth from the animal skin with possible further depth increase. Fig. 2 demonstrates good correlation for non-invasive *in vivo* external scanning of the rat by the probe and *ex vivo* MP quantification in different rat's organs. The results allow long-term *in vivo* study of MP redistribution among different organs for the same animal. The MP dynamics was non-invasively recorded in different organs of a live rat as shown in Fig. 3. The developed devices are promising for investigation of concentration evolution of various MP inside animals' bodies, for selection of biodegradable MP, etc. The possibility of magnetic immunoassay (MIA) realization directly inside a human body (Fig. 4A) is of our special scientific interest. We believe that the localization of antibody-targeted MP by the probe is promising for diagnostics of cancer, atherosclerosis or other diseases. The results demonstrate that the developed detection methods combined with magnetic nanolabels can substitute the radioactive labeling in many applications, e.g. in evaluation of targeted drug delivery (Fig. 4B), due to high sensitivity to MP and lack of sensitivity to linear dia- or paramagnetic materials.

Fig.1. Rat near the induction probe.

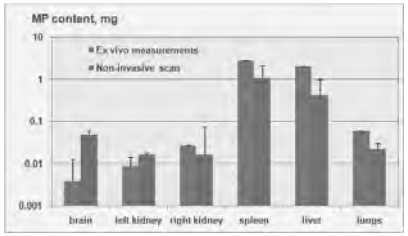

Fig.2. Results of *in vivo* scanning and *ex vivo* MP quantification in different organs

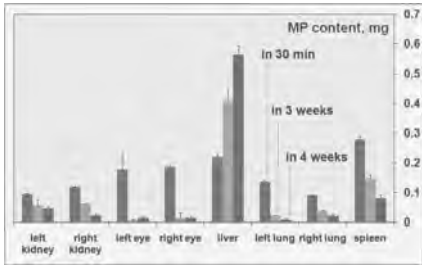

Fig.3. Long-term MP redistribution among rat's organs

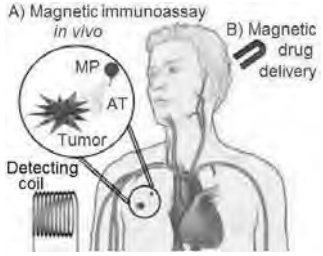

Fig. 4. MIA *in vivo* and drug delivery

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Calibration phantom for quantitative tomography analysis of biodistribution of magnetic drug carriers.

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Ferrofluids are being investigated for minimally invasive cancer treatment procedures such as magnetic drug targeting (MDT) and magnetic hyperthermia (MHT) with the aim of treating the cancer locally, minimizing the side effects. Micro-computed tomography (μCT) has been introduced as adequate technique for non-destructive 3-dimensional analysis of biological samples enriched with magnetic nanoparticles [1].

As an example of biological tissue specimen measured with synchrotron beam and x-ray tube an ex-vivo artery model after magnetic drug targeting [2] is presented in Figure 1. The main quality difference between the left image (synchrotron) and right image (X-ray tube) is the spatial density discrimination. In case of monochromized synchrotron radiation the data sets can be evaluated quantitatively, not so if a polychromatic X-ray tube is used. For complex multi-phase samples such as biological tissue enriched with magnetic nanoparticles the beam hardening artifacts cause severe problems for quantitative density determination. This problem requires a proper calibration of the polychromatic tomography equipment enabling the analysis of the data in a semi-quantitative way.

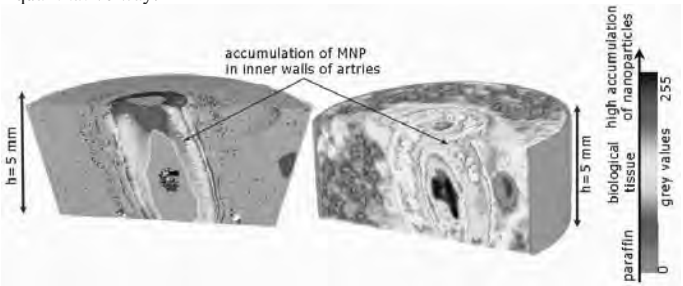

Figure 1: left: Cross-section of an artery segment measured with monochromized synchrotron beam.
right: Cross-section of the same artery segment measured with polychromatic x-ray tube.

For this purpose a phantom system has been designed. These phantoms consist of polyurethane (PU) gel containing different amount of magnetic nanoparticles. As the attenuation of the beam also depends on the thickness i.e. the path length of the beam transmitting the object the reference sample has been defined to a rectangular trapeze. Thus, with one phantom the information about the nanoparticle concentration as well as the attenuation in dependence of the path length can be determined. Recent results on progress of calibration of polychromatic tomography equipment will be presented.

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Magnetic relaxation imaging using a fluxgate based scanner

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We aim to use a 2-dimensional magnetic relaxation (MRX) imaging technology to examine the growth of human stem cells, derived from bone marrow, inside plastic cell culture bags as described in [1]. The inner surface of these bags has been modified by atmospheric pressure plasma treatment and MRX imaging is applied to measure the homogeneity and the long term stability of these modifications. For it, functionalized magnetic nanoparticles bind to the modified surfaces and act as specific markers. The advantage of using MRX is that one can distinguish between bound and unbound particles located in the same sample volume. MNPs bound to the inner surface of the bags can be distinguished from the mobile ones by analyzing the relaxation curves using a phenomenological model described in [2]. The sample is moved by an x-y-shifting table and positioned under an assembly consisting of a fluxgate magnetometer and a magnetizing coil. The setup is depicted in Fig 1. The image result can be obtained with two different methods: A simple but yet effective approach is to calculate the amplitude difference of two points on the relaxation curve and to use this information to form a pixel on a graphic display, the second and more advanced method is to use a curve fit to describe the relaxation curves mathematically. With this approach, the differentiation between bound and unbound particles can be performed quantitatively. A sample image of freeze-dried and thus immobilised MNPs forming our institute's logo 'emg' measured using two fluxgates and the first imaging method is depicted in Fig. 2.

Fig. 1: Photograph of the measurement setup.

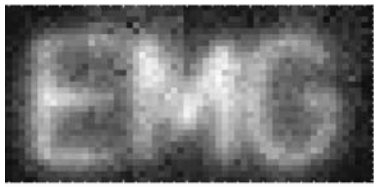

Fig. 2: Sample image of freeze-dried MNPs obtained by simultaneously scanning with two fluxgates.

Since for each measurement point, the sample has to be magnetized and the relaxation curve has to be recorded, it takes relatively long measurement times. One single complete measurement cycle can last up to 5 seconds. A scan area of about 4x4 cm^2 (2500 single measurements) can take up to 20 hours for measurement completion. This is a critical time for cell culture bags containing cells which have to be tempered and fumigated. By reducing the magnetization time by the factor of more than two and decreasing the time for relaxation measurement accordingly, the measurement time of the sample area of 4x4 cm^2 could be reduced to 4 hours without a significant loss of image resolution. This time could be furthermore reduced by implying a second fluxgate sensor. To eliminate crosstalk between the sensors their distance was optimized. Also the scan line was carefully selected to decrease the sensor overlap. The most crucial point was the demand of having two almost identical fluxgates concerning both sensitivity and noise level.

This work is financially supported by the BMWi under contract number 16INO548.

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Intracellular iron oxide nanoparticle coating stability determines nanoparticle usability and cell functionality

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Iron oxide nanoparticles are routinely exploited as T_2/T_2^* contrast agents[1]. One of the hottest topics in this biomedical research area is the non-invasive imaging of pre-labelled stem or therapeutic cells upon transplantation in an animal model of interest[2]. To this end, commercial particles such as Endorem® are frequently employed, although the particles display several characteristics which makes them less suitable for in vitro labelling[3].
In the present work, the effects on cell physiology of in-house produced cationic magnetoliposomes (MLs), i.e. 14-nm diameter iron oxide cores each individually enwrapped by a lipid bilayer containing 3.33% of distearoyltrimethyl ammoniumpropane (DSTAP)[4] - a cationic lipid - are compared with the effects of Resovist (carboxydextran-coated), Endorem (dextran-coated) and VSOP (citrate-coated) iron oxide particles. It is shown that intracellular stability of the coating molecules is of primordial importance. The results in vitro show that citrate-coated particles are degraded very fast, whereas dextran-coated ones are more stable, but still less than the lipid-coated MLs. The degradation of the particles can be shown by the increase in free ferric ions, and the distorted r1/r2 ratio of the particles, hampering their use for long-term imaging. Cells labelled with the different particles reaching similar amounts of intracellular iron, show increases in reactive oxygen species and transferrin receptor expression in C17.2 neural progenitor cells and impeded functionality of PC12 rat pheochromocytoma cells (Fig 1). The extent of these effects is in line with the degradability of the particles in vitro. The MLs appear to be the most stable particles and further show a high persistence of the label in continuously proliferating C17.2 cells. When exposed to phospholipases, which degrade lipid structures, the outer lipid layer is easily degraded, but the inner lipid layer is strongly resistant due to an unfavourable orientation of the lipids. This leads to monolayer coated iron oxide cores which tend to aggregate in an aqueous environment, both in vitro and in cultured cells. The clustered particles have a much stronger cumulative magnetic dipole and result in a significant shortening of transverse relaxation. This makes MLs ideal MR contrast agents as their small size enables efficient cellular uptake[5], and the subsequent intracellular clustering enhances their MR contrast generation.
In conclusion, the results indicate the type of coating material used is highly important with regards to maintaining cell functionality and stability of the label. Further characterization of cell-nanoparticle interactions is both warranted and needed[1].

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Fig. 1: Neurite outgrowth of PC12 cells in response to nerve growth factor for control cells (a), and cells prelabelled for 24 h with MLs (b), Endorem (c), Resovist (d) and VSOP (e). Scale bars: 25 µm.

Magnetic Nanoneedles for Optofluidic Applications

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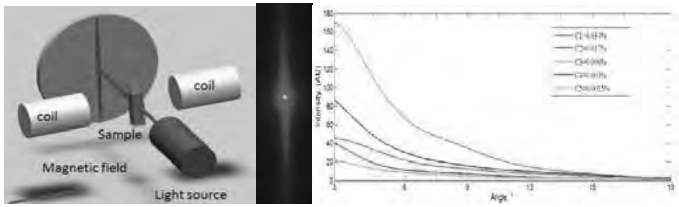
Imaging in micro and nanofluidics is a challenge: the sizes of micro and nanochannels are so small that the installment of additional optical and mechanical switches is almost impossible. We suggest to control the light beam by using the existing fiber optics and adding a functional lens which would expand/contract the light on demand. In previous work we showed that the shape of the laser beam passing through a colloid with suspended Fe_3O_4 nanoparticles can be altered by varying an applied magnetic field. Taking advantage of the light diffraction caused by magnetic chains, the light beam can be stretched/compressed in a predictable manner.(1). This effect can be used for illumination in biomedical and optofluidic devices. Magnetic nanowires and nanotubes appear to be the best candidates for optofluidic applications, because they are stable in rotating magnetic field and magnetostatic interactions between the nanoneedles are stronger. We will discuss the method of formation of rotating diffraction patterns illuminating larger area on demand. Moreover, we will show that magnetic nanoneedles can create one-dimensional periodic diffraction gratings in microfluidic channels. This diffraction gratings can be used for illumination and spectral expansion of the white light. Some biomedical applications of these effects will be discussed in the talk.



A B C

Figure 1. A) Schematic of the experimental setup for observation of diffraction patterns, the arrow shows the direction of magnetic field; B) Diffraction pattern from a magnetic colloid based on needle-like magnetic nanoparticles C) Intensity of light in diffraction pattern for different nanoparticle concentration.

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Talk 62

Effect of the core size of monodisperse superparamagnetic nanoparticles on their relaxometric enhancing properties for MRI

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Most commercially available superparamagnetic nanoparticles (SPMNPs) that are used in the biomedical field for cell labeling and tracking by MRI exhibit the disadvantage of being heterogeneous in shape and size and of often consisting of ultra small iron oxide cores (~ 5 nm), limiting their magnetic properties and hence their contrast enhancing properties.[1] Homogeneously composed and shaped nanoparticles have the advantage that they have the potential for quantitative and multi-phase MR imaging.[2]

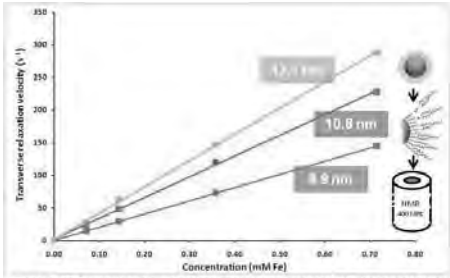

Figure 1: Transverse relaxation velocities versus Fe concentration

$TEM_{bare\ SPMNPs}$ (nm)	8.9 ± 2.4	10.8 ± 1.6	13.1 ± 1.2
$DLS_{with\ lipids}$ (nm)	50	72	68
r_2 ($mM^{-1}s^{-1}$)	202	318	402

Table 1: Properties of the SPMNPs: TEM size of the iron oxide core, DLS size of the lipid-coated particles, and transverse relaxivity ratio r_2.

To overcome these drawbacks, we have chosen to synthesize SPMNPs through the thermal decomposition method.[3] This method relies on the LaMer model to ensure small, spherical and narrow size distributed SPMNPs. To achieve an increase in core size we used the seed mediated growth method.[3]
Using this approach we obtained monodisperse, highly crystalline iron oxide nanoparticles of 8.9 ± 2.4, 10.8 ± 1.6, and 13.1 ± 1.2 nm. The SPMNPs were thoroughly characterized using transmission electron microscopy (TEM), dynamic light scattering (DLS) and vibrating sample magnetometry (VSM) (Table 1). Due to the synthesis method requiring high boiling hydrophobic solvents, the SPMNPs have a hydrophobic coating of oleic acid. A change in coating is required for biomedical applications and was successfully achieved by applying a ligand addition of DSPE-lipids to the SPMNPs with only minor differences in the hydrodynamic size of the SPMNPs. The water dispersible SPMNPs were further characterized in a 400 Mhz (9.4 T) Bruker NMR Spectrometer. The transverse relaxivity ratio (r_2) was measured for different concentrations of SPMNP suspensions using a Carr-Purcell-Meiboom-Gill sequence. Relaxivity ratio's increased with increasing core size of the SPMNPs. 8.9 nm particles gave rise to an r_2 value of 202 $mM^{-1}s^{-1}$, 10.8 nm particles had an r_2 of 318 $mM^{-1}s^{-1}$ and 13.1 nm particles showed an r_2 value of 402 $mM^{-1}s^{-1}$ (Figure 1). The r_2 values for the 10.8 and 13.1 nm SPMNPs are quite larger in comparison with the r_2 value of a commercial SPMNP such as Endorem (r_2 = 212 $mM^{-1}s^{-1}$). These results suggest that the thermal decomposition method and seed mediated growth method are highly suited to produce high quality monodisperse SPMNPs of different sizes showing outstanding relaxivity enhancing properties.

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Talk 63

Cylindrical Magnetic Nanoshells for Multispectral MRI

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Chemically synthesized magnetic nano- and microparticles are widely used in magnetic resonance imaging (MRI) as contrast agents and as labels for cell-tracking[1]. Recent work has suggested the possibility of producing also top-down microfabricated magnetic structures that can function as MRI contrast agents[2,3]. As one example, exploiting the enhanced geometrical definition possible with top-down fabrication techniques, previous work showed how spectral information could be encoded into the geometry of double-disk magnetic microstructures that could be spatially imaged and spectrally distinguished with standard MRI and nuclear magnetic resonance (NMR) techniques, respectively. Here we report on a new magnetic nanostructure, a hollow, cylindrical, nanoshell geometry that similarly allows for the geometrical encoding of unique radio-frequency signatures through controlled changes to cylinder materials, lengths, radii and wall-thicknesses. The figure shows an example of such microfabricated cylindrical shells together with initial data showing a frequency-shifted NMR peak. While the hollow cylindrical structures are intrinsically simpler than previously demonstrated composite double-disk structures, their microfabrication demands unconventional techniques. Accordingly, we discuss a newly introduced fabrication technique based on local back-sputtered redeposition on photolithographically prepatterned substrates[4]. The technique allows not only for the fabrication of highly-monodisperse cylindrical magnetic nanoshells, but should be readily adaptable to various other nanoparticle structures.

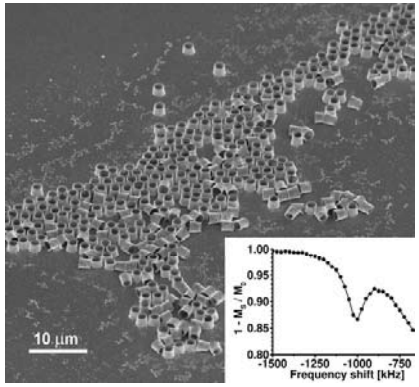

Figure: Scanning electron micrograph (SEM) of microfabricated hollow cylindrical shells together with frequency-shifted NMR peak produced by such shells when submerged in water.

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On-chip generation and manipulation of magnetic w/o and o/w droplets

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We report the generation and downstream manipulation of magnetic droplets in continuous flow. Using small stationary magnets, ferrofluid droplets were (i) split into enriched and depleted daughter droplets and (ii) deflected across a chamber for sorting based on applied field and magnetite loading.

Droplets have been studied extensively within lab-on-a-chip devices.[1] Operations such as merging, splitting and sorting have been achieved by dedicated channel design or electric forces. The magnetic manipulation of droplets could be advantageous as magnets can be positioned externally. Furthermore, magnetic forces are generally independent of pH and ionic strength. However, so far, magnetic droplets have received relatively little attention with most investigations conducted in millimetre tray systems by manual pipetting of magnetic particle suspensions.[2-5] Here, we explore the on-chip continuous flow generation of magnetic droplets followed by downstream continuous flow splitting or deflection.

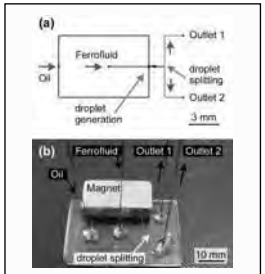

Fig 1: (a) Chip design for magnetic droplet generation featuring droplet generation and splitting zone. (b) Experimental setup with NdFeB magnet.

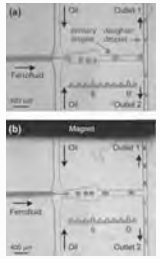

Fig 2: (a) Equal magnetite content without magnetic field, (b) enriched and depleted daughter droplets with magnetic field.

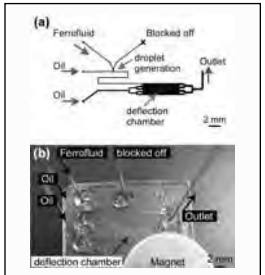

Fig 3: (a) Chip design featuring T-junction for generation and 2 mm wide chamber for deflection of droplets. (b) Experimental setup with disc magnet.

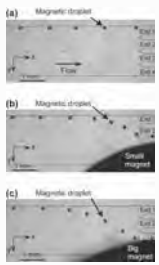

Fig 4: (a) No droplet deflection in absence of magnetic field. (b-c) Deflection at different magnetic field strengths.

Droplet splitting: The chip (fig. 1) featured a design for droplet formation and a splitting junction. Ferrofluid (EMG 507) and oil were pumped at 0.7 μL min^{-1} and 5 μL min^{-1}, respectively. In the absence of a magnetic field (fig. 2a), ferrofluid droplets were formed and found to split into two equal daughter droplets. When a magnet was placed next to the channel, the magnetite concentrated in one half of the droplet (fig. 2b) leading to enriched and depleted daughter droplets. This behaviour was investigated for a variety of flow rates, magnetic fields and for aqueous as well as organic ferrofluids.

Droplet deflection: Ferrofluid droplets (EMG 705) were generated at a T-junction (fig. 3) at flow rates of 2 μL h^{-1} (ferrofluid) and 26 μL h^{-1} (oil). In the absence of a magnetic field (fig. 4a), the droplets were found to follow the direction of laminar flow and exited the 2 mm wide deflection chamber directly opposite the inlet. When a magnetic field was applied, ferrofluid droplets were pulled towards the magnet. The extent of deflection was studied as a function of magnetic field strength (fig 4b,c) as well as magnetite loading.

We have thus demonstrated the continuous flow magnetic manipulation of droplets as a feasible method for operations such as splitting and sorting. Magnetic depletion and enrichment could be used as part of a digital microfluidic system in which magnetic functionality can be added and removed as required. Deflection across the flow chamber could be used for guiding droplets towards different outlets as desired for particular downstream applications.

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Equilibrium and nonequilibrium aggregation of Superparamagnetic colloids

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The aggregation process of superparamagnetic colloids is an essential step in low gradient magnetophoretic separation[1]. Under conditions of low magnetic gradients, typical magnetophoretic velocity of individual superparamagnetic beads are rather slow[2] ($\sim$ 1 mm per hour in a 20 T/m gradient). Under specific circumstances, beads are observed to aggregate forming long chains which move at high speeds under low magnetic gradients, making the low-magnetic separation process feasible and efficient[1].

Here we study theoretically the conditions for aggregation of superparamagnetic colloids, by using brownian dynamics simulations. Aggregation is found in conditions in which the energy associated to the particle-particle magnetic interaction is much higher than the thermal energy k_BT (fig. 1). Depending on the magnetization of the colloids, we have found either equilibrium or nonequilibrium aggregation. In the first case, aggregates are formed in short time scales (0.1 s for 100 nm size colloids) and after that initial regime, the number of aggregates of different sizes is constant over time (in spite of being an externally applied magnetic field) as corresponds to an equilibrium situation. To the best of our knowledge, this *equilibrium aggregation* regime under an external field has not been previoulsy identified neither in experiments nor in simulations. This could be due to the relatively narrow conditions of magnetization and sizes of magnetic beads under which this regime holds. The second case (irreversible aggregation) is more commontly found for large beads with high magnetic saturation. In this case, the size of the aggregates always increase over time and the average aggregate mass as a function of time is found to follow a power law.

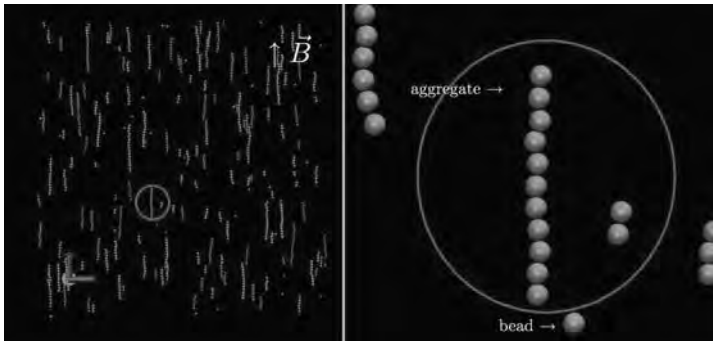

FIG. 1: Snapshots from a simulation corresponding to a $100nm$ superparamagnetic beads in water under an external magnetic field along the z axis. On the left, we show how several aggregates of different length are formed for values of the particle-particle magnetic interaction about $15k_BT$. On the right, a detail of the picture showing an aggregate formed during the simulation run. The VMD software[3] has been used to generate these images from simulation data.

This work is supported by the Spanish Government (grants FIS2009-13370-C02-02 and PET2008-02-81-00), Generalitat de Catalunya (2009SGR164) and SEPMAG Tecnologies. The Supercomputing resources employed in this work were provided by the CESGA Supercomputing Center, Spain. We acknowledge discussions with Ll. Martinez and A. Montras (SEPMAG Tecnologies).

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Circulating tumor cell detection by magnetic depletion of normal blood cells

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A negative magnetic cell enrichment technology was used to detect circulating tumor cells, CTCs, in the peripheral blood of head and neck squamous cell carcinoma cancer patients (1). The volume of 10 mL to 20 mL blood was donated to the study with the written patient consent using research protocols approved by the Institutional Ethics Review Board. The sample was depleted of erythrocytes by lysis and further depleted of leukocytes by adding pan-leukocyte marker antibody (CD45) and the magnetic bead colloid (Stem Cell Technology Inc., Vancouver, B.C., Canada) followed by magnetic separation in quadrupole magnetic flow sorter (QMS). Consistent with previous reports, CTC were positively identified if: (a) they contained a nucleus based on DAPI stain, (b) stained positive for cytokeratins, and (c) have a high nuclei to cytoplasmic ratio. In addition, for a blood sample to be considered positive for CTCs, the enriched sample must be positive for epithelial growth factor receptor, EGFR, as measured by RT-PCR. While most of the blood samples were obtained during surgery, a number were taken prior to and during surgery. In all of the pre and post surgery paired samples, significant numbers of CTCs were detected. Of 32 blood samples, 63% contained CTCs and the number of CTCs identified per mL of blood collected ranged from 0 to 214. The final purity ranged from 1 CTC in 9 total cells to 1 CTC in 20,000 total cells, the final purity being both a function of the number of CTCs and the performance of the specific enrichment procedure. A number of these enriched samples were observed under confocal microscope in addition to the microscopic observations under traditional wide-field fluorescent microscope. As expected, the FITC stained cytokeratins appeared in the cytoplasm and the average size of these positively stained cells on the cytospin smear preparations was in the range of 8-12 µm. Current studies involve the investigation if cancer stem cell and mesenchymal markers are present on these CTCs and correlations of patient outcome to the number and type of CTC present.

1. Balasubramanian, P., Yang, L., Lang, J. C., Jatana, K. R., Schuller, D., Agrawal, A., Zborowski, M., and Chalmers, J. J. (2009) Confocal images of circulating tumor cells obtained using a methodology and technology that removes normal cells. *Molecular Pharmaceutics* 6, 1402-1408

Improved Designs of a Microfabricated Magnetic Sifter for Biomolecule and Cell Purification

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Magnetic separation of proteins and cells is a method of growing importance in biology and medicine. Previously, we reported preliminary designs for a novel magnetic separation device, the magnetic sifter.[1,2] The magnetic sifter (Fig. 1) utilizes parallel fluid flow through a dense array of slots in a magnetically soft membrane. Early designs with very narrow slots suffered from low capture efficiencies and inefficient flushing of captured magnetic nanoparticles, inspiring us to explore modified structures. We have used finite element modeling to show that the magnetic field and field gradient, and therefore capture force, experienced by magnetic capture probes is sufficiently large when the slot width is increased from 4 µm to 40 µm. The increase in slot width is experimentally found to improve flow uniformity, reduce slot clogging, and allow for efficient rinsing of the sifter following capture. The result is higher capture efficiencies, excellent reproducibility, and complete release of captured magnetic particles.

Fig. 2 shows separation results from three consecutive separations of MACS nanoparticles with a sifter containing 40 µm square-shaped slots. The average capture efficiency is 77 ± 0.2%, and the average elution efficiency of captured magnetic nanoprobes is 99 ± 2.7%. The flow rate for these experiments was 1 ml/hr. Efforts are underway to increase the sifter area, currently 20 mm^2, to increase volume throughput without an increase in linear velocity of capture probes and the associated reduction in capture efficiency. The increased slot size of the sifter also enables its use for applications in flow-through separation of magnetic cell. Separations have been carried out with magnetically labeled human umbilical vein endothelial cells (HUVEC). The planarity of the sifter allows for observation of the separation process with a microscope (Fig. 3). Optical observations of the capture process indicate capture efficiencies higher than 90% at a flow rate of 1 ml/hr.

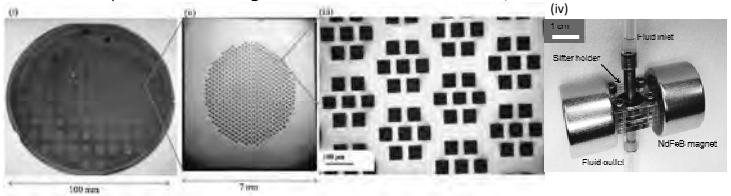

Fig. 1. (i) Fabricated wafer containing 120 sifter dies. (ii) Sifter die with magnetic material deposited on surface. (iii) Optical micrograph of 40 µm square holes. (iv) Separation assembly.

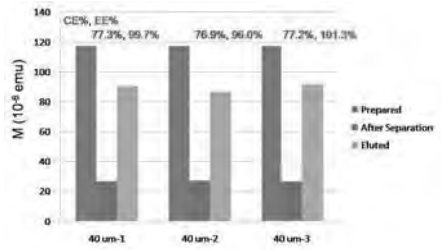

Fig. 2. Separations with the magnetic sifter, showing capture efficiencies (CE) and elution efficiencies (EE) calculated by measuring the magnetic content (y-axis) of the MACS solution before and after passing through the sifter, and of the solution used to wash the sifter (eluted). Separations and elutions were carried out with 200 µL of MACS at 1 ml/hr and 200 ul of PBS at 20 ml/hr, respectively.

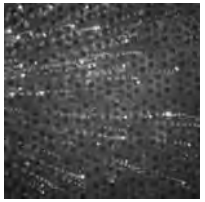

Fig. 3. Optical micrographs show trajectories of magnetically labeled HUVEC. Multiple exposures are shown in red, while the final frame is shown in green. Trajectories of captured cells end in yellow (red + green images merged).

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Colloidal transport and separation on magnetic garnet films

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2) Departament de Quımica Fısica, Universitat de Barcelona, Martı i Franques 1, Barcelona, 08028, Spain.

The variety of chemical and biological functionalities of colloidal particle surfaces turns colloidal particles into an important tool in the medical, biochemical and biophysical field. Colloidal particles are being used for novel forms of DNA-sequencing[1], optical bar-coding[2], as carriers of biomolecules and proteins in microfluidic channels, and as fluorescent markers in microrheological experiments of the cytoskeleton. One important type of colloidal particles are paramagnetic colloids, i.e. colloids that contain a paramagnetic core made of superparamagnetic grains surrounded by a polymer shell. These particles can be manipulated using magnetic fields. Magnetic manipulation of paramagnetic particles in the human body is limited to fields with spatial variations on the macroscopic scale. These limitations, however, do not exist on a lab-on-a-chip; where magnetic fields can be created that vary on the colloidal length scale. We use magnetic fields of magnetic garnet films that are heterogeneous on the colloidal scale. They enable simple controlled manipulations of a large assembly of colloidal particles, with individual control over each particle. We explore the multitude of colloidal transport modes that can be triggered in heterogeneous magnetic fields by using homogeneous time dependent external magnetic field modulations. We show that the use of magnetic garnet film patterns adds new avenues of transport, separation and distribution of micro material to the microfluidic arsenal that will be useful when a large assembly of colloids needs to be manipulated simultaneously.

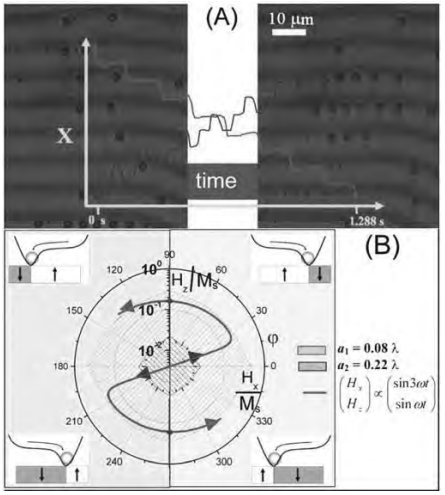

Figure 2. (A) Polarization microscope images of a garnet film with small 0.5 µm red) and large 2.8 *µm*, blue) particles. Trajectories of both particles are superimposed. The motion occurs in discrete steps that lead to a net motion in opposite directions separating the particles. (B) Separation mechanism: The external magnetic field modulations occur along the green line. The discrete hopping of the large (small) particles occurs above the blue (red) thresholds. Hopping directions are indicated by small schemes in each quadrant.

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Pietro Tierno, Francesc Sagués, Tom H. Johansen and Thomas M. Fischer *Colloidal transport on magnetic garnet films* Phys. Chem. Chem. Phys**. 11,** 9615-9625 (2009)

Microfluidic Chip-Based Immunomagnetic Detection of Circulating Tumor Cell

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Detection of Circulating Tumor Cells (CTCs) in patient blood has been focused on as a strong potential diagnostic tool for early cancer detection [1]. Immunomagnetic separation is among the successful methods employed [2,3]. Recent development in microsystems has also allowed for capture of these rare cells [4]. Here we describe a microfluidic chip-based immunomagnetic detection of CTCs. Throughput can be improved through the combination of the well-practiced immunomagnetic separation techniques and optimized engineered design of the microfluidic device.

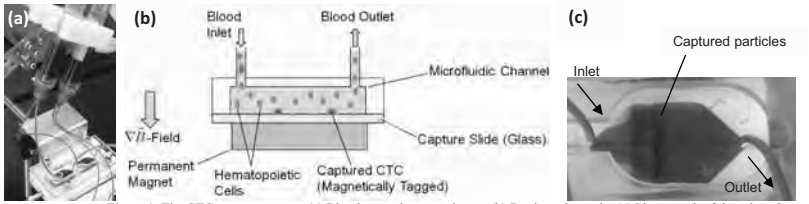

Figure 1. The CTC capture system. (a) Blood screening experiment. (b) Device schematic. (c) Photograph of the microchannel.

Figure 1 shows an illustration of our CTC capture system. CTCs in the blood are labeled with gold-shell magnetic nanoparticles, functionalized with anti-EpCAM antibodies through thiol interactions [5]. As the blood sample is pumped through the microchip (Fig.1(a)), particles as well as labeled CTCs are collected by a permanent magnet allocated below the microfluidic chip (Fig.1(b)). Captured magnetic particles can be seen in the photograph shown in Fig.1(c).

Samples for preliminary experimentation consisted of either PBS or whole blood spiked with cultured SKBr3 (breast cancer cell) or PC3 (prostate cancer cell). Capture rates of up to 90% and 86% were demonstrated with PBS and whole blood, respectively. Figure 2 is an example of CTC screening in patient blood. The cell is fluorescently stained against cytokeratin (protein found in epithelial tissue, positive test), CD45 (found on leukocytes, negative test), DNA (found in cell nucleus, positive test), showing successful capture of a CTC. In conclusion, the proposed capture system is simple and can be easily applied to several configurations of magnetic nanoparticles and antibodies.

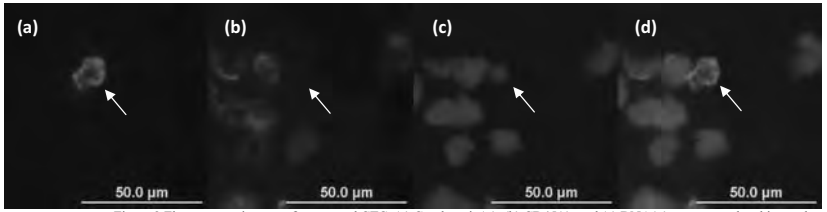

Figure 2.Fluorescence images of a captured CTC. (a) Cytokeratin(+), (b) CD45(-), and (c) DNA(+) were tagged and imaged. (d) Image reconstructed from (a)-(c).

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Talk 70

Nanoparticle Size and Surface Charge Determine Formation of Protein Corona, Cellular Uptake and Magnetic Resonance Imaging Properties

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Engineered magnetic nanoparticles (MNPs) are currently emerging as promising tools in numerous applications for medical diagnostics and therapy, for example as contrast agents in magnetic resonance imaging (MRI) and drug delivery systems [1]. Cellular therapies using immune cells, such as dendritic cells (DCs), are increasingly applied in clinical trials to study cellular migration and/or deposition in conjunction with treatment outcome using MRI [2].

DCs serve as sentinels of the immune system through acting as professional antigen presenting cells that initiate host adaptive immune responses [3]. They reside in sites of primary antigen entry and therefore are the first cells to be involved in antigen scavenging, processing, and subsequent T-cell priming. Importantly, DCs are expected to perceive MNPs as foreign antigens [4], thus providing the capability to biologically sense MNP surface chemistry.

Magnetic nanoparticles dispersed in biological fluids associate with biopolymers such as proteins in a manner that depends largely on MNP surface characteristics [5]. Thus, an understanding of how this "protein shell" influences cellular uptake and ultimately MRI imaging properties would greatly aid in developing improved nanoparticle formulations.

In this study, we systematically examined the influence of total serum components on nanoparticle properties such as hydrodynamic diameter and surface charge, and evaluated possible impacts on DC responses as well as MR imaging properties. For this purpose, we engineered magnetic nanoparticles on the basis of layer-by-layer technology to vary surface charge, poly-electrolyte composition and overall particle size [6]. Labeling of MNPs in a protein-rich environment was compared to DCs treated under serum-free conditions as depicted in Figure 1. We examined uptake kinetics via magnetic separation, intracellular iron content, and transmission electron microscopy. Phenotypical analyses were performed employing flow cytometry and contrast agent properties using MRI. We expect this study to be important for MNP usage in MRI that i) involve labeling of cells with MNPs *in vitro* in a biomacromolecule-rich environment, such as serum, and ii) MNP uptake into cells *in vivo* following systemic administration of nanoparticles.

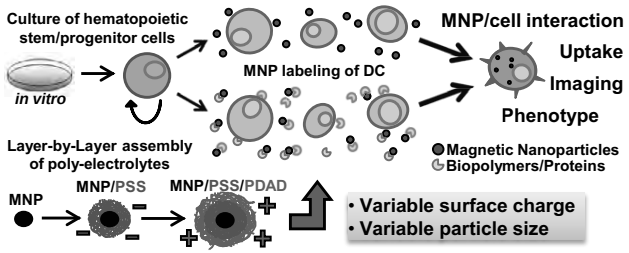

Figure 1: Schematic representation of experimental design.

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Talk 71

Saturday, May 29, 2010				
7:30	**Registration desk opens**			
8:30	Krishnan, Kannan	**Tutorial III** on Magnetic Things We All Should Know	Seattle, U.S.A.	Tutorial 3
	Session 12: Biological Applications I - *Chair: Shan Wang (U.S.A.)*			
9:00	Antosova, Andrea	Magnetic fluids have ability to decrease amyloid aggregation associated with amyloid-related diseases	Kosice, Slovakia	Talk 72
9:15	Ho, Vincent	Magnetic cell labelling and manipulation in 2D and 3D cell cultures	Cambridge, U.K.	Talk 73
9:30	Bakandritsos, Aristides	Development of Functional Magnetic Targeted Carriers for Cationic Drugs	Patras, Greece	Talk 74
9:45	Laurencin, Mathieu	Human Erythrocytes Covered with Magnetic Core–Shell Nanoparticles as Multifunctional Drug Carriers	Paris, France	Talk 75
10:00	Palfreyman, Justin	Magnetic Barcoded Microcarriers for Biomolecular Labelling Applications	Cambridge, U.K.	Talk 76
10:15	**Coffee break / exhibitors**			
	Session 13: Biological Applications II - *Chair: Jeff Anker (U.S.A.)*			
11:00	Riegler, Johannes	A simplified mathematical model for targeted magnetic delivery of cells using an MRI scanner	London, U.K.	Talk 77
11:15	Shoshi, Astrit	GMR-based real-time cell endocytosis monitoring of magnetic particles	Vienna, Austria	Talk 78
11:30	Sureshkumar, Manthiriyappan	Multifunctionalized magnetic bionanocomposites for biotechnology applications	Taipei, Taiwan	Talk 79
11:45	Thanh, Nguyen TK	Superparamagnetic Fluorescent Nickel-Enzyme Nanobioconjugates: Synthesis and Characterization of a Novel Multifunctional Biological Probe	London, U.K.	Talk 80
12:00	Torres-Lugo, Madeline	Examination of the Enhanced Potentiation of Combined Cisplatin Treatment with Magnetic Fluid Hyperthermia	Mayaguez, U.S.A.	Talk 81
12:15	Rozhkova, Elena	Biofunctionalized Magnetic-Vortex Microdiscs for Biomechanical Cell Actuation	Argonne, U.S.A.	Invited talk 5
12:55	**Lunch**			
	Session 14 : Biosensors - *Chair: Frank Ludwig (Germany)*			
14:00	Dempsey, Nora	Micro-structured hard magnetic films for lab-on-chip applications	Grenoble, France	Talk 82
14:15	Donolato, Marco	On chip detection and manipulation of biological entities carried by magnetic beads via domain wall conduits	Como, Italy	Talk 83
14:30	Enpuku, Keiji	AC susceptibility measurement of magnetic markers for liquid phase detection of biological targets	Fukuoka, Japan	Talk 84
14:45	Kinnunen, Paivo	Growth Measurements of Individual Bacteria with a Magnetic Bead Rotation Sensor	Ann Arbor, U.S.A.	Talk 85
15:00	Li, Yuanpeng	Biomarker quantification in unprocessed human sera by a competition-based nanomagnetic protein assay and high-magnetic-moment nanoparticles	Minneapolis, U.S.A.	Talk 86
15:15	Østerberg, Frederik	Chip-based measurements of Brownian relaxation of magnetic beads using a planar Hall effect magnetic field sensor	Lyngby, Denmark	Talk 87
15:30	Sivagnanam, Venkataragavalu	Study of spatio-temporal immunofluorescence on magnetic bead patterns in a microfluidic channel	Lausanne, Switzerland	Talk 88
15:45	**Closing Comments and Announcement of the NEXT MEETING: Wolfgang Schuett / Jian Ping Wang**			
16:00	**Meeting ends**			

Magnetic fluids have ability to decrease amyloid aggregation associated with amyloid-related diseases

Andrea Antosova[1], Katarina Siposova[2], Martina Koneracka[1], Vlasta Zavisova[1], Zuzana Daxnerova[3], Peter Kopcansky[1]*, Zuzana Gazova[1]

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Amyloidoses are a group of incurable human protein diseases including pathologies like Alzheimer′s and Parkinson′s diseases, cataracts, and type II diabetes, characterized by the self-assembly of normally soluble protein into amyloid aggregates localized in various part of the body[1]. It is generally accepted that amyloid assemblies, varying from oligomers and pores to fibrils, are the main cytotoxic species and their reduction is beneficial[2]. However, a few studies are related to the effect of nanoparticles on amyloid aggregation of proteins. Recently we found that Fe_3O_4–based magnetic nanoparticles are able to interact with lysozyme amyloids *in vitro*[3]. The obtained results initiated us to investigate the effect of four magnetic fluids on amyloid aggregation of hen egg white lysozyme as model amyloidogenic proteins. The typical amyloid character of lysozyme amyloid aggregates was confirmed by spectroscopic and microscopy techniques. Magnetic particles were prepared by co-precipitation of ferric and ferrous salts in alkaline medium. Freshly prepared magnetic particles were dispersed in water (MF1, MF2, MF4) or physiological saline solution (MF3) and stabilized with perchloric acid (MF1) or sodium oleate (MF2, MF3, MF4). BSA (MF2) or Dextran (MF4) was added as a modifying agent. The interaction of MFs with lysozyme amyloid aggregates was detected by Thioflavin T fluorescence method (ThT assay) sensitive to amount of amyloid aggregates. We have found that MFs are able to interact with amyloid fibrils *in vitro* resulting into reduction of amyloid aggregates. The incubation of lysozyme amyloid aggregates with studied MFs caused extensive decreasing of the amount of amyloid aggregates (from 50 to 90 % detected for MF1 to MF4) in comparison to that observed for non-treated aggregates. The depolymerizing effect of MFs was confirmed by electron microscopy. The obtained results indicate that presence of MFs led to a disassembly of lysozyme amyloid aggregates. These features make MFs of potential interest as therapeutic agents against amyloid-related diseases.

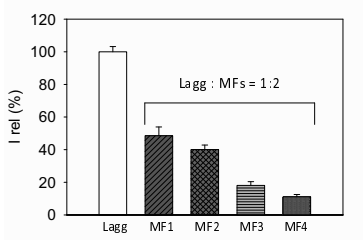

Figure: Amount of lysozyme amyloid aggregates (Lagg) in presence of magnetic fluids (MF1 - MF4) detected by ThT assay. Lysozyme amyloid aggregates (10 μM = 0.147 mg/ml) were 24h incubated with MF in ratio Lagg:MFs = 1:2 Data are normalized to the fluorescence signal observed for Lagg .

Acknowledgement

This work was supported within the projects Nos. 26220220005, 2622012033 in frame of Structural Funds of European Union, Centre of Excellence of SAS Nanofluid and VEGA 0079, 0056 and 0077.

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Magnetic cell labelling and manipulation in 2D and 3D cell cultures

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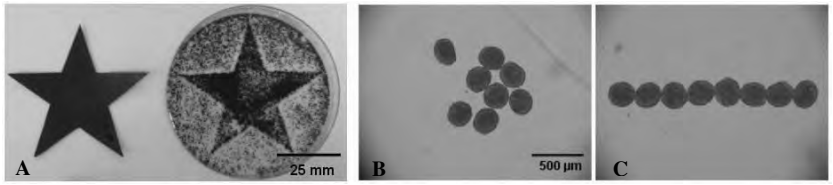
Magnetic labelling of mammalian cells has numerous applications in cell separation and sorting, cell patterning in tissue engineering, cell-based therapies, mechanotransduction studies and magnetic resonance imaging. Current labelling techniques usually require cellular internalisation of magnetic materials and this is hard to control actively and dependent on temperature and incubation time. To expedite the process, a simple and precise technique for magnetically labelling mammalian cells was developed in which cell membrane proteins were first biotinylated and then bound to streptavidin paramagnetic particles. Characterisation studies were carried out to examine and analyse this labelling method. The results showed that the degree of magnetic labelling could be precisely controlled by adding different amounts of paramagnetic particles and the labelling is effective, quick and independent of incubation time. The particles bound to the cell surface were subsequently internalised and studies showed that this labelling method did not have any observable drastic effect on cell viability and proliferation. The labelled cells were measured to have significant magnetic moments in an applied field. They could be targeted and positioned precisely with magnetic field gradients. Highly defined cell patterns were achieved using magnetically labelled HeLa, TE671 cells and human monocytes. Spatially segregated HeLa and TE671 cells were also successfully co-cultured on the same plate using this technique. Multi-layering of labelled cells could also be achieved, as shown by scanning electron microscopy. The labelled cells were further cultured to generate magnetic multicellular spheroids. These spheroids could be easily and quickly separated magnetically without the need for centrifugation. They could also be patterned using magnetic fields in a few seconds and the patterned spheroids then fused together to form a larger tissue construct. This cell labelling methodology can be adapted to a wide variety of mammalian cell types. The labelled cells can then be manipulated in two dimensional (2D) cell culture or as three dimensional (3D) magnetic multicellular spheroids.



(A) Magnetic cell patterning using a star shaped template. (B) Random distribution of magnetic multicellular spheroids. (C) Magnetic patterning of spheroids.

Development of Functional Magnetic Targeted Carriers for Cationic Drugs

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One of the major challenges in contemporary drug administration technologies is the targeted delivery of drugs, in order to suppress the systemic distribution and, in turn, the ensuing undesirable side-effects. A possibly effective strategy towards this goal is the entrapment of the drug in magnetic carriers.[1] Here, the preparation of such carriers is reported through alkaline condensation of a single ferrous precursor into iron oxides in the presence of functional polymers. Three different polymers were used: **i)** poly(sodium styrene) sulfonate (PSS),[2] **ii)** carboxymethyl cellulose (CMC)[3] and **iii)** poly (carboxylate-ether) graft co-polymers (PC-PEG). Uncoated, plain magnetite colloids were also prepared with the typical co-precipitation route for comparative reasons. Light scattering results indicate that the stability in salted media is generally enhanced, but only the PEGylated system is endowed with stability even at [NaCl] above the human blood plasma levels (Fig.1a). Monitoring the interactions with human blood plasma also indicated the facile "recognition" of the uncoated magnetic nanoparticles, evidenced by their hydrodynamic diameter (D_h) increase (Fig. 1b), unlike their PEGylated counterparts (Fig. 1c). Mag-CMC and Mag-PC-PEG carry on the polymeric corona an abundance of carboxylates that can bind cationic drugs such as doxorubicin. Drug loading experiments with MagCMC (Fig.1d), show a high uptake capacity and loading efficiency. It is noteworthy that the uptake, when expressed in mol of Dox per mol of carboxylates, is higher than the expected 1:1 molar ratio and approximately 1.3 mol/mol. This can be explained as a result of hydrophobic interactions between the respective moieties of the drug and of the polymer'sbackbone. Similar results were obtained with Mag-PC-PEG. In order to obtain evidence that the drug-carrier interactions do not affect the activity of Dox, HT-29 cancer cells were incubated with Dox-loaded carriers. Only a minor reduction on the cytotoxicity was observed, as compared to free Dox, possibly due to a fraction of Dox that remained bound and did not enter the cells.

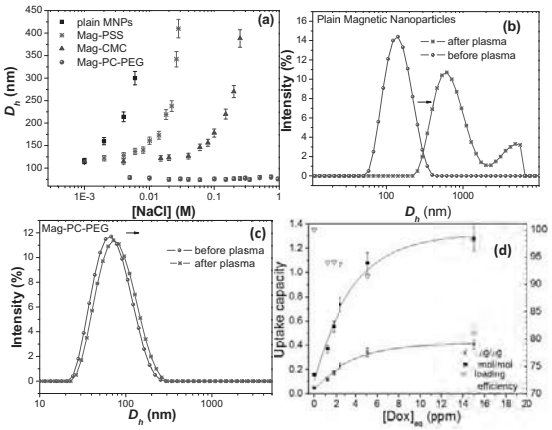

***Figure 1**. (a) Colloidal stability dependence upon [NaCl], (b) and (c) assays for carrier-protein interactions in human blood plasma and (d) uptake capacity for doxorubicin and loading efficiency (%).*

The *in vitro* results suggest that biocompatibility and entrapped-drug activity should not be an issue with regard to future biomedical applications, thus further encoura-ging the currently ongoing research on the *in-vivo* application of the Mag-PC-PEG in mice for tumor treatment with magnetic targeting.

1) a) Häfeli, U., Schütt, W., Teller, J., Zborowski, M. *Scientific and Clinical Applications of Magnetic Carriers*, Plenum Press, New York 1997. b) Lin, M.M., Kim, D.K., El Haj, A.J., Dobson, J. *IEEE Transactions on Nanobioscience* **7**, 298, (2008).
2) Bakandritsos, A., Bouropoulos, N., Zboril, R., Iliopoulos, K., Boukos, N., Chatzikyriakos, G., Couris, S. *Advanced Functional Materials* **18**, 1694 (2008).

Human Erythrocytes Covered with Magnetic Core–Shell Nanoparticles as Multifunctional Drug Carriers

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We describe the preparation of new biological hybrid entities resulting from the irreversible adsorption of Core-Shell Magnetic Nanoparticles (CSMNs) on human Red Blood Cells (hRBCs). We propose to use the well-known interaction between amorphous silica and the main constituent of the biological membrane, i.e., phosphatidylcholine (PC) to obtain multifunctional drug carriers. We prove that hRBCs covered with magnetic nanoparticles are good candidates for magnetic vectorization and bioconjugation. Electron, fluorescence and confocal microscopy were used to characterize the magnetic hRBCs (Figure 1). In addition, no hemolytic activity (hemoglobin release) was detected for magnetic hRBCs after 5 hours at 37°C (figure 2). As a conclusion magnetic hRBCs are good candidates for imaging and drug delivery.

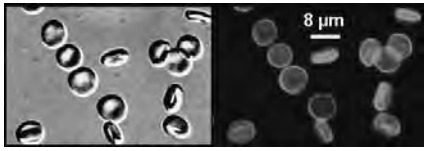

Figure 1 : DIC (Differential Interference Contrast) (left) and fluorescence (right) micrographs of magnetic hRBCs. The green fluorescence is provided by the FITC entrapped in the shell of CSMNs.

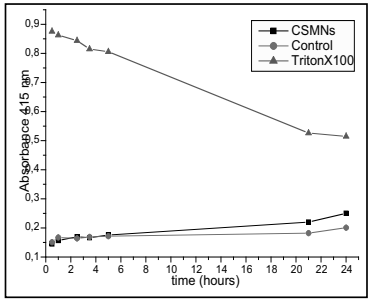

Figure 2 : Evolution of absorbance at 415 nm (released hemoglobin) of control hRBCs (negative control: 0%), hRBCs lysed by 1% Triton X-100 detergent (positive control: 100%), and magnetic hRBCs in the same washing buffer at 37°C.

Magnetic Barcoded Microcarriers for Biomolecular Labelling Applications

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Magnetic microcarriers combined with the ability to encode information have a number of unique benefits over the conventional optical based microcarriers used for multiplexed assays, such as Quantum Dots. These include (i) write and re-writeability leading to applications in combinatorial chemistry for reaction tracking; (ii) coding capacity that scales exponentially with the number of magnetic elements; (iii) magnetic detection generates an unambiguous electronic output; (iv) microfluidic integration without complicated optics offers a hand-held lab-on-a-chip platform; (v) one design can offer all the codes, so well-suited for mass fabrication.

Our group is working on two different architectures of magnetic tag, one is a stack of magnetic ellipses, interleaved with copper spacers [1]. The other consists of a row of magnetic elements encapsulated in a bio-compatible SU-8 polymer backbone [see figure]. We shall present an overview of this architecture and its multi-step fabrication process by photolithography, thermal evaporation and ion milling. A novel chemical functionalization strategy has been developed to regenerate a chemically active surface following the milling step. This chemical strategy can also be used to release the tags into solution without the traditional sacrificial aluminium layer, and spacer molecules have also been investigated to increase binding efficiency.

Shape anisotropy is used to coercivity-tune each magnetic element, so that each switches between two stable magnetisation states at a particular critical applied field strength. This allows the tags to be written with a non-local magnetic field applied to the whole tag, so is compatible for in-flow writing applications (such as split 'n' mix). Following an assay, the tags are flown through a microfluidic channel over an embedded TMR (tunnelling magnetoresistance) sensor, which can read the signature of individual tags flowing past – preliminary data for 5-bit barcodes will be shown. Optical trapping is one of many techniques that are being explored to control the movement of tags in flow, and preliminary data will also be shown.

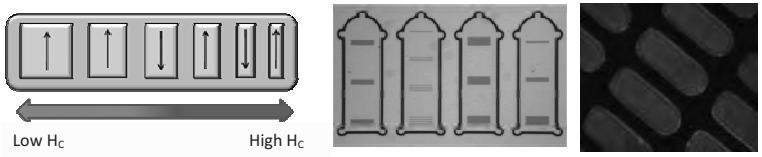

Figure: [left] Schematic of multi-coercivity magnetic elements encapsulated in a polymer shell. [centre] optical microscope image of 3 and 4-bit designs, with "mickey mouse ears" for optical trapping. [right] SU-8 backbones after chemical etching and reactivation, coupled to the TAMRA fluorophore.

[1] J.J. Palfreyman, J.A.C. Bland *et al.* "Digital Biomagnetism: Electrodeposited multilayer magnetic barcodes", *JMMM*, **321** (10) 1662-1666 (2009)

A simplified mathematical model for targeted magnetic delivery of cells using an MRI scanner

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Introduction

One of the major challenges for cell transplantation therapies is the spatial localization and tracking of cells over time. Magnetic targeting of cells has been demonstrated previously using permanent magnets[1], while magnetic resonance (MR) imaging is conventionally used for *in-vivo* cell tracking in preclinical models. High performance MR gradient coils produce homogeneous magnetic field gradients that penetrate the whole body. Work by other groups has shown that these field gradients are strong enough to move magnetic objects inside the scanner[2-4] and that magnetic delivery and tracking of objects can be combined.

In this work we demonstrate the feasibility of targeted cell delivery by steering magnetically labelled cells using MR imaging gradients in a vascular bifurcation flow model. We also present a simplified mathematical model for magnetic cell delivery in an MRI scanner. Our initial findings support the potential value of MRI for improved targeting of intravascular cells.

Methods

Experiments were performed using a custom designed vascular bifurcation model (Figure 1), connected to an infusion pump (Harvard Instruments PHP2000). The bifurcation was placed into the centre of a 9.4T experimental scanner (Varian, 60 mm bore size, gradients: rise time 5 T m^{-1} ms^{-1}, max. 1T/m), with the direction of flow parallel to B_0 (Z). Human mononuclear cells were labelled with micrometer sized super paramagnetic iron oxide particles, Bangs Particles (diameter: 1.5 µm). 0.6 ml of labelled cells ($2x10^6$ cells/ml) or ($4x10^6$ cells/ml) were infused at flow rates of 0.3 ml/min to 2.5 ml/min leading to a mean velocity of 1 cm/s to 8 cm/s whilst gradients (amplitude 500mT/m) were applied in the X direction, perpendicular to the direction of flow. Cell suspensions leaving each bifurcation outlet tube (volume 0.25ml each) was collected and cell concentrations were estimated using a heamocytometer. Gradient echo images were acquired using the following parameters: TE 1.24 ms, TR 100 ms, FA 30°, FOV 50x30 mm, Matrix 192x128.
A simplified mathematical model was constructed which takes magnetic force, drag force, inflow distribution and shape of cell aggregates into account. We compared predictions of this model against experimental data to confirm its validity.

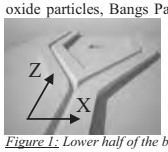
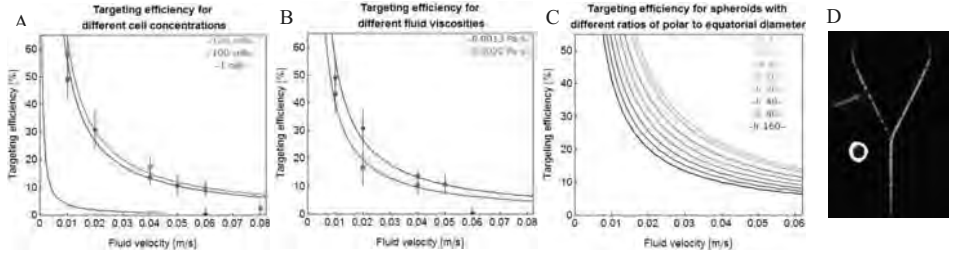


Figure 1: Lower half of the bifurcation model used in these experiments. The inner diameter of the tube was 0.8mm and the bifurcation angle was 30^0.

Results

Following application of the magnetic field gradient from the MRI system, there was an up to 55% increase in the number of cells reaching the outlet to which the gradients were directed. **Figure A** shows experimental results for 2 million cells/ml (*blue dots*) and 4 million cells/ml (*green dots*). An increase in flow rates lead to decreased targeting efficiency. In Figure A, the theoretical prediction for no aggregation is shown by the *purple line* while the *blue* and *green lines* show the targeting efficiency for the aggregation of 100 and 120 cells respectively. It was necessary to incorporate cell aggregation into the model in order to explain the difference between the theoretical (*purple line*) and the experimental data (*green and blue dots*). However, keeping the number of cells per aggregate constant leads to a discrepancy at higher flow rates. For **Figure B**, 2 million cells/ml were suspended in 3% serum (*red dots*) and 50% serum (*red dots*). Continues lines show the theoretical predictions for a viscosity of 0.0013 Pa*s (*blue*) and 0.0022 Pa*s (*red*). This data indicates that increasing the viscosity leads to a decrease in targeting efficiency. **Figure C** shows the effect of different polar to equatorial diameter ratios for spheroid like cell aggregation. **Figure D** shows a gradient echo image indicating reduced signal intensity in the exit branch to which the cells where directed (*red arrow*).



Conclusions

Our results show that an MRI scanner can be used to steer cells into the desired direction in a vascular bifurcation model. Additionally we show that cell aggregation is an important factor to explain our experimental results. Our theoretical model indicates the level of aggregation (approx. 100 cells) necessary to explain the experimental data and shows the effect of different geometrical arrangement on the theoretical targeting efficiency. We also show the possibility of using MR imaging to confirm targeting success. These preliminary findings provide evidence to support the potential of magnetic targeting of cells using MRI for future clinical applications, allowing image guided targeted delivery of cells and other therapeutic agents to sites of the body which cannot be reached with external permanent magnets.

References:

[1] P. Kyrtatos et al. JACC Cardiov Interv 8 (2009), pp. 794-802 **[2]** N. J. Darton et al., Nanotechnology **19**, 395102 (2008) **[3]** S. Martel et al., Applied Physics Letters **90**, 114105 (2007). **[4]** J. B. Mathieu, S. Martel, Biomedical Microdevices **9**, 801 (2007).

GMR-based real-time cell endocytosis monitoring of magnetic particles

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The presented Magnetic-Lab-on-a-Chip-System[1] (MAGLab-System) provides a platform for on-chip cell analysis. This includes magnetic manipulation and magnetoresistive-based detection of labeled cells. Both cell manipulation and detection are carried out by employing superparamagnetic particles (Beads). Magnetically labeled cells are manipulated by superimposing magnetic gradient fields from small local coils and homogeneous fields from Helmholtz coils. Both attractive and repulsive forces can be applied, leading to a configuration which allows for computer control of three dimensional (3D) movements. Bead detection is realized by giant magnetoresistive (GMR) sensors which are embedded in a silicon chip and provide electronic signals proportional to the bead surface coverage of the sensors.

In this presentation we will focus on GMR-based real-time monitoring of cell endocytosis (uptake). After immobilizing magnetic beads onto a GMR-sensor surface, human fibroblast cells (NHDF = Normal Human Dermal Fibroblasts) are seeded and grow until a confluent layer is formed over the entire sensor surface. While the cells start to engulf and internalize the beads in transport vesicles derived from the cell membrane, the distance between the beads and the sensor increases, which results in a decrease of the magnetic stray field strength and, thus, the sensor signal. By controlling the size and surface biology of the magnetic particles, we explore the complex kinetics of cellular uptake mechanisms and thereby mimic cellular responses to changes in the environment.

a) b)

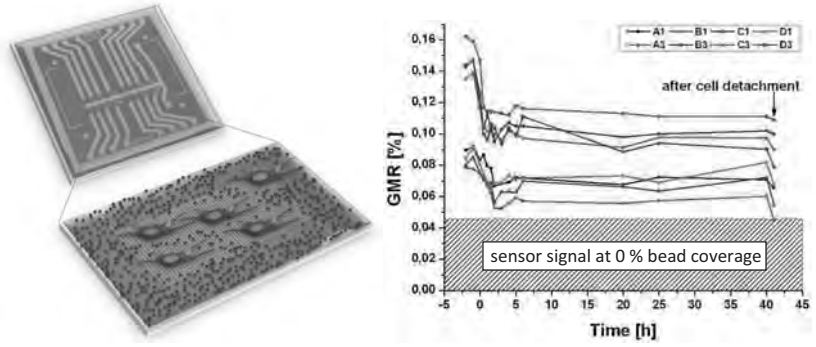

Figure 1: On-Chip Cell Analysis: a) Cells grown onto bead covered sensors. The bead transfer into the cell causes a change of the bead-sensor distance leading to a GMR signal change. b) Corresponding GMR response during uptake process showing the kinetic of cell endocytosis over a period of time.

[1] J. Schotter, A. Shoshi and H. Brückl, Journal of Magnetism and Magnetic Materials 321(2009)1671–167

Talk 78

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The use of bionanocomposites is emerging as relatively modern field of scientific research. A new environment friendly "green" scheme is proposed for the preparation of multifunctionalized magnetic nanocomposites. Finely blended bacterial cellulose (BC) nanofiber is mixed with ferrous and ferric ions and then addition of base generates magnetic-cellulose fibers (MBC). Polydopamine coating methodology is introduced to create multifunctionality surface of MBC act not only acted as mimic peroxidase, but also used as enzyme immobilization matrix. Glucose oxidase (GOX) was successfully immobilized and H_2O_2 generated by the GOX reaction was effective removed by the mimic peroxidase activity of the nanocomposite. Silver ions could be reduced by polydopamine surface (PMBC) as Ag nanoparticles and incorporated into the magnetic nanocomposite. Ag nanoparticles integrated nanocomposites (PMBC-Ag) are demonstrated as effective antibacterial agent against Gram positive and negative organisms. The possibility of sterilizing culture medium using Ag deposited nanocomposite is also addressed. The as-prepared samples morphology, magnetic properties and chemical nature have been characterized. The simple, inexpensive, multifunctional material with superparamagnetic nature can be used as potentially useful in a wide variety of biotechnology applications and believed this approach can be extend to other biomaterials.

Keywords: *Bionanocomposites; Bacterial cellulose (BC) nanofibers; Mimic peroxidase; Glucose oxidase (GOX) immobilization; Ag nanoparticle; Antibacterial activity;*

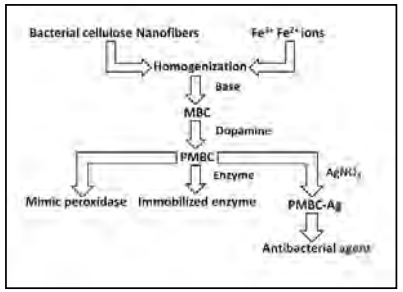

Fig.1 Process digram

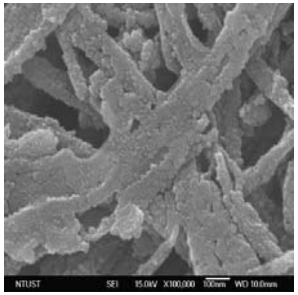

Fig. 2 FE-SEM micrograph of PMBC

Talk 79

Superparamagnetic Fluorescent Nickel-Enzyme Nanobioconjugates: Synthesis and Characterization of a Novel Multifunctional Biological Probe

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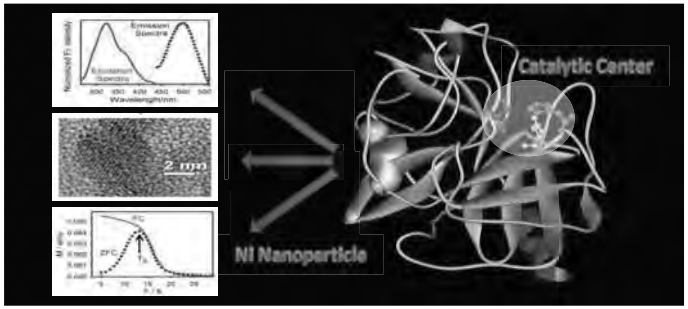
We report, for the first time, the synthesis of superparamagnetic and fluorescent nickel (Ni) nanoparticles (consisting of a single material) directly conjugated to an enzyme, bovine pancreatic α-chymotrypsin (CHT) by chemical reduction in aqueous solution. The structural charaterization of Ni-CHT nanobioconjugates was done by UV-VIS absorption/photoluminescence spectroscopy and high-resolution transmission electron microscopy. The temperature dependence of the magnetization M(T) taken in zero field cooling and field cooling conditions, exhibits the main features of superparamagnetism. Circular dichorism studies were performed to monitor the structural perturbation to the structure of the enzyme after conjugation with nickel nanoparticles (Ni NPs). The functional integrity of the enzyme conjugated to Ni NPs was investigated by monitoring the enzymatic activity of Ni-CHT conjugates by using UV-VIS absorption spectroscopy and compared with the unbound enzyme under similar experimental conditions. To confirm the conjugation of Ni NPs to CHT, we carried out the Förster resonance energy transfer (FRET) studies using a fluorescent probe, 4-nitrophenyl anthranilate (NPA), known to bind at the enzymatic active site of CHT, as the donor (D) and Ni-NP-bound CHT as the acceptor (A). Our studies also demonstrated that the FRET from the donor NPA to acceptor Ni NPs in CHT can be monitored to follow the D-A distance and hence the protein structure during thermal unfolding. Such a multifunctional superpapramagnetic, fluorescent and biologically active nano-bioconjugate may find its relevance in nanoparticle-based diagnostic and therapeutic applications.



Talk 80

Examination of the Enhanced Potentiation of Combined Cisplatin Treatment with Magnetic Fluid Hyperthermia

Madeline Torres-Lugo (madeline@ece.uprm.edu), Orlando Soto, Amalchi Castillo, Janet Mendez, Hector Rodriguez-Luccioni, and Carlos Rinaldi
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Synergistic potentiation of platinum containing antineoplastic agents has been shown with regional hyperthermia, but this modality faces significant clinical challenges. On the other hand, hyperthermia induced by magnetic nanoparticles has the advantage of delivering thermal energy at the nano-scale, preventing side effects commonly observed in whole body and regional forms of hyperthermia. Previous work[1] has demonstrated that combined Magnetic Fluid Hyperthermia (MFH) and Cisplatin (CIS) treatment caused a significant synergistic potentiation when compared to a similar treatment using hot water hyperthermia. Such differences could be the result of an increase in membrane permeabilization due to mechanical disruption of the cell membrane by MFH. To further investigate such phenomena, this work pursued the preliminary assessment of the potentiation effects of MFH in the absence of the CIS active transport mechanism, also known as hCRT1. For this purpose, the Caco-2 cell line was employed. The active CIS transporter hCRT1 was saturated using Copper (Cu) (2μM) prior and during treatment. Cytotoxic effects of Cu were tested (IC_{50} ~150μM). Results indicated that the concentration employed did not cause cytotoxic effects. To confirm the saturation of the active transport mechanism using the aforementioned concentration, the CIS IC_{50} with and without Cu was calculated. Results indicated that the CIS IC_{50} in the absence of Cu was 68μM whereas in the presence of the ligand the value significantly increased the IC_{50} to 126 μM. Combined MFH experiments with CIS (5μM) were conducted with and without the presence of Cu for 30 min at 41°C (17.4kA/m, 239kHz). Cells were suspended (500,000 cells in 2.5 mL) in culture media with CIS, Cu, and dextran coated ferrite-based magnetic nanoparticles (70nm, 0.6mg Fe/mL). Further CIS exposure was allowed after MFH for an additional 2h. This additional exposure was performed as recent work[1] demonstrated that this sequence provided the highest reduction in cell viability when either MFH or hot water HT was performed. At this point, CIS was removed; cells were seeded on 75cm^2 culture flasks, and allowed to recuperate for 48hrs. Results indicated that the aforementioned CIS concentration and the application of MFH for 30min at 41°C did not cause any significant reduction in cell viability. Interestingly, a significant reduction in cell viability was observed when MFH was applied in the presence of Cu (see figure). Such reduction indicates that application of MFH results in other CIS transport mechanisms besides the hCRT1 mechanism. These preliminary studies suggest that passive transport of CIS could be enhanced by MFH.

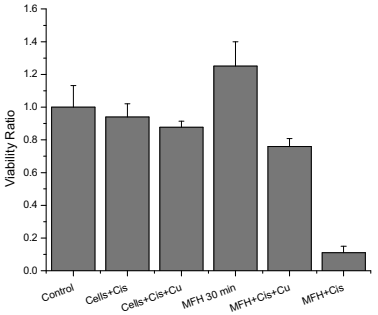

Figure 1. Cell Viability of Caco-2 Cells after exposure to combined MFH, CIS and Cu for 30 min at 41°C.

[1]Jason S. Lee, Héctor L. Rodríguez-Luccioni, Janet Méndez, Anil K. Sood, Carlos Rinaldi, and Madeline Torres-Lugo, *Hyperthermia Induced by Magnetic Nanoparticles Improves the Effectiveness of the Anticancer Drug cis-Diamminedichloroplatinum,* submitted, 2010

Talk 81

Magneto-mechanical actuation for targeted destruction of cancer cells

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Nanomagnetic materials offer exciting avenues in life sciences for probing cell mechanics and activating mechanosensitive ion channels, as well as for advancing cancer therapies. Most experimental works so far have used superparamagnetic materials. Our approach is based on interfacing cells with lithographically defined microdisks that possess a spin-vortex ground state, also known as "soft magnets". These disks are composed of metal permalloy (Fe 20% Ni 80%) core encapsulated into biocompatible gold shell. Owing to the presence of 3-*d* transition metals with high values of the saturation magnetization these disks are able to induce a high magnetomotive force. In addition, due to the unique fluxed magnetic spin arrangement these particles do not magnetically interact with each other; therefore, disks do not agglomerate. Gold shell preserves the particles surface from potentially toxic transition metals leakage and also allows routine coupling chemistry for functionalization of on the disks surface with biomolecules for specific biorecognition.

When an alternating magnetic field is applied the microdisks vortices shift, creating an oscillation, which transmits a mechanical force to the cell [1]. The spin-vortex-mediated stimulus creates two dramatic effects: compromised integrity of the cellular membrane detected via LDH-leakage cells viability assay, and initiation of programmed cell death (nuclear DNA nicking). A low frequency field of a few tens of hertz applied for only ten minutes was sufficient to achieve 90% cancer-cell destruction *in vitro*. We estimate that the spin-vortex induced forces are in the ~10s pN range, whereas > ~100s pN forces are needed to physically rupture the cell membrane. On the other hand, forces as small as few pN were reported to be sufficient to activate an existing single ion channel or induce formation of membrane pores. In both cases interruption of the barrier function of the cell membrane, even gentle and temporary, can result in altering of the molecular transport into and out of cells, therefore altering intra cellular machinery function. We experimentally demonstrated that the magnetic vortex-based mechanical stimulus applied to the cell leads to induction of intracellular Calcium ions signaling triggering apoptosis or "programmed call death" pathways. Because reduced sensitivity of cancer cells toward apoptosis leads to inappropriate cell survival and malignant progression, selective induction of apoptosis is of clinical importance for the anticancer strategies.

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Micro-structured hard magnetic films for lab-on-chip applications

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Micro flux sources producing magnetic fields which are spatially modulated at the micron scale can be used for lab-on-chip applications involving the trapping / levitation / manipulation of magnetic species. The magnetic force on a magnetic particle submitted to the field of a flux source depends on both the field and the field gradient and thus the force per unit volume increases as the size of and distance from the source decreases. The use of permanent magnets to produce the flux offers a number of specific advantages. It favours autonomy and stability. Micro-patterned soft magnetic materials require the application of an external magnetic field while micro-coils operating in DC mode produce much smaller fields in the mT range. The challenge in fabricating micro-permanent magnet based sources is first of all to produce hard magnetic films of the appropriate thickness (1-100 µm) and we have demonstrated that triode sputtering is suitable for the large-area deposition of high performance hard magnetic films (NdFeB, SmCo) in thick film form on Si substrates [1]. The second challenge is to laterally pattern the films on the scale of 1-100 µm. In this paper we will report on the use of two techniques for the micro-patterning of such films: (i) topographic patterning using standard micro-fabrication techniques [2] and (ii) thermo-magnetic patterning [3]. Details of the preparation, patterning and characterisation (e.g. fig. 1a,b) of the micro-magnets will be given. Examples of the application of these micro-magnets will also be presented (e.g. fig. 1c).

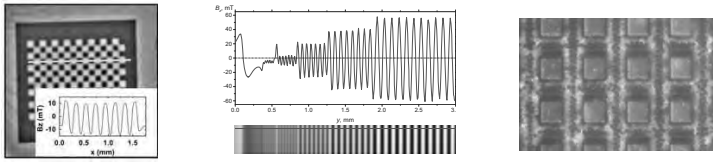

Figure 1: Scanning Hall Probe Microscope images / profiles of the stray magnetic fields produced 25 µm above theromagnetically (a) and topographically (b) patterned hard magnetic NdFeB films; (c): optical image of polystyrene beads (10 µm) in a paramagnetic buffer solution in levitation above a topographically patterned hard magnetic film.

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On chip detection and manipulation of biological entities carried by magnetic particles via domain walls conduits

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A novel method, based on magnetic domain wall conduits, for the manipulation of nanometric magnetic particles in suspension with active control of position and time, is presented. The method combines two dimensional translation, rotation and trapping of single magnetic particles along multiple trajectories with a digital control at the micro and nano scale and compatibility with lab-on-chip applications.
Magnetic nanoparticles are captured by the stray field of a magnetic domain walls (DWs) in nanoscale magnetic strips (conduits) and their transport and release is obtained via precise control over DW nucleation, displacement, and annihilation processes through application of external magnetic fields. This can be obtained in conduits made of rectilinear segments (zig-zag), thus implementing a stepper motor for the digital motion of magnetic particles, or in curved conduits allowing for a continuous motion with precise control of the particle position with a precision of the order of 100 nm. Pulsed magnetic fields with duration of 100 ms in the case of the zig-zag conduits, or continuous rotating fields in the case of circular rings, are applied through an electromagnet monitoring in real time the beads displacement using an optical microscope.

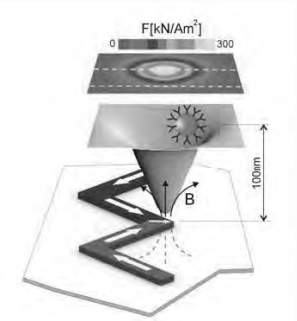

Fig. 1: Zig-zag conduit and potential well for a magnetic bead, originating from the stray field from the DW.

As an example of biological application we demonstrated the manipulation of magnetic beads coated with streptavidin, protein A and fluorescent antibodies (Anti-streptavidin-Cy3), as well as pairs of beads bound by chemical affinity between streptavidin and fluorescent antibodies[1]. The manipulation of magnetic labelled yeast cells on our platform is also shown thus demonstrating a cellular control on the micron scale. All those experiments are carried out in a polymethylmethacrylate microfluidic cell to ensure complete compatibility with existing lab on a chip technology.
Different shapes, sizes and materials of the nanowires are object of our on-going research including conduits with bifurcations and micro-sized conduits for controlled trapping and releasing magnetic beads inside a microfludic channel. We discuss preliminary results and the possibility of integrating in the system one or more sensors of DWs and magnetic particles described in a previous work [2]. They essentially consist in a portion of the DW conduit, e.g., a corner, flanked by conductive contacts for detecting electrically the presence of a DW and a magnetic bead thanks to the anisotropic magnetoresistance effect.
In prospective, this capability is unique compared to other technologies [3,4] and paves the way to the realization of networks of conduits with externally programmable functions and continuous monitoring of the desired process.

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[2] M. Donolato, M. Gobbi, P. Vavassori, M. Cantoni, V. Metlushko, B. Ilic, M. Zhang.,S.X. Wang and R. Bertacco, *Nanotechnology* ***20,*** 385501 (2009) P. Vavassori, V. Metlushko, B. Ilic, M. Gobbi, M. Donolato, M. Cantoni, R. Bertacco, *Appl. Phys. Lett*, ***93***, 203502(2008)
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AC Susceptibility Measurement of Magnetic Markers for Liquid Phase Detection of Biological Targets

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Ac susceptibility measurement of magnetic markers in solution was performed for the liquid phase detection of biological targets. The principle of detection is schematically shown in Fig. 1. The targets are fixed on the surface of large polymer beads, and the magnetic markers couple to the targets. In this case, the Brownian relaxation time of the bound markers becomes much longer than that of the free markers. It becomes τ_p=100 s when the polymer beads with diameter of d_p=6.7 μm was used, while the relaxation time of the free markers with diameter of d=110 nm becomes τ=0.51 ms. This difference caused the difference in the frequency dependence of the susceptibility between them. As a result, we can detect the bound markers by the decrease of the susceptibility of the markers. Using the method, we detected biotins that were conjugated on the surface of the polymer beads (Spherotech Inc, USA) with avidin-coated Fe_3O_4 markers (R&D Systems, U.S.A.). Changes of the susceptibility caused by the binding reaction between them were measured with a magneto-resistive sensor. The imaginary part of the susceptibility χ" was measured at f=250 Hz and excitation field of 2 mT. In Fig. 1(b), relationship between the decrease of the susceptibility $\Delta\chi$" and the number N_p of the polymer beads is shown for different incubation times. As shown, good relationship was obtained between them. We could detect the polymer beads as small as N_p=1800. Since it is expected that 6700 biotins are conjugated on the single polymer bead and the sample volume was 60 μl, sensitivity of the system was estimated as high as 3.3×10^{-16} mol/ml in terms of molecular-number concentration. Binding process was also studied from the dependence of the signal on the incubation time, which indicated typical reaction time of 15 min.

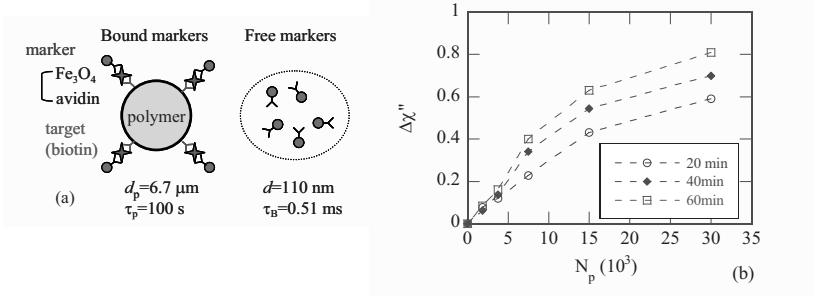

Fig. 1. (a) Principle of liquid phase detection using large polymer beads to immobilize the bound markers. (b) Relationship between the decrease of the susceptibility $\Delta\chi$"=[χ"(N_p=0)-χ"(N_p)]/χ"(N_p=0) and the number N_p of the polymer beads for different incubation times.

Growth Measurements of Individual Bacteria with a Magnetic Bead Rotation Sensor

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Microbiological studies are often conducted on the population level, and the heterogeneity within the cell population is a subject that is still actively studied. In this regard, accurate methods for measuring individual bacterial cell growth are needed to relate single cell growth to population based growth. Here, we demonstrate an asynchronous magnetic bead rotation (AMBR) biosensor with the capability to measure bacterial growth on the single cell level. AMBR biosensors consist of a superparamagnetic micro bead coated with antibodies specific to the antigen of interest.[1] After immunomagnetic separation, the beads are placed in a rotating magnetic field, where they experience a constant torque, and therefore their rotation rates have a simple dependence on the volume and shape of the bead complex. To measure individual bacterial growth, beads with single *E. coli* attached were isolated, and their rotation rates were observed on an optical microscope (Figure 1). The AMBR sensor rotational rate was estimated to be sensitive to 0.1 femtoliter changes in the volume of a bacterium, which corresponds to a roughly 300 nm change in the cell length. This exceeds the volume measurement accuracy of a diffraction limited optical microscope. The presented AMBR method allows for biomedical sensor applications that depend on volume changes in the sub femtoliter range. Further development of a simple off-microscope device would enable parallel high throughput studies of single cell growth and cell response to environmental variables.[2] The sensitivity can be further improved by increasing the bead frequency, where a higher exerted drag also enables additional applications, which will also be discussed in the presentation.

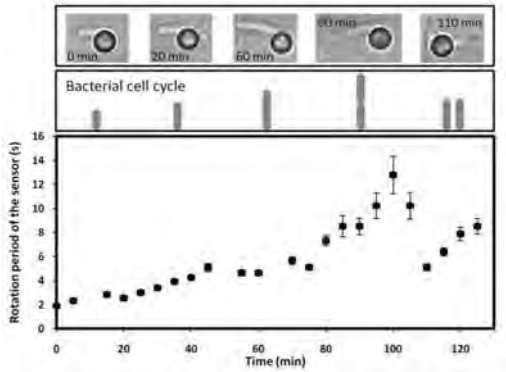

Figure 1: AMBR sensor data showing the growth of an individual *E. coli* cell. The data points show the rotation rate of the sensor bead. The top box visualizes the growth phases of the bacteria showing the division and the reattachment of the daughter cell. A schematic presentation is given in the middle box. Note that at time 100 min the cell divides and reattaches in a different angle, changing the form factor of the sensor.

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Biomarker quantification in unprocessed human sera by a competition-based nanomagnetic protein assay and high-magnetic-moment nanoparticles

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*corresponding author: Jian-Ping Wang (email: jpwang@umn.edu)

This study reports the first realization of specific and accurate quantification of a ultra low-abundance serum protein in **unprocessed human sera**, employing our novel giant magnetoresistive (GMR) biosensing system with uniform high-magnetic-moment nanoparticles [1,2] and a competition based detection scheme [3].

The combination of GMR sensors and magnetic nanoparticles has attracted much attention as a promising alternative since GMR sensors have several potentially unique merits such as portability, low cost, rapid detection, and high signal to noise ratio. Nevertheless, most GMR biosensor research has focused on proof of concept, model studies, or detection of spiked serum samples. Specific detection and accurate quantification of protein biomarkers in unprocessed human serum samples is missing. In this work, we demonstrate, for the first time, the accurate quantification of interleukin-6 (IL-6, an ultra low-abundance protein in serum) with great specificity, in unprocessed human sera, employing our competition-based GMR biosensing system.

Based on the detecting principle, the sensitivity of GMR biosensing system strongly depends on the distance d between the magnetic particle and sensor since the magnetic signal drops off as $1/d^3$. Current GMR detection generally employs a sandwich-based approach (Fig. 1A and Fig. 1C). This increases the distance between the nanoparticle and sensor, compared to two-layer approach (Fig. 1B and Fig. 1C), thus compromising detection sensitivity. In addition, the signals detected by this sandwich-based approach may not necessarily provide a one-to-one correspondence to biomarker concentration, limiting its usage to unequivocally quantify proteins from unprocessed serum samples. To address these challenges, we propose a two layer detection scheme (one analyte and one antibody only) (Fig. 1B, Fig. 1C), where the magnetically labelled analyte competes the bonding sites with unlabelled analyte. Such an approach minimizes the distance of the magnetic nanoparticles to the GMR sensor, which theoretically enhance the signal over the sandwich-based approach by 3.4 to 39 times, depending on the size/orientation of antibody and protein. In addition, the signal detected by the two layer competition-based approach scheme corresponds to one specific biomarker concentration.

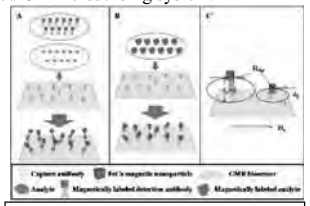

Fig. 1. First competition based nanomagnetic protein assay for biomarker detection.

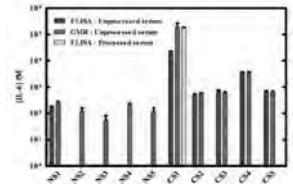

Fig. 2. Comparison of quantifications of IL-6 molecules in unprocessed human serum samples using ELISA and competition-based GMR biosensor. Heathy: NS1-NS5; Cancer: CS1-CS5

The concentrations of IL-6 in unprocessed human serum samples were then quantified using this novel competition-based two-layer detection scheme. Ten human serum samples (five healthy and five cancer patients) were evaluated. High-magnetic-moment nanoparticles labelled IL-6 molecules were mixed with unprocessed human serum sample and applied on the GMR biosensors. We demonstrated the successful quantification of IL-6 levels in all the unprocessed serum samples with great specificity (ELISA failed four), suggesting a higher sensitivity and specificity in our device (Fig. 2). Excitingly, the levels of IL-6 in the five sera from lung cancer patients are all significantly higher than those in the sera from healthy individuals, preliminarily supporting IL-6 as a potential lung cancer biomarker.

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Chip-based measurements of Brownian relaxation of magnetic beads using a planar Hall effect magnetic field sensor

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When a magnetic bead with a permanent magnetic moment is exposed to an alternating field, it will be able to follow the field by a physical rotation up to the Brownian relaxation frequency, $f_B=(k_BT)/(6\pi\eta V_h)$, where k_B is Boltzmann's constant, T is the absolute temperature, η is the viscosity of the fluid and V_h is the hydrodynamic volume of the bead.[1] Measurements of the complex, frequency dependent magnetization $m=m'-im''$ are used to characterize the Brownian relaxation, which gives rise to a peak in the out-of-phase magnetization m'' at $f=f_B$. Measurements of changes in f_B upon binding of biomolecules to the bead will give a quantitative measure of the amount of biomolecules, which averages over the entire sample volume. Until now, measurements of the Brownian relaxation behavior have been carried out in ac susceptometers based on inductive coil or SQUID detection.[1,2]

Here, we demonstrate a completely new approach, where a magnetic field sensor based on the planar Hall effect (PHE)[3] is used to detect the Brownian relaxation behavior. The alternating magnetic field exciting the magnetic beads is provided from the bias current passed through the sensor, and thus no external magnetic fields are required. The PHE sensor is integrated in a microfluidic channel into which a suspension of nanomag-D beads with a diameter of 130 nm is injected. Fig. 1 shows the sensor geometry. The response due to magnetic beads can be measured by use of lock-in technique. Fig. 2 shows the in-phase and out-of-phase 2nd harmonic responses V_2' and V_2'' upon application of an ac bias current I measured without magnetic beads and measured after injection of a bead suspension at the indicated temperatures. It can be shown theoretically that V_2' is proportional to m'' and that V_2'' is linearly related to m'. This prediction is confirmed by the data in Fig. 2, where a peak is observed in the V_2' data corresponding to f_B and the V_2'' data show the decrease with f expected for Brownian relaxation. The temperature dependence in Fig. 2 is consistent with changes in f_B due to the temperature dependence of the viscosity of water. Thus, we have performed the first demonstration of measurements of the Brownian relaxation behavior of magnetic beads by use of a microscopic sensor operating at room temperature integrated in a microfluidic channel. This opens a new field of applications of microscopic magnetoresistive biosensors.

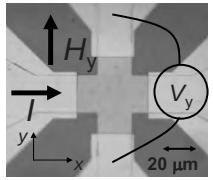

Fig. 1 Micrograph of the planar Hall effect sensor. The sensor is sensitive to the field H_y in the y-direction. A positive current I in the x-direction gives rise to a magnetic field near the sensor in the -y-direction. Magnetic beads give rise to a field in the +y-direction acting on the sensor.

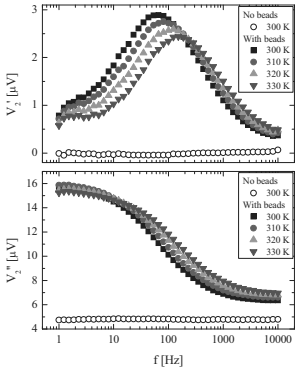

Fig. 2 The top and bottom parts shows 2nd harmonic in-phase and out-of-phase responses measured without beads and with a suspension of 130 nm nanomag-D beads at the indicated temperatures. The data were corrected for an instrumental phase shift. The amplitude of the applied current was 20 mA.

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Talk 87

Study of spatio-temporal immunofluorescence on magnetic bead patterns in a microfluidic channel

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Taking advantage of the specific binding of an antibody (Ab) to its antigen (Ag), the immunoassay is currently the predominant analytical technique for the specific and accurate detection of a broad variety of analytes in medical and biotechnological applications [1,2]. Before, we realized a sandwich immunoassay on streptavidin-coated magnetic beads patterned inside a microfluidic channel [3,4].
In the present work, we study the spatio-temporal immunofluorescence developed on magnetic bead dot patterns that are exposed to a continuous flow of fluorescent Atto-488-biotin in PBS-BSA (1%) as a target Ag. The fluorescent signal on the magnetic bead surface not only indicates the Ag-Ab association, but also reflects the exact exposure of the Ag to the local bead pattern. An analytical diffusion model is proposed to study the fluorescence distribution along the microchannel and is compared to the experiment. It demonstrates that the target Ag, by diffusion, gradually gets depleted from the flow, when the latter passes over subsequent magnetic bead positions. The highest fluorescence intensity is observed on the dot pattern which is most upstream in the flow, and the fluorescence decreases gradually along the microfluidic channel. According to the proposed model and experimental results, the fluorescence signal is a decreasing function of the position of the bead pattern and the flow rate.
We think our method holds great promise for studying a wide range of other types of Ag-Ab interactions and affinity constants, using magnetic bead patterns that are immobilized at well-controlled positions in a microchannel.

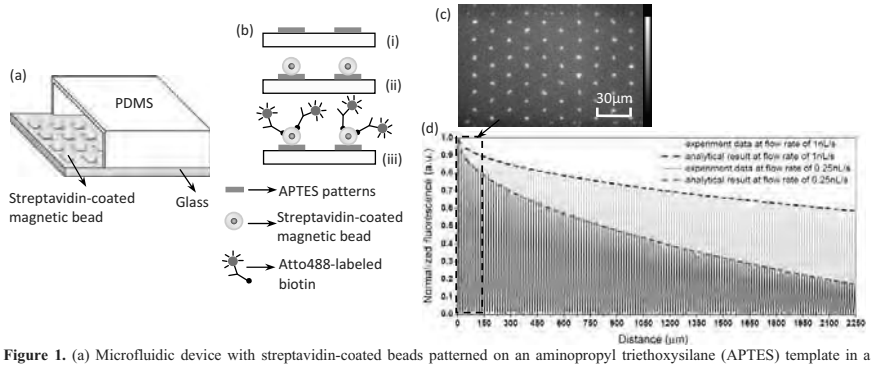

Figure 1. (a) Microfluidic device with streptavidin-coated beads patterned on an aminopropyl triethoxysilane (APTES) template in a microfluidic channel. (b) Schematic illustration of the direct immunoassay formation on the streptavidin-coated bead patterns. The glass substrate is (i) patterned with APTES, (ii) subsequently incubated with streptavidin-coated beads; (iii) Atto-488-labeled biotin is the fluorescent target Ag (100 pg/mL in PBS-BSA(1%)) captured from the flow. (c) Fluorescence micrograph, acquired after performing the direct immunoassay on the beads, showing the most upstream 10 columns of dot patterns. (d) Fluorescence intensity profile along 150 subsequent bead patterns after performing the direct immunoassay at two different flow rates. The first peak and the last peak correspond to the single dot patterns present in the first and the 150th column downstream the flow. The black line and the grey line present the experimental fluorescence profiles obtained at a flow rate of 1 nL/s and flow rate of 0.25 nL/s, respectively. For comparison, the dotted lines refer to the model calculation at 1 nL/s and 0.25 nL/s flow rate, respectively.

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Talk 88

8th International Conference on the Scientific and Clinical Applications of Magnetic Carriers - Rostock, Germany

Poster Presentations (in alphabetical order of the first author)

#	First Author	Poster Title	City, Country
1	Afshar, Rana	3-Dimensional Focusing of Magnetic Particles in a Microfluidic Chip	Lausanne, Switzerland
2	Ahrentorp, Fredrik	Sensitive high frequency AC susceptometry of magnetic nanoparticle systems	Göteborg, Sweden
3	Ally, Javed	Recent Advances and Fundamentals of Magnetically Targeted Aerosol Particles in the Airways	Edmonton, Canada
4	Anandakumar, S.	Magnetic Carriers Translocation in Microfluidics	Daejeon, South Korea
5	Araujo, Ana	Mössbauer characterization of magnetite/polyaniline magnetic nanocomposite	Recife, Brasil
6	Arias, Jose L.	Formulation and characterization of maghemite/poly(D,L-lactide-co-glycolide) (core/shell) nanoparticles	Granada, Spain
7	Asin Pardo, Laura	Biodistribution study of magnetic and fluorescent nanoparticles within dendritic cells and their effect in the maduration stage	Zaragoza, Spain
8	Asín Pardo, Laura	Cell Internalization of Magnetite Nanoparticles by Dendritic Cells for Intracellular Magnetic Hyperthermia.	Zaragoza, Spain
9	Astalan, Andrea	The use of high frequency AC susceptometry in magnetic hyperthermia	Paris, France
10	Augusto, Paulo	Arsenic removal from water by magnetic separation: a review and a case study using magnetic aggregrates and magnetic stabilized bed	Salamanca, Spain
11	Avdeev, Mikhail	Aggregate structure in biocompatible aqueous magnetic fluids with steric and electrostatic stabilization	Moscow, Russia
12	Aviles, Misael	Design of magnetic collagen gels and their effect in cell behavior	Evanston, IL, USA
13	Balaji, Deepak	Surface coating dependent selection of Superparamagnetic Nanoparticles for whole cell isolation	Leuven, Belgium
14	Balasoiu, Maria	Small angle neutron scattering methods in magnetic nanoparticles diagnostics	Dubna, Russia
15	Basina, Georgia	Synthesis of Water-Soluble Inverse Spinel Ferrites (FeFe2O3 and MnFe2O4) by a Modified Polyol Process as Contrast Agents in Medical Magnetic Resonance Imaging	Athens, Greece
16	Basly, Brice	Suspensions of dendronised iron oxide nanoparticles for biomedical applications	Strasbourg, France
17	Batalha, Iris	Biopolymeric magnetic particles for bioseparation purposes	Caparica, Portugal
18	Baumann, Romy	In vitro Investigations for Magnetic Lung Drug Targeting:Influence of Particle Size of Magnetite Nanoparticles on the Deflection in Magnetic Fields	Greifswald, Germany
19	Beaucorps, de, Caroline	Innovative Nanoprobes for Magnetic Immunoassays: Elaboration and Characterization	Paris, France
20	Benelmekki, Maria	Contribution to the study of Ferrite nanobeads: Synthesis, characterization and investigation of Horizontal Low Gradient magnetophoresis behaviour and reversible aggregation	Braga, Portugal
21	Bokharaei, Mehrdad	Production of Monosized Microspheres Using Flow Focusing	Vancouver, Canada
22	Bregar, Vladimir	Magnetization state in magnetic nanoparticle agglomerates	Ljubljana, Slovenia
23	Brusentov, N.	Combined MRI-adaptive Magneto-thermo-polychemotherapy for Improved Cancer Treeatment	Moscow, Russia
24	Büttner, Markus	Investigation of the connection of magnetic active core sizes and hydrodynamic diameters of a magnetically fractionated ferrofluid	Jena, Germany
25	Burdinski, Dirk	Liposomal Tracers for Magnetic Particle Imaging (MPI)	Eindhoven, Netherlands
26	Bustamante, Rodney	Magnetic fluids of maghemite-poly(vinylpyridine) nanoparticles as multifuncional platform for theragnostic	Zaragoza, Spain
27	Byrne, Fiona	Application of Magnetic Nickel Nanowires to macrophage cells and osteoblast cells: biocompatibility study and clinical future perspectives	Dublin, Ireland
28	Carneiro, Marcella	Cytotoxicity effect of rhodium (II) citrate loaded magnetic nanoparticles and magnetoliposomes in breast cancer and normal breast cells	Brasilia, Brasil
29	Chandra, Sudeshna	Design of a glucose amperometric biosensor by novel arginated Fe3O4 nanoparticles	Mumbai, India
30	Chen, D.-X.	Size distribution and magnetic structure of iron-oxide nanoparticles studied by measuring magnetization curves at various temperatures	Bellaterra, Spain
31	Chen, Yun	Magnetic manipulation of actin orientation, gliding and polymerization using nano-sized iron oxide particles	Bethesda, MD, USA
32	Chiriac, Horia	Preparation method for magnetite and continuous solid solutions of magnetite-maghemite	Iasi, Romania
33	Chiriac, Horia	Detection of disease-related DNA through metallic nanowires	Iasi, Romania
34	Chiriac, Horia	Fe/Fe2O3 and Fe/Fe3O4 core-shell nanoparticles for biomedical applications	Iasi, Romania
35	Chuev, Mikhail	Interpretation of Mössbauer spectra of magnetic nanoparticles delivered into mouse spleen	Moscow, Russia
36	Chung, Ting-Hao	Preparation of Magnetic Styrene-Based Polymeric Microspheres: Influence of Incubation Time and Concentration of Magnetic Nanoparticles	Chiayi, Taiwan
37	Clarke, Sarah	Magnetic Polymer Nanocomposite Materials for Targeted Drug Release in Cancer.	Dublin, Ireland
38	Cordo, Paloma	Nanoparticles of Molybdenum Chlorophyllin Photosensitizer and Magnetic Citrate-Coated Cobalt Ferrite complex available to Hyperthermia and Photodynamic Therapy clinical trials	Ribeirão Preto, Brasil
39	Craciunescu, Izabell	Functionalized fluorescent magnetic nanoparticles for biomedical application	Cluj-Napoca, Romania
40	Creixell, Mar	Targeted magnetic nanoparticles for magnetic fluid hyperthermia.	Mayagüez, Puerto Rico
41	Cummins, Zach	Manipulating Ferrofluid at a Distance: Magnets Pushing and Dynamic Control	College Park, MD, USA
42	Delyagina, Evgenya	PEI 600 Da conjugated to magnetic nanobeads as a non-viral vector for gene delivery	Rostock, Germany
43	Dennis, Cindi	Fallacy of the Conventional Method for Determination of the Blocking Temperature of Magnetic Nanoparticle Systems	Gaithersburg, MD, USA
44	Dong, Guijun	Functinalized paramagnetic iron oxide nanoparticles (PIONs) for the application of targeted muscle damage therapy	Jinan, China
45	Dutz, Silvio	Fractionation of Magnetic Microspheres in a Microfluidic Spiral	Jena, Germany
46	Dutz, Silvio	Magnetic Properties of Iron Oxide Multicore Nanoparticles Classified by Asymmetric Flow Field-Flow Fractionation	Jena, Germany
47	Dzarova, Anezka	How synthetic and bacterial magnetic nanoparticles influence human leukocyte activity	Košice, Slovakia
48	Eberbeck, Dietmar	Effect of MNP-core size and aggregates of MNP on the signal amplitude for Magnetic Particle Imaging	Berlin, Germany

8th International Conference on the Scientific and Clinical Applications of Magnetic Carriers - Rostock, Germany

Poster Presentations (in alphabetical order of the first author)

#	First Author	Poster Title	City, Country
49	Emadi, Masoomeh	Immobilization of Thiadiazole Derivatives in Magnetite With Mesoporous Silica Shell; Application to Heavy Metal Removal from Biological Sample	Marvdasht, Iran
50	Epherre, Romain	Glycine nitrate process for the elaboration of manganite nanoparticles as possible self-regulating mediators for hyperthermia	Pessac, France
51	Erglis, Kaspars	Three dimensional dynamics of ferromagnetic swimmer	Riga, Latvia
52	Erne, Ben	Colloidal Nanoparticle Interactions in Liquid Environment Quantified by Cryogenic Electron Microscopy	Utrecht, The Netherlands
53	Estevez, A	Preparation, characterization and testing of alginate based magnetic carriers and others for arsenic removal of water	Salamanca, Spain
54	Falqueiro, André	Preparation and characterization of Selol-loaded magnetic nanocapsules for hyperthermia cancer therapy	São Paulo, Brasil
55	Fantechi, Elvira	Influence of cobalt content on the hyperthermal efficiency of ferrite nanoparticles	Sesto, Italy
56	Ferguson, R Matthew	Size-dependent magnetic relaxation in magnetite nanoparticles: lessons for Magnetic Particle Imaging	Seattle, WA, USA
57	Foca-nici- Ciurlica, Ecaterina	Oleate coated magnetic cores based on magnetite, Zn ferrite and Co ferrite nanoparticles; preparation, physical characterization and biological impact on Helianthus annuus photosynthesis	Iasi, Romania
58	Focanici-Ciurlica, Ecaterina	Comparative cytogenetic study on magnetic nanoparticle toxicity in plants	Iasi, Romania
59	Fox, Eoin	Phase transfer of Magnetic Iron-Oxide Nano-Particles using phosphate based ligands	Dublin, Ireland
60	Frimpong, Reynolds	Synthesis Conditions, Interactions and Their Effect on the Anisotropy	Lexington, KY, USA
61	Fuhrer, Roland	Highly magnetic nanocomposite actuator for artificial muscle applications	Zurich, Switzerland
62	Gaboyard, Manuel	Activ-Adembeads : A new ready-to-use tool for sensitive magnetic beads-based immunoassays and biosensors	Zurich, Switzerland
63	Garcia, Monica	DMSA-coated magnetic fluid clearance by mouse liver	Brasilia, Brasil
64	Gassner, Anne-Laure	Multiplug magnetic beads trapping in a capillary with a magnets necklace	Lausanne, Switzerland
65	Gomez, Jorge	Quantitative FMR measurements of Magnetic Immunoassays	São Paulo, Brazil
66	Gorobets, S	MFM characterization of magnetic nanoparticles for yeast cell labling	Kyiv, Ukraine
67	Gorschinski, Angelika	A synthesis to magnetic metal nanoparticles and dual-functional microspheres	Karlsruhe, Germany
68	Guilherme, Luciana	Encapsulation of rifampicin and nanosized magnetic particles within biocompatible polymeric nanocapsules	Brasilia, Brasil
69	Guo, Chen	Temperature-Responsive Magnetite/PEO-PPO-PEO Block Copolymer Nanoparticles for Controlled Drug Targeting Delivery	Beijing, China
70	Gutiérrez, Marlen	Magnetic zeolite as carrier of proteins	Santiago, Chile
71	Habulin, Maja	Activity of cholesterol oxidase immobilized onto magnetic nanoparticles	Maribor, Slovenia
72	Hajdú, Angéla	The role of the hydrophilic coverage on magnetic core in MRI contrast enhancement at different field strengths	Szeged, Hungary
73	Hecht, Ariel	The AMBR assay: A novel biosensor based on magnetic torque	Ann Arbor, MI, USA
74	Heidsieck, Alexandra	Efficient Targeting of Magnetic Nanoparticle Complexes in the Cardiovascular System	Munchen, Germany
75	Heim, Erik	Fluxgate magnetorelaxometry of superparamagnetic nanoparticles for hydrogel characterization	Braunschweig, Germany
76	Herrmann, Inge	Therapeutic blood purification using functionalized core/shell nanomagnets	Zurich, Switzerland
77	Hershberger, Stefan	Scalable Magnetic Designs to Achieve Comparable Capture Rates and Capture Efficiency across Multiple Vessel Diameters	Abbott Park, IL, USA
78	Hirota, Noriyuki	Observation and Numerical Simulations of the Self-Organization Phenomena of Feeble Magnetic Particles due to the Induced Magnetic Dipole Interactions	Tokyo, Japan
79	Horák, Daniel	Modified magnetic poly(2-hydroxyethyl methacrylate-co-glycidyl methacrylate) microspheres for bacterial DNA isolation	Brno, Czech Republic
80	Horng, Herng-Er	High-sensitivity immunomagnetic reduction assay on tumor biomarkers using high-Tc superconducting quantum interference device	Taipei, Taiwan
81	Horska, Katerina	Large scale magnetic separation of Solanum tuberosum lectin from potato starch industry waste water	Ceske Budejovice, Czech Republic
82	Hu, Kuowei	pH-dependence of Biodegradable Silica Nanotubes toward Oral Delivery of Drugs and MR Imaging Contrast Vectors: in situ Etching and Chelating of Gd3+ from Gd(OH)3 Nanorods	Tainan City, Taiwan
83	Hu, Lili	Intrinsic peroxidase-like activity of Bacterial magnetic nanoparticles	Beijing, China
84	Huang, Chih-Chia	Truncated octahedral magnetite nanoparticles: size-controlled synthesis, characterization, and potential application in MR imaging	Tainan City, Taiwan
85	Huber, Dale	Synthesis, Functionalization, and Magnetic Characterization of Magnetite Nanoparticles for Applications in Nano-medicine	Albuquerque, NM, USA
86	Ibrahim, Mounir	Loading Erythrocytes with Magnetite Nanoparticles via Osmotic Pressure Induced Cell Membrane Pores	Crawley, Australia
87	Jaiswal, Manish	Temperature triggered drug release, in vivo biodistribution and biocompatibility studies of poly(N-isopropylacrylamide)-chitosan based magnetic nanohydrogels	Mumbai, India
88	Ji, Yanqin	Preparation and Characterization of Magnetic Polymer Microspheres for the Separation of Radioactive Strontium	Beijing, China
89	Jing, Ying	Optimization of heating performance assisted by dynamic coercivity analysis for magnetic hyperthermia	Minneapolis, MN, USA
90	Jozefczak, A.	Heating ability of magnetite labeled single-walled nanotubes	Poznań, Poland
91	Juan, Eduardo	Closed-Loop Temperature Control of Cobalt Ferrite Ferrofluids Using Continuous Modes Controllers	Mayagüez, Puerto Rico
92	Karadeniz, Hakan	Electrochemical biosensor for detection of DNA hybridization using indicator-based and indicator-free magnetic assays	Izmir, Turkey
93	Karl, Stephan	High gradient magnetic fractionation for the detection of malaria transmitting gametocytes	Crawley, Australia
94	Kauffmann, Paul	Diamagnetic trapping of cells above micro-magnets	Grenoble, France
95	Kekalo, Katsiaryna	Synthesis and characterization of magnetite-containing particles with dextran/chitosan coating	Minsk, Belarus
96	Kettering, Melanie	Internalization and cytotoxicity effects of different iron oxide magnetic nanoparticle coatings on tumour cells	Jena, Germany
97	Khandhar, Amit	Systematic protocol for optimizing monodispersed magnetite nanoparticles for combined applications in biomedical imaging and therapy	Seattle, WA, USA

8th International Conference on the Scientific and Clinical Applications of Magnetic Carriers - Rostock, Germany

Poster Presentations (in alphabetical order of the first author)

#	First Author	Poster Title	City, Country
98	Khurshid, Hafsa	Polysiloxane DiblockCopolymer Coated Iron Nanoparticles as Excellent MRI Contrast Enhancement Agents	Newark, DE, USA
99	Kim, D	Magnetic nano-micelles for simultaneously triggered drug delivery and hyperthermia	Argonne, IL, USA
100	Kimpe, Katrien	The use of MagnosphereTM MS300/Tosyl paramagnetic beads in HCV and EBV assay formats.	Leuven, Belgium
101	Kitenbergs, Guntars	Thermal fluctuation effects in magnetophoresis of superparamagnetic microbeads	Riga, Latvia
102	Koch, Martin	On the Question of Magnetization	Copenhagen, Denmark
103	Koehler, Fabian	Magnetic EDTA: Coupling heavy metal chelators to metal nanomagnets for rapid removal of cadmium, lead and copper from contaminated water	Zurich, Switzerland
104	Kolesnichenko, Vladimir	Synthesis, colloid and surface chemistry of metal oxide nanoparticles	New Orleans, LA, USA
105	Komissarova, Lubov	Development of Magnetic Operated Preparations for Boron Neutron Capture of Tumour Therapy	Moscow, Russia
106	Kralj, Slavko	Silica-coated fluorescent magnetic nanoparticles marked with monoclonal antibodies for breast-cancer targeting	Ljubljana, Slovenia
107	Kumar, Ananda	Reduced non-specific tissue heating with dielectric shielding for magnetic nanoparticle-mediated thermal therapy for metastatic prostate cancer	Baltimore, MD, USA
108	Lak, Aidin	Influence of synthesis parameters on magnetization and size of iron oxide nanocrystals	Braunschweig, Germany
109	Lapresta-Fernandez, Alejandro	Magnetic core-shell fluorescent pH nanosensors	Jena, Germany
110	Larsen, Esben	Size-dependent Accumulation of PEGylated Coated Magnetic Iron Oxide Nanoparticles in Tumours	Aarhus, Denmark
111	Latha, Subbiah	Development and assessment of sucralfate magnetic nanosuspension for targeted ulcer therapy	Tamil Nadu, India
112	Latha, Subbiah	Formulation, design, development, optimisation and evaluation of azithromycin magnetic nanoemulsion	Tamil Nadu, India
113	Latorre-Esteves, Magda	Effects of Nanoparticle Surface Charge On Cellular Uptake and Mechanism of Internalization In Vitro	Mayagüez, Puerto Rico
114	Leonowicz, Marcin	Structure and properties of magnetic beads for biological sensor applications	Warsaw, Poland
115	Lerche, Dietmar	Characterization of dispersion properties of magnetic fluids derived from nano particles by multi-sample analytical centrifugation	Berlin, Germany
116	Li, Aihua	A novel method for rapid detecting Salmonella enteritidis and Escherichia coli O157 by immuno-magnetic capture / double-tagging PCR / enzyme immunoassay	Beijing, China
117	Liao, Mei-Yi	Kirkendall effect for fabrication of hollow MnF2O4 nanostructures as contrast efficacy T2 imaging agents	Tainan, Taiwan
118	Liao, Shu-Hsien	Characterization of oscillation dynamics of magnetic nanoparticles at ultra-low frequencies	Taipei, Taiwan
119	Liebi, Marianne	Magnetoresponsive Lanthanide Doped Phospholipid Vesicles	Zurich, Switzerland
120	Lin, Yu-Sheng	Preparation of Silica Coated Superparamagnetic Nanoparticles and Its Application in Immobilization of Endo-Xylanase	Chiayi, Taiwan
121	López, Jorge	Investigation of the magnetic behaviour in Fe3O4 ferrofluid	Rio Branco, Brazil
122	Ludwig, Frank	Characterization of magnetic core-shell nanoparticle suspensions using ac susceptibility for frequencies up to 1 MHz	Braunschweig, Germany
123	Lunov, Oleg	Thermal Destruction on the Nanoscale: Cell Membrane Hyperthermia with Functionalized Magnetic Nanoparticles	Ulm, Germany
124	Mahmoudi, Morteza	Interaction of Partially Iron Saturated Human Transferrin with Superparamagnetic Iron Oxide Nanoparticles	Tehran, Iran
125	Makovec, Darko	Superparamagnetic clusters as carriers for an anatase photocatalyst	Ljubljana, Slovenia
126	Malinge, Pauline	Antibody and Fc-fusion Protein Purification using Protein A/G coated Beads	Geneva, Switzerland
127	Manina, Elena	Biomedical examination of Fe-Co oxide nanoparticles for local ferromagnetic hyperthermia	Minsk, Belarus
128	Manju, Saraswathy	Functionalization of magnetic nanoparticles using curcumin conjugates: A multidrug carrying vehicles for targeted drug delivery applications.	Trivandrum, India
129	Mardinoglu, Adil	Mathematical Modelling of the Magnetic Carrier Collection Efficiency with Magnetisable Stent Implant in a Mechanically Stretched Vessel. Future Application of Implant Assisted Magnetic Drug Targeting.	Dublin, Ireland
130	Marten, Gernot	Magnetoresponsive Biocatalysts	Düsseldorf, Germany
131	Masrour, Rachid	Physical properties and giant magnetoresistance in perovskite	Fez, Morocco
132	Matoussevitch, Nina	Au55-clusters and magnetic nanoparticles for biomedical applications	Kehl, Germany
133	Maurizi, Lionel	New method of synthesis of SPIONs: in vitro and in vivo biological applications as a MRI contrast agent	Dijon, France
134	McFadden, Meghan	Magnetic Beads in Mixture Screens for Modulators of Protein-Protein Interactions	Hamilton, Canada
135	Mefford, Thompson	Size effects of polymer-nanoparticles systems in magnetic hyperthermia	Clemson, SC, USA
136	Mesarosova, Monika	The impact of surface modifications on the biological activity of superparamagnetic magnetite nanoparticles.	Bratislava, Slovakia
137	Montràs, Anna	Bio-activated magnetic beads for a cost effective isolation of biological molecules: Fast separation and reusability using Low Field Gradient Magnetophoresis	Barcelona, Spain
138	Moore, Lee	Coupling of planar Halbach array to a step-SPLITT channel for continuous sorting of magnetic microspheres	Celveland, OH, USA
139	Morjan, Ion	Recent advances in the synthesis and characterization of magnetic iron oxide nanoparticles prepared by laser pyrolysis	Bucharest, Romania
140	Müller, Eberhard	Investigations on Controlling the Crystal Modification of Fe2O3 Nanoparticles Produced by CO2 Laser Vaporization	Freiberg, Germany
141	Nadejde, Claudia	Magnetite submicron particles coated with rifampicin and fluorescent chlortetracycline for drug delivery applications	Iasi, Romania
142	Nadejde, Claudia	Double-layered magnetic nanoparticles for biological applications	Iasi, Romania
143	Nan, Alexandrina	Application of click chemistry for functionalization of polypyrrole coating the magnetic nanoparticles	Cluj-Napoca, Romania
144	Nejadmoghaddam, Mohammad Reza	Extraction and purification of Nanomagnetic particles from bacteria	Tehran, Iran
145	Newman, Brant	Endothelialization of Magnetic Graft Materials using SPM-labeled Endothelial Cells	Rochester, MN, USA
146	Nikitin, Maxim	Study of magnetic particle in vivo degradation by Mössbauer spectroscopy	Moscow, Russia
147	Nikolaev, Boris	Preparation of magnetic iron oxide nanoparticles in water-in-oil micro-emulsion stabilized by nonionic surfactant Tween 81 for MRI diagnostics	St. Petersburg, Russia

8th International Conference on the Scientific and Clinical Applications of Magnetic Carriers - Rostock, Germany

Poster Presentations (in alphabetical order of the first author)

#	First Author	Poster Title	City, Country
148	Nunes, Eloiza	Colloid preparation of maghemite nanoparticles functionalized with dirhodium(II) citrate	Goiânia, Brazil
149	Oh, Sunjong	Magnetic labels detection in paper chromatography using planar hall resistance sensor	Daejeon, South Korea
150	Oliveira, Tiago	Interaction of J774A1 macrophages with iron oxide nanoparticles coated with dextran	São Paulo, Brasil
151	Olivetti, Elena	Functionalised magnetic nanoparticles for nucleic acids separation in plant disease diagnostics	Torino, Italy
152	Orlov, Alexey	Optimization of Immunoassays based on Magnetic Nanolabels by Optical Biosensor Picoscope™	Moscow, Russia
153	Ortega, Daniel	Nanoscale imaging of magnetically loaded stem cells as diagnostic and therapeutic vectors for lung cancer	London, England
154	Ozturk, Hande	Growth of block copolymers from the surface of magnetic nanoparticles	Istanbul, Turkey
155	Papa, Anne-Laure	Functionalized titanate nanotubes as potential carriers in nanomedicine	Dijon, France
156	Payer, Petra	Synthesis and Clinical Application of Magnetic Nanoparticles	Jena, Germany
157	Pecova, Michaela	Fast and Efficient Proteolysis Using Trypsin Immobilized on Magnetic Nanoparticles Isolated from Magnetotactic Bacteria	Olomouc, Czech Republic
158	Peeters, Sara	Real-time PCR to study the sequence specific magnetic purification of DNA	Leuven, Belgium
159	Pina, Ana	Immobilization of enzyme bovine enterokinase on biopolymeric magnetic shells	Caparica, Portugal
160	Pivetal, Jérémy	Trapping of magnetically-labelled liposomes on flat micro-patterned hard magnetic films	Ecully, France
161	Pondman, Kirsten	Trapping of spherical and elongated magnetic particles in microfluidic systems	Enschede, The Netherlands
162	Pouponneau, Pierre	FeCo nanoparticles encapsulated into biodegradable microparticles for targeted liver chemoembolization	Montréal, Canada
163	Prabhakaran, Shanmugam	Synthesis and Antibacterial Activity of Multifunctional Fe3O4–Ag Heterodimer Nanoparticles	Mumbai, India
164	Pyshnyi, Michael	Visualization of magnetic microparticles in blood vessels using synchronous ultrasonic Doppler imaging.	Moscow, Russia
165	Raikher, Yuriy	Theory of the magneto-inductive hyperthermia in a rotating field	Perm, Russia
166	Richter, Heike	Multichannel magnetorelaxometry in vivo monitoring of magnetic nanoparticle quantity for thermal ablation studies	Berlin, Germany
167	Rinaldi, Carlos	Nonlinear Energy Dissipation of Magnetic Nanoparticles in Oscillating Magnetic Fields	Mayagüez, Puerto Rico
168	Rodriguez, Anselmo	RF susceptibility of magnetic nanoparticles system with application in Biomedicine	Rio Branco, Brazil
169	Rodriguez, Guillermo	Fast detection of Legionella pneumophila in cooling towers by immunomagnetic microspheres	Castellón, Spain
170	Roig, Anna	Iron oxide nanoparticles in microporous silica shell presenting enhanced r2 relaxivity values	Bellaterra, Spain
171	Rossier, Michael	Selective noble metals extraction from dilute, acidic streams using functionalized carbon-coated nanomagnets	Zurich, Switzerland
172	Rusu, Viorel	MagnaFy: the use of iron oxide nanoparticles enabling MRI visibility of medical devices	Aachen, Germany
173	Sadeghiani, Neda	Effects of Magnetohyperthermia on Ehrlich Solid Tumor	Brasilia, Brasil
174	Saez-Fernandez, Eva	Multifunctional magnetic nanomedicine based on poly(e-caprolactone)	Granada, Spain
175	Safarikova, Mirka	Magnetic affinity starch adsorbent for the purification of cyclodextrin glucanotransferase	Ceske Budejovice, Czech Republic
176	Schaetz, Alexander	Homogeneous Catalysts immobilized on Carbon Coated Cobalt Nanoparticles	Zurich, Switzerland
177	Scheucher, Elisabeth	Magnetic optical sensor particles for pH measurement	Graz, Austria
178	Schneider, Thomas	Towards stem cell sorting by differential surface marker expression	Cleveland, OH, USA
179	Schreiber, Eveline	Comparison of Mitoxantrone and Nanoparticle Distribution after Magnetic Drug Targeting in an ex vivo Bovine Artery Model	Erlangen, Germany
180	Schrittwieser, Stefan	Optical relaxation measurements of novel hybrid nanoparticles for homogeneous biosensing	Vienna, Austria
181	Seino, Satoshi	Surface Modification of Gold/Iron-oxide Composite Nanoparticles by Phosphorylcholine Groups	Osaka, Japan
182	Silva, Gabriela	Synthesis and application of manganese dioxide coated magnetite for removal of As(III) from contaminated waters	Belo Horizonte, Brazil
183	Sinn, Irene	Fabrication and Characterization of Morphologically and Magnetically Uniform Janus Microspheres	Ann Arbor, MI, USA
184	Sobik, Martin	Stable suspensions of Fe-filled carbon nanotubes	Enschede, The Netherlands
185	Socoliuc, Vlad	Biocompatible water based magnetic nanofluids: colloidal stability investigations by light scattering methods	Timisoara, Romania
186	Sorokina, Olga	Estimation of the Oblongness of Aggregates of Magnetic Particles Formed in Static Magnetic Field Using ESR spectroscopy	Moscow, Russia
187	Stanca, Sarmiza	Fluorescent magnetic nanoparticles for the delivery of biomolecules	Jena, Germany
188	Stolarczyk, Jacek	Directed assembly of magnetic nanoparticles for biomedical applications	Dublin, Ireland
189	Stolbov, Oleg	Field-controlled deformations and volume changes of magnetopolymeric microcapsules	Perm, Russia
190	Strbak, Oliver	Metabolites signal changes induced by magnetosomes during MR experiment	Brno, Czech Republic
191	Subramanian, Natesan	Formulation development and In-vitro characterization of chitosan magnetic nanoparticles containing artesunate for targeted delivery to breast cancer	Tamilnadu, India
192	Sudha, Vishnubhotla	Hard Magnetic Barcode nanowires for biosensing applications	Daejeon, South Korea
193	Tang, Tao	Biocompatibility of bacterial magnetic particles in mice	Beijing, China
194	Tasci, T. Onur	Utilization of AC and DC magnetic fields for focused magnetic fluid hyperthermia and magnetic particle fractionation	Salt Lake City, UT, USA
195	Thanh, Nguyen TK	Synthesis and Characterization of Magnetic Nanoalloys from Bimetallic Carbonyl Clusters	London, England
196	Thanh, Nguyen TK	Tracking transplanted neural progenitor cells in spinal cord slices by MRI using CoPt nanoparticles as a contrast agent	London, England
197	Thom, Kathleen	Preliminary investigations on labelling of the vaccine adjuvant Al(OH)3 with Resovist® for magnetic resonance tracking	Greifswald, Germany
198	Tietze, Rainer	Distribution pattern of chemotherapeutics: Distinctions of systemic application versus magnetic nanoparticle guided delivery in vivo	Erlangen, Germany

8th International Conference on the Scientific and Clinical Applications of Magnetic Carriers - Rostock, Germany

Poster Presentations (in alphabetical order of the first author)

#	First Author	Poster Title	City, Country
199	Tomasovicova, Natalia	Radiation stability of the PEG stabilized biocompatibile magnetic fluid	Kosice, Slovakia
200	Torres, Teo	Magnetic core-shell nanoparticles as MRI contrast agents: biodistribution in an in vivo animal model	Zaragoza, Spain
201	Torres, Teobaldo	Influence of the average size and polydispersion on the Specific Power Absorption in CoFe2O4 Nanoparticles.	Zaragoza, Spain
202	Tsedev, Ninjbadgar	Water Dispersible Magnetic Nanoparticle Clusters and their Application as Contrast Agent	Dublin, Ireland
203	Vega Alvarez, Sascha	In vivo Nanoparticle Toxicity Trials in Drosophila	Mayagüez, Puerto Rico
204	Veintemillas-Verdaguer, Sabino	Production of iron oxide carbon nanocomposites by laser pyrolysis: Application as MRI contrasts	Madrid, Spain
205	Veverka, Miroslav	Magnetic heating experiments with nano-crystalline Co0.4Zn0.6Fe2O4	Prague, Czech Republic
206	Veverka, Pavel	Extracellular and intracellular magnetic heating by core-shell La0.75Sr0.25MnO3@SiO2 nanoparticles	Prague, Czech Republic
207	Victora, Randall	Optimization of Magnetic Anisotropy and Applied Fields for Hyperthermia Applications	Minneapolis, MN, USA
208	Vilos, Cristian	Design and synthesis of magnetite- and antibiotic-loaded Poly(3-hydroxybutyric acid-co-hydroxyvaleric acid) (PHBV) super paramagnetic nanoparticles.	Santiago, Chile
209	Visbal-Onufrak, Michelle	A Parametric Study of Specific Absorption Rates in Magnetic Nanoparticles for Magnetic Fluid Hyperthermia	Mayagüez, Puerto Rico
210	Vlaskou, Dialechti	Magnetic microbubbles as mediators of gene delivery through Ultrasound-activation	Berlin, Germany
211	Vojtisek, Martin	DNA hybridisation on magnetic particles in continuous flow	Hull, England
212	Volmer, Marius	Improving the detection sensitivity of magnetic micro beads by spin valve sensors	Brasov, Romania
213	Voltaire, Efim	Treatment of purulent wounds using suspensions of magnetite microparticles.	Sukhum, Abkhazia
214	Wagner, Kerstin	Synthesis and investigation of cell interaction of magnetic nanoparticles with catechol-containing shells	Jena, Germany
215	Waleczek, Martin	Iron-Boron Nanowires for Applications to Boron Neutron Capture Therapy	Hamburg, Germany
216	Wang, Feng	Fabrication of Functionalized Magnetic Mesoporous Silica Nanoparticles for Laccase Adsorption and Biocatalysis	Beijing, China
217	Wang, Hanjie	Multifunctional Magnetic Polymeric Liposomes for the Delivery of Anticancer Drug Paclitaxel	Tianjin, China
218	Wang, Miao	A novel approach of transferring oleic acid capped iron oxide nanoparticles to aqueous phase	Xi'an, China
219	Weis, Christian	Magnetic resonance imaging of tumor cell migration in animals	Erlangen, Germany
220	Williams, Stephen	Refinement of magnetic nanoparticle drug carriers using the mechanism of quadruple magnetic field-flow fractionation	Cleveland, OH, USA
221	Wotschadlo, Jana	Short-term application of magnetic core-shell nanoparticles – Effect on immune cells	Jena, Germany
222	Yang, Hong-Chang	Immumagnetic assay of bio-targets using bio-functional magnetic nano-particles and high-Tc SQUID-detected nuclear magnetic resonance	Taipei, Taiwan
223	Yang, Shieh-Yueh	Immunomagnetic reduction assay on chloromaphenicol extracted from shrimp	Taipei, Taiwan
224	Yoshida, Takashi	Nonlinear Behavior of Magnetic Fluid in Brownian Relaxation	Fukuoka, Japan
225	Zablotskii, Vitalii	High-Field Gradient Permanent Micromagnets for Targeted Drug Delivery with Magnetic Nanoparticles	Pamplona, Spain
226	Zborowski, Maciej	Magnetic pressure as a scalar representation of field effects in magnetic suspensions	Cleveland, OH, USA
227	Zhang, Jixi	Core-shell structured mesoporous /superparamagnetic composite spheres for targeted thrombolysis	Shanghai, China
228	Zhiqiang, Yang	Detecting Magnetically Modulated Fluorescent Probes In Turbid Media	Clemson, SC, USA

3-Dimensional Focusing of Magnetic Particles in a Microfluidic Chip

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Biochemically functionalized magnetic particles may be used for analyte capture and purification in microfluidic lab-on-a-chip systems[1]. In particular, the switchable magnetization of superparamagnetic particles offers interesting options for particle retention and manipulation on-chip[2-3] . A detection method based on a cytometric principle, like the discrimination and high throughput counting of single particles in a microfluidic flow, presents an interesting statistical approach for analyte detection and quantification in modern bio-assays. Using such optical methods, which mostly are based on laser light coupling into a microchannel and scattered light detection, requires a highly focused particle stream. For accurate detection, we present here a novel on-chip magnetic manipulation device, allowing 3-dimensional focusing of magnetic particles in a microfluidic chip by combining magnetic and fluidic forces.

A schematic view of our microfluidic chip, comprising two microfluidic channels and two magnetic micropoles positioned close to the channel walls, is shown in Fig. 1a. The microfluidic channels are made from polydimethylsiloxane (PDMS). The magnetic system consists of an external coil that magnetizes a pair of soft magnetic poles VACOFLUX 50 (cross-section of 50x100 μm^2 at the tip end) through a magnetic yoke. The tip positioning is asymmetric with respect to the channel walls. These magnetic tips focus the magnetic field across the microchannel in a highly confined area. The 3-D focusing protocol starts with the capture of a well-defined amount of magnetic particles in a localized area on one of the channel sidewalls (dosing). Controlled demagnetization of the tips releases the plug forming a confined stream of individual particles. This is schematically shown in Fig. 1b. Due to the symmetric field configuration in the z-direction, the stream of particles is aligned at half of the channel height. The secondary flow from a side channel entering the main channel further downstream pushes the particle stream to the middle of the channel (y-direction). Fig. 1c shows a microscopic image of the released particles stream (flow rate 0.6 mm/sec, particle size 1 µm). Confocal microscopic imaging confirms that the particles are focused within 10 % of the centre position of the channel.

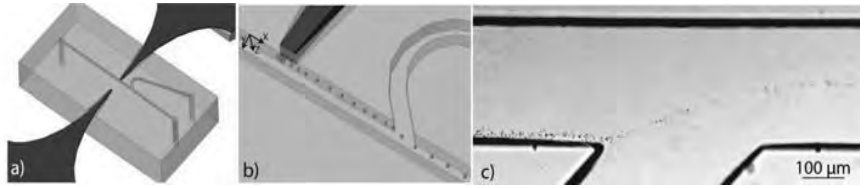

Figure 1: a) Schematic microfluidic chip with a pair of soft-magnetic poles adjacent to the microfluidic channel (b) Schematic view of the release of a magnetic bead plug and the formation of a focused particle stream; (c) photograph showing the deviation of the focused particle stream (particle size 1 µm, channel width 150 µm) towards the middle of the main channel after introduction of a secondary flow via a side channel.

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Poster 1

Sensitive high frequency AC susceptometry of magnetic nanoparticle systems

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AC susceptometry is used in many applications concerning magnetic nanoparticles such as magnetic biodetection [1], magnetic hyperthermia [2] or studies of particle stability [3]. In order to gain as much knowledge as possible of a magnetic nanoparticles system, it is of great importance to both have high magnetic sensitivity and high frequency excitation to cover all the magnetic relaxations that are present, for instance the Brownian and Néel relaxation. We have earlier showed the results of the highly sensitive DynoMag® system, which is capable of measuring dynamic magnetic properties from a few Hz up to 250 kHz. In order to reach the Néel relaxation frequencies for small maghemite particles, below app. 10 nm, we started to develop a high frequency AC susceptometer within the framework of the European project, FP7-214137-Nano3T. Today, this instrument is capable of measuring dynamic magnetic properties from 25 kHz to 10 MHz with high magnetic sensitivity. In combination with the DynoMag® system we can thus cover the frequency range from a few Hz up to 10 MHz with high sensitivity. One example of measurements where we used both AC susceptometers (from 10 Hz to 250 kHz using the DynoMag® system and from 25 kHz to 10 MHz using the high frequency AC susceptometer) can be seen in figure 1. From the results we can fit the data to a single-core Debye model and the results of the particle size (11.5 nm) are well matched with VSM analysis (11.2 nm). In the presentation we will give more examples of applications and present the key-points in order to develop a high frequency range AC susceptometer based on induction technique with a high sensitivity.

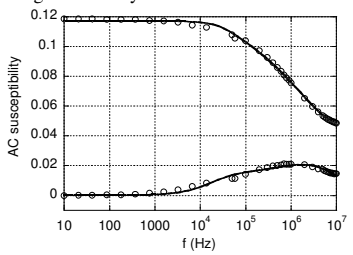

Figure 1. AC susceptibility of magnetic nanoparticles of maghemite dispersed in water versus frequency, from 10 Hz to 10 MHz using the two AC susceptometers. There is a good agreement between the two measurements. The solid line represents the experimental data fitted to a single-core Debye model.

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Poster 2

Recent Advances and Fundamentals of Magnetically Targeted Aerosol Particles in the Airways

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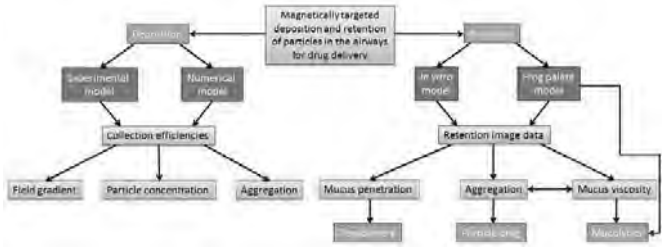
From a fundamental perspective to develop a targeting system for aerosolized magnetic particles in the lung, the issues of particle deposition and retention need special attention. Unlike delivery of magnetic particles in circulatory system the particles must be captured from a gaseous phase flowing in relatively large ducts rather than liquid flow in narrow veins. This means that collection efficiencies will be significantly different between the two systems. Furthermore, the airway clearance mechanism is designed to remove foreign particles, but there is no such defense mechanism in the circulatory system. This presents a challenge which requires understanding of particle aggregation at the target site and drag force operating on individual particles and aggregates before a robust clinical solution can be developed. The figure below provides the structure of this talk which will present our findings from investigations using *in vitro* and *ex vivo* models as well as numerical studies to better understand the targeting requirements in the airways via the inhalation route.

Our findings regarding deposition indicate that a simplified numerical approach will be sufficient to emulate the experimentally observed deposition with reasonable accuracy, i.e. collection efficiencies are found to be within 12% of each other. To maximize the collection efficiency, particles should be removed from all regions of the aerosol flow, e.g. by applying the magnetic field from various directions. The other key finding was that due to clearance mechanism of the lung, simulated by an adapted frog palate model, particle retention requires stronger magnetic forces than deposition, so the focus of development should be on retention. The frog palate model also proved to be a useful technique as in conjunction with *in vitro* experiments, it was shown that particle retention at two levels is dependent on the viscosity of the mucus: (1) Viscosity affects the drag force exerted on the particles/aggregates, i.e. driving force to remove the particles from target site, at the mucus lining the airways. (2) Indirectly viscosity also affects the magnetic field needed to retain particles, since larger aggregates need smaller magnetic force to be retained, and larger aggregates are formed when viscosity is lower. Application of mucolytics was found to be very helpful for retention of particles at target site as it considerably lowers the mucus viscosity or can disrupt clearance mechanism in case of N-acetylcysteine. Our recent findings regarding the effect of surfactants on particle drag show that surfactants greatly increase drag force, and mixed surfactants (such as lung surfactants) can particularly limit particle aggregation which can hinder retention at target site.



Poster 3

Magnetic Carriers Translocation in Microfluidics

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Abstract: We have designed, fabricated and demonstrated a micro system for controlled translocation and detection of functionalized magnetic beads using lithographically patterned soft magnetic microstructures attached with ultrasensitive Planar Hall Resistance sensor. The main aim of our micro system is to provide a novel method and corresponding device for transportation of functionalized magnetic beads on to the Planar Hall Resistance sensor surface for single magnetic bead detection. The patterned soft magnetic NiFe pathways can generate different stray magnetic fields due their structural geometry when they are subjected to the external rotating magnetic field. The inhomogeneity of the stray magnetic fields in the NiFe pathways can attract the partially magnetized magnetic beads and govern the translational back and forth magnetic bead motion, when the external magnetic field rotates in clock wise and counter clock wise direction.

The magnetic beads of 2.8 μm size (Dynabead® 280) that were placed on the NiFe pathways in the fluidic chamber are shown to be transported on to the Planar Hall Resistance sensor and subsequent single magnetic bead detection is performed. The device is usable to transport functionalized micro beads for sensitive detection of biochemical reactions or assay for future integrated lab-on-a-chip systems for medical diagnostic purposes. The micro system mainly points to application in magnetic biochips used in diagnosis, and molecular studies, and can be applicable whenever there is a need of performing nanoscale or microscale transport.

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Poster 4

Mössbauer characterization of magnetite/polyaniline magnetic nanocomposite

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Aniline surface coated Fe_3O_4 nanoparticles has been successfully synthesized by UV irradiation varying the time and the acid media (HCl, HNO_3, or H_2SO_4). XRD (Figure 1) patterns are consistent with the crystalline structure of magnetite. Nevertheless, for UV irradiation time longer than 2h, extra XRD lines reveal the presence of goethite. The mean crystallite size of uncoated particles is estimated to be 25.4 nm, meanwhile that size is reduced to 11.2 nm for the UV irradiated sample in HCl medium for 4h. Mössbauer spectra of uncoated nanoparticles reveals the occurrence of thermal relaxation at room temperature meanwhile, the 77K-Mössbauer spectrum suggests the occurrence of electron localization effect similar to that expected in bulk magnetite (Figure 2). The Mossbauer spectra of UV irradiated sample in HCl medium during 4h, confirms the presence of the goethite phase. For this sample, the thermal relaxation is more evident, since the room temperature spectrum shows larger spectral area for the nonmagnetic component due to the smaller crystallite size. Meanwhile, the 77K-Mössbauer spectrum suggests the absence of the electron localization effect above 77K.

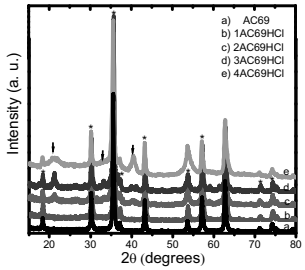

Figure 1

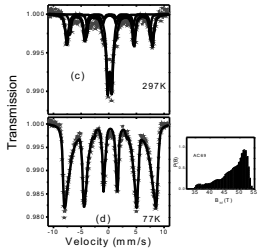

Figure 2

Poster 5

Formulation and characterization of maghemite/poly(D,L-lactide-*co*-glycolide) (core/shell) nanoparticles

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Introduction: Magnetically controlled drug targeting is currently one of the most promising possibilities to actively (selectively) deliver anticancer agents to the tumor site. This means that the drug carrier will be guided to specific cells in a manner that differs from its normal biodistribution, selectively delivering antitumor molecules to the diseased site without a concurrent increase in its level in healthy tissues. With the aid of a magnetic field, those magnetic colloid can improve the outcome of chemotherapy by allowing: *i*) the maximum fraction of the delivered active agent to react exclusively with the cancer cells without adverse effects to the normal cells; and *ii*) preferential drug distribution to the cancer cells, limiting systemic drug concentrations and, importantly, avoiding normal tissue clearance [1, 2]. In this work, we describe the synthesis and characterization of magnetic nanoplatforms composed of a magnetic core of maghemite (γ-Fe_2O_3) and a shell of the biodegradable poly(D,L-lactide-*co*-glycolide) (PLGA).

Materials and methods: Nanomaghemite was prepared by oxidation of nanomagnetite (mean diameter: 12 ± 3 nm) at 90º C for 30 min by ferric nitrate (600 mL, 0.34 M) [3]. The synthesis of maghemite/poly(D,L-lactide-*co*-glycolide) (γ-Fe_2O_3/PLGA) (core/shell) nanoparticles was carried out by following the emulsion solvent evaporation technique [4], except that the aqueous phase was a γ-Fe_2O_3 suspension (0.3 %, w/v). Briefly, a water-in-oil emulsion was prepared by mixing (19500 rpm, 30 min) an appropriate volume of a γ-Fe_2O_3 aqueous suspension (0.3 %, w/v) containing polyvynil alcohol (0.3 %, w/v) with 10 mL of a 1 % (w/v) PLGA in ethyl acetate. The ethyl acetate content was reduced to a very minimum by using a rotary evaporator. The suspension of magnetic nanocomposites was then subjected to a cleaning procedure that included repeated cycles of magnetic sedimentation and redispersion in water. The presence of the shell, the particle size and the width of the size distribution were determined by Photon Correlation Spectroscopy (PCS) and High Resolution Transmission Electron Microscopy (HRTEM). Fourier transform infrared spectrometry data were used for the characterization of the chemistry of the three types of particles (γ-Fe_2O_3, PLGA and core/shell nanoparticles). The surface electrical properties were analyzed by means of zeta potential (ζ) determinations as a function of pH and ionic strength. The surface properties of the composite particles were studied by contact angle measurements of standard liquids (water, formamide, and α-bromonaphthalene) on pellets of the particles.

Results and discussion: Following the synthesis routine, γ-Fe_2O_3/PLGA nanoparticles of suitable and moderately monodisperse size were obtained (mean particle size: 140 ± 15 nm). The infrared spectra of the three types of particles demonstrated that all the bands of the polymer are present in the spectrum of the composite particles, a clear indication that the shell observed in HRTEM was indeed PLGA. The efficiency of this coating was first demonstrated by comparing the zeta potential (ζ) of core/shell particles with those of the nucleus and of pure polymeric particles (also synthesized for this work). In fact, the zeta potential (ζ)-pH and ζ-ionic strength trends of the magnetic composites were similar to the ones for PLGA and clearly different to the ones of the magnetic core, this suggesting the efficiency of the coating. The fact that the surface properties of the γ-Fe_2O_3/PLGA nanoparticles mimic those of the polymer was further confirmed by thermodynamic analysis. We demonstrated the similarities between the polymer and magnetic core/shell nanoparticles, and the differences with the magnetic core: the originally hydrophilic γ-Fe_2O_3 changes to hydrophobic when coated by PLGA.

Conclusions: In this work we have shown that it is possible to reproducibly coat nanomaghemite with a shell of poly(D,L-lactide-*co*-glycolide). Although the existence of the polymeric shell is observable under the electron microscope, the efficiency of the coating was demonstrated by the chemical characterization and the surface analysis of the γ-Fe_2O_3/PLGA nanoparticles compared to that of their components.

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Poster 6

Biodistribution study of magnetic and fluorescent nanoparticles within dendritic cells and their effect in the maduration stage

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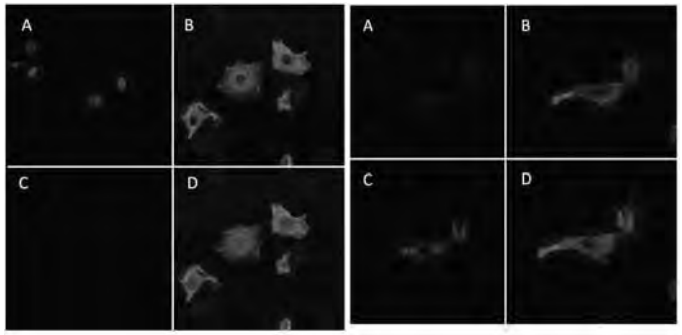
Dendritic cells (DCs) play a crucial role initiating the immune adaptative response mediated by T cells. DCs reside in peripheral organs in an immature state, characterized by their high capacity to incorporate a wide array of antigens. Once they incorporate and process the antigens, maturation process starts carrying on several changes at phenotypical and functional level. Mature dendritic cells migrate to lymph nodes to activate T cells that recognize the antigens. More recently, it has been described that DCs are able to incorporate not only biologic antigens, but inorganic compounds has been uptaken by these cells. This ability to incorporate several kinds of antigens and/or particles presents these cells as an optimal target for transport, vectorization and delivery studies. Nanotechnology has appeared in the last years as an alternative method to improve therapeutic, diagnostic and prognostic protocols. Magnetic NPs (MNPs), due their physical and chemical properties, are often used in biological studies.

We have studied a set of imaging and microscopy techniques, as transmission and scanning electron microscopy and confocal microscopy to visualize NPs uptaken by DCs, using two commercially available (Micromod GmbH) samples: magnetic and fluorescent nanoparticles, both with hydrodynamic radius of 250nm. Energy-dispersive x-ray spectroscopy (EDX) analysis was performed in order to detect iron within the cells. With regard to the maturation of the dendritic cells we have studied the uptake nanoparticles capacity to induce it, to that end, expression of membrane molecules (CD14, CD40, CD83, CD86, CD1a, DC-SIGN) have been studied by fluorescent activated cell sorting (FACS) resulting in a nanoparticles concentration dependent maturation stimulus.



Confocal images of DCs (left) and DCs with NPs (right). A- nucleus died with DRAQ5, B- Tubulin, C- Red channel: NPs are marked with a Rhodamine derivate and D- overlay

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Cell Internalization of Magnetite Nanoparticles by Dendritic Cells for Intracellular Magnetic Hyperthermia.

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Magnetic nanoparticles (MNPs) are being increasingly used for targeting eukaryotic cells as a way for labeling,[1], assessment of cytotoxicity [2] or DNA transfection protocols. [3] Targeting specific tumor sites with drug-loaded MNPs is also a promising strategy to fight proliferation of metastatic cells, but it is now recognized that the reticulo-endothelial system (RES) is a major obstacle since it detects and phagocytes NPs, preventing their therapeutic function. Dendritic cells (DCs) obtained from myelomonocytic progenitors, and primed with tumor antigens have shown antitumoral activity when inoculated in animal models [4] and humans [5].The injection of MNP-charged DCs into the blood system appears as a valuable MNP-delivery strategy for tumor targeting, since the cargo could be delivered inside a 'biological' unit from the same organism and therefore no RES action against these carriers is expected. The MNPs vectorized in this way could be used as heating agents for magnetic hyperthermia (MHT) therapy, and efficiently kill tumors to which a sufficient numbers of MNPs have been delivered. We present experiments on the internalization of magnetite-based MNPs into dendritic cells in order to assess a) the final location of the particles; b) the viability of the cultured cells from their mononuclear cells progenitors, and c) the effectiveness of alternating magnetic fields as source of intracellular heat for MHT *in vitro* protocols. We found that magnetite nanoparticles of ca. 12 nm covered with dextran and both $COOH^-$ and NH_2^+ on their surface were efficiently incorporated by DCs, showing minimum effects on the DCs viability, i.e., the fraction of viable cells on days 0,1,2,3 and 4 after NPs incorporation was not significantly affected by the internalization of Fe_3O_4 NPs. After separating the MNPs/loaded cells by centrifugation in density gradient, the resulting material was analyzed by electron and confocal microscopy and magnetic measurements. It was found that NPs are internalized in lysosomes, providing a large magnetic signal. A simple method using room temperature magnetometry was applied to quantify the incorporation of MNPs into DCs. Using commercial Fe_3O_4 nanoparticles (Micromod GmbH) of 12 nm, we found that mature DCs are able to incorporate magnetic material in the range of 1-10 pg/cell after 24 h of incubation, depending on particle surface. Applying 30 minutes of AC magnetic field (260 kHz, 16 mT) on magnetite-charged DCs cells resulted in 40 % of cell death, as reflected by flow cytometry (anexine-propidium iodide staining) analysis. Our results suggest that loading DCs with proper number of MNPs could be a promising strategy for improved vectorization and hyperthermia therapy in cancer treatment.

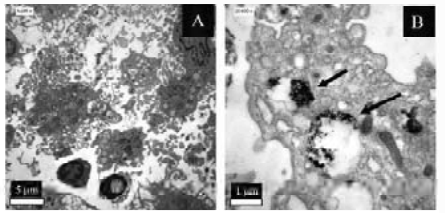

Figure 1 *Electron microphotographs of DCs without (A) and with (B) MNPs. Intra-lysosomal NPs aggregates inside lysosomes are observed (black arrows). Without NPs, the content of lysosomes is seen as homogeneously white.*

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The use of high frequency AC susceptometry in magnetic hyperthermia

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Magnetic hyperthermia using magnetic nanoparticles is a rapidly growing research area for cancer treatment [1] and today, clinical trials are carried out with promising results. Magnetic hyperthermia using magnetic nanoparticles relies on the fact that the particle system absorbs heat when it is subjected to an alternating magnetic field at a specific frequency. The amount of heat absorbed depends on the magnetic loss of the magnetic nanoparticle system. In order to optimize the heat absorption of the nanoparticle system the size of the particles has to be fitted to the used hyperthermia frequency and the size distribution has to be as low as possible. The magnetic nanoparticle systems have to be analyzed using vibrating sample magnetometry (VSM), transmission electron microscopy (TEM) and specific loss power (SLP) measurements. In order to determine the magnetic loss spectra in a large frequency range for a nanoparticles system it is very straightforward to use high frequency AC susceptometry that covers the hyperthermia frequency range. From the AC susceptometry data (the magnetic loss e. g. the imaginary part of the AC susceptibility) can be used to estimate the SLP value at a specific frequency and field amplitude [2]. Within the European project, FP7-214137-Nano3T on hyperthermia, we have developed a sensitive high frequency AC susceptometer. With this system we can calculate the SLP values at a specific frequency in the frequency interval between 25 kHz and 10 MHz. In table 1 below we have listed the calorimetrically measured SLP values using a high field- and frequency generator system (410 kHz, with field amplitudes 11 kA/m and 24 kA/m) and the estimated SLP values from the AC susceptometry analysis for g-Fe2O3 particles with average diameter of 11 nm and small dispersity. As can be seen there are discrepancies between the two methods when determining the SLP values, especially at high field amplitudes. This can be explained by the fact that the estimation from the AC susceptometry data relies on a linear field dependency of the magnetization and the hysteresis loop is elliptically shaped. None of these statements are valid at higher fields. Also the magnetic loss spectrum is temperature dependent. In the presentation we will show how to compensate for this in order to estimate better SLP values from AC susceptometry data.

Table 1. Measured SLP values in W/g, using the high field generator system and estimated SLP values from AC susceptometry data for the four magnetic nanoparticle systems.

Sample	Measured @ 11 kA/m	Measured @ 24 kA/m	Estimated @ 11 kA/m	Estimated @ 24 kA/m
1	97	250	166	792
2	96	242	170	809
3	72	200	116	550
4	72	197	120	577

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Arsenic Removal from Water by Magnetic Separation: A Review and a Case Study Using Magnetic Aggregates and Magnetic Stabilized Bed

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Abstract

Arsenic is an important element, specially when focusing the health issues, due to its toxicity and carcinogenic characteristics. Arsenic is also an important contaminant of water, specially in the water used for human consumption, and, if in-taken continuously, represents an hazard for public health. In fact, it is well-known that high levels of arsenic in drinking water lead, in long-term, to skin diseases and to skin, lungs, kidneys and bladder cancer.

Several processes to achieve the elimination of arsenic from water, were developed and applied, being the main ones based on precipitation-coagulation techniques, separation by membranes, ion-exchange processes, and adsorption; each one of these methods presents advantages and disadvantages which will be reviewed and detailed in this paper.

We have developed a new process technology for arsenic removal, based on the adsorption of arsenic by magnetic aggregates that are stabilized in a Magnetic Stabilized and Fluidized Bed. In this paper details of this process and a description of the results obtained so far, will be given.

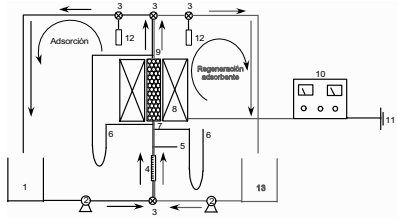

Figure 1 - MSFB experimental setup used for the removal of arsenic by stabilized magnetic aggregates.

Aggregate structure in biocompatible aqueous magnetic fluids with steric and electrostatic stabilization

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Most of biomedical applications of magnetic fluids (MFs) require magnetic nanoparticles to be non-toxic, chemically stable, uniform in size and well-dispersed in aqueous media. Besides the several unique problems of particular applications, the colloidal stability of MFs is critical in general, i.e stabilization of magnetic particles under physiological conditions is of great practical importance. Particle aggregation must be excluded in magnetic field during application with the reference to the risk of clots in blood vessel. For water (the basis of biological media) the synthesis of systems with individual magnetic particles coated by a surfactant shell or ionic molecules, which would provide the steric or electrostatic stabilization, respectively, in physiological conditions, is still a problem. Despite comparatively high volume fractions of magnetic material (φ_m>1%), which can be dispersed in these systems with keeping relatively long-term stability, the systems are often not free of aggregates.

In the presented work we compare two classes of water-based MFs with respect to aggregate structure. In the first case (type 1), the stabilization of magnetite nanoparticles (characteristic size of about 10 nm) is achieved by purely sterical stabilization using double layers of short mono-carboxylic (lauric and myristic) acids [1]. In the second case (type 2) magnetite nanoparticles are coated by ionic (citric and polyacrylic) acids [2]. The aggregate presence is detected by dynamic light scattering, while their inner structure is analyzed by small-angle neutron scattering (SANS) with application of the contrast variation (H_2O/D_2O mixtures, Fig.1). The principle difference in the density of aggregates, as well as in water access to magnetite for the two stabilization types is revealed.

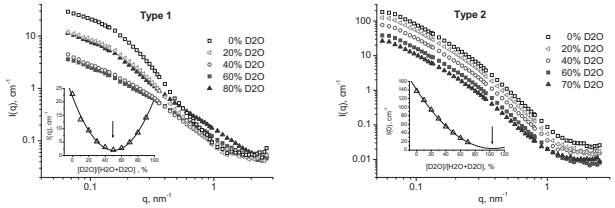

Fig.1. SANS curves at several contrasts for two types of MFs. Insets show changes in forward scattering intensity and match points (arrows).

The work has been done in the frame of the project Helmholtz-RFBR (HRJRG-016). The research was also supported by the European Commission under the 6th Framework Program through the Key Action: Strengthening the European Research Area, Research Infrastructures. Contract nr: RII3-CT-2003-505925, GKSS, Germany and by the Romanian Authority for Scientific Research through the *Nanomagpoli* research project.

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Poster 11

Design of magnetic collagen gels and their effect in cell behavior

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Tissue engineering strategies are used to enhance the native ability of the body to heal. Many strategies are based on the use of biomaterial scaffolds to provide mechanical support and promote cell infiltration, combined with the delivery of tissue inductive factor and cell transplantation. It is known that the microenvironment to which cells are subjected regulate their structure and function. Cells are able to probe the properties of the substrate and translate this into chemical signals that impact cell function. Moreover, cells are capable of depositing extra cellular matrix proteins to modulate the properties of the substrate, thus altering their microenvironment. However, in vitro cell culture models do not to take into account the dynamic properties of the substrate that cells experience in vivo. The ability to change the properties of the microenvironment as a function of time can lead to enhanced culture systems. This is promising for stem cell culture, which can be directed to differentiate into a specific cell types and then transplanted for functional recovery. Stem cell differentiation is carried out by the addition of soluble factors in the culture media that direct cell fate. However, the mechanical factors are important and are not usually looked upon. With this in mind we are incorporating magnetic particles within a hydrogel for cell culture applications. By subjecting the culture system to an external magnetic source we can impart localized forces in the cell vicinity. The magnitude of the magnetic field can be varied over time to study the dynamic effects of localized forces on cell behavior. In order to do this we have incorporated magnetic microparticles within collagen gels. The properties of the gels and the magnetic particle content are varied within the system. Static studies are carried out seeding the cells on the gels with a permanent magnet placed close to the cells during culture. Initial studies were carried out with fibroblast cells evaluating cell survival and morphology using a stain for actin filaments. Studies of cell survival, proliferation, and morphology will be presented for fibroblast and mouse embryonic stem cells.

Poster 12

Surface coating dependent selection of Superparamagnetic Nanoparticles for whole cell isolation

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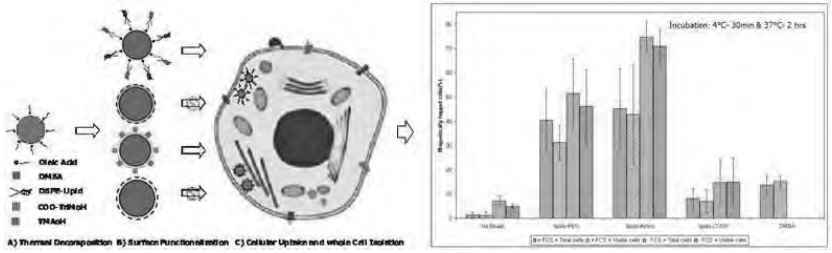
Superparamagnetic nanoparticles (SPMNPs) are inorganic nanocrystals characterized by superparamagnetic properties, high surface to volume ratio and size controlled physical properties which can be fine-tuned depending on the application of interest. Nanoparticle-cell interaction play key role in applications such as hyperthermia, imaging, drug delivery, whole cell and subcellular compartmental isolation. In particular for whole cell and subcellular manipulations, nanoparticle-cell surface interaction is the critical step and is mainly governed by three major physiochemical properties such as size, shape and surface coating. Hence it is crucial for SPMNPs to retain its intrinsic properties during the cellular interaction and thereby making these SPMNPs feasible for whole cell and subcellular manipulation. Here we present surface coating dependent selection of SPMNPs for whole cell and sub-cellular isolation.



In this study, SPMNPs of size Ø10nm have been synthesized by thermal decomposition method. Obtained SPMNPs were monocrystalline Fe_3O_4 with narrow size distribution and superparamagnetic (no hysteresis) with high mass magnetization value (~60emu/g). However SPMNPs were hydrophobic due to oleic acid coating. Hence SPMNPs were functionalized with various water-dispersible surface coating by using two alternative functionalization methodologies: a) *Ligand exchange*: DMSA, TMAOH & COO-TriMAOH were used to replace oleic acid coating. b) *Ligand addition*: DSPE-Lipids were introduced on top of oleic acid coating. Functionalized SPMNPs were further characterized for its physical properties showing retention of superparamagnetic properties and slight increase in size characterized using dynamic light scattering (DLS). Further, DMSA and DSPE-Lipid functionalized SPMNPs showed medium stability without any change in size-dependent physical properties, while TMAOH and Carboxyl-TriMOH coated SPMNPs showed high instability by agglomerating in medium. Thus biocompatibility based selection of DMSA and DSPE-Lipid coated SPMNPs was performed for whole cell isolation application in HeLa cells. Interesting, we also observed surface coating and time -dependent variation in SPMNPs cellular uptake and magnetic cell isolation efficiencies. As a future perspective due to its unique surface functionality, these SPMNPs show great potential for subcellular compartmental isolation like plasma membrane and endosomes.

Small angle neutron scattering methods in magnetic nanoparticles diagnostics

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Design, manufacture and characterization of materials are nowadays in the top of any activity connected to the development of new technologies. There is no single experimental technique that can provide us with all the information we need to know about materials. Different techniques, based on different physical processes, provide different information, and as the materials under study become ever more complex, it becomes crucial to study them using multiple, complementary experimental technique.

The great penetrating power of the neutron makes it a powerful probe of the microscopic nature of condensed matter which reveals key insights [1-5]. Small angle neutron scattering is an effective method of studying fundamental as well as technological problems in the nanoparticle systems research. Also, as the values of nuclear and magnetic scattering cross sections are of comparable magnitude, the neutron scattering is providing an ideal tool for measuring both the atomic and magnetic structure of the matter.

In this presentation, we discuss some aspects of using small angle neutron scattering techniques in the study of magnetic nanoparticles dispersed in liquid and polymeric matrix.

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Synthesis of Water-Soluble Inverse Spinel Ferrites ($FeFe_2O_3$ and $MnFe_2O_4$) by a Modified Polyol Process as Contrast Agents in Medical Magnetic Resonance Imaging

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Over the last decades there has been a growing interest in the synthesis of nanophase magnetic nanoparticles because of their potential applications in many scientific and technological areas including biomedical from drug location, magnetic hyperthermia and magnetic resonance imaging. For most common applications, selection of magnetic nanoparticles is preferentially towards the use of inverse spinel ferrites such as $MnFe_2O_4$, Fe_3O_4 and $CoFe_2O_4$ because of their unique magnetic properties at nano scale sizes, their multiple functionalities and their contrast enhancement in Magnetic Resonance Imaging (MRI).

In this study, inverse spinel ferrites of $FeFe_2O_3$ and $MnFe_2O_4$ were prepared by a modified polyol route in commercially available polyethylene glycols (PEGs) with low molecular weight. The reaction takes place in the presence of pluronic-F-127, a relatively nontoxic water-soluble di-functional block copolymer surfactant terminating in primary hydroxyl groups. This polymer plays the role of morphogenetic and surface functionalized agent using $Fe(acac)_3$, $Mn(acac)_2$ and Fe(II)lactate hydrate as inorganic precursors at temperatures above 210 ^oC under reflux conditions. The crystal structure of the prepared nanomaterials confirmed by X-ray diffraction (XRD) and Fourier Transform Infra-Red Spectroscopy (FT-IR) established the surface functionalization. The particles are well dispersible in water and after exchange with water soluble andcitric acid diammonium salt, they transformed into very stable (for months) colloidal solutions. The as-synthesized magnetic particles exhibit a superparamagnetic behavior, with room temperature magnetization values 45 and 65 emu/g for the magnetite and manganese ferrites, respectively. These magnetization values are quite high when considered the small size of the particles (~ 9 nm and ~ 11 nm, respectively) and the non-magnetic layer of organic loading (~20% wt.).

Both $FeFe_2O_3$ and $MnFe_2O_4$ solutions demonstrated the capability of inducing imaging contrast in T2 weighted MRI. Such MRI contract introduced by the superparamagnetic $FeFe_2O_3$ or $MnFe_2O_4$, typically characterized as signal drops as shown in Figure 1, is attributed to the effect of shortening the transverse relaxation time (T2) of water. It appears that at a given concentration $FeFe_2O_3$ presents stronger T2 shortening effect and higher MRI contrast than $MnFe_2O_4$.This modified polyol process is found to be a simple route appropriate for the synthesis of stable ferrofluids of crystalline Mn and Fe ferrite nanoparticles with superparamagnetic behavior, suitable for biomedical applications such as contrast agents for MRI.

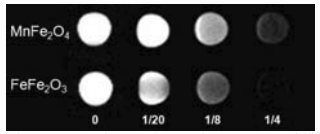

Figure 1. Both $FeFe_2O_3$ and $MnFe_2O_4$ exhibit T2 weighted MRI contrast, evidenced by inducing MRI signal drops comparing to pure water. This contrast effect is Fe concentration dependent.

Poster 15

SUSPENSIONS OF DENDRONISED IRON OXIDE NANOPARTICLES FOR BIOMEDICAL APPLICATIONS

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Superparamagnetic iron oxide nanoparticles (SPION) with appropriate surface coating have been widely used for numerous *in vivo* applications such as magnetic resonance imaging contrast enhancement, tissue repair, immunoessay, hyperthermia, drug delivery. In this field, most work has been achieved improving the materials biocompatibility but only a few investigations and developments have been carried out in improving the quality of the magnetic nanoparticles, their size distribution and their shape and in studying the effect of their functionalization on their structural and magnetic properties.

Iron oxide nanoparticles with sizes around 12 nm have been synthesized by two methods: the co-precipitation of iron chlorides by a base and by the thermal decomposition of iron stearate. After co-precipitation, the stripped iron oxide nanoparticles are stable in water suspension at pH below 5 or above 7 (NP_{cop}) (IEP = 6.8). The thermal decomposition method leads to nanoparticles covered with oleic acid molecules in an organic solvant (NP_{td}). These both nanoparticles have then been covalently coated with a hydrophilic polyethyleneglycol-based dendron having a phosphonic acid as a focal point.[1] The functionalization step has been determined to obtain stable suspension of iron oxide nanoparticles in water and osmolytic conditions.

For nanoparticles synthesized by co-precipitation, the grafting has been demonstrated to occur at pH 5 by interaction of negatively charged phosphonate groups with hydroxyl and positively charged groups at the iron oxide surface.[2] Their corresponding suspension's isoelectric point was shifted towards lower pH as the amount of dendron increased leading to stable suspension at pH = 7.[3] For nanoparticles obtained by thermal decomposition, a ligand exchange process including a phase transfer in water has been optimized/perfected.

The optimisation of grafting conditions has conducted to very stable water suspensions of iron oxide nanoparticles at pH=6.8. Both functionalized nanoparticles have been carefully characterized (XRD, TGA, IR, TEM, Elemental analysis, UV-visible, Zeta potential). The grafting step has been shown to preserve the magnetic properties of the iron oxide nanoparticles due to super-super exchange interactions through the phosphonate group.[1] Finally, the relaxation properties of the colloidal suspensions have been studied in order to evaluate the possible use of these materials as MRI contrast agents. Indeed, NMR measurements revealed significantly reduced water proton relaxation times T_1, T_2 under a 1.5T magnetic field with a quite satisfactory ratio r_2/r_1 of 25 for nanoparticles obtained by co-precipitation. These values are lower for iron nanoparticles synthesized by thermal decomposition and these have been related to their structural and magnetic properties.

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Poster 16

Biopolymeric magnetic particles for bioseparation purposes

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Gum Arabic (GA) is a highly branched polysaccharide isolated from *Acacia senegal* and *Acacia seyal* trees, widely used in the food and pharmaceutical industries. Recent studies indicate its potential in controlled drug delivery systems, carriers for the microencapsulation of oils and other bioactive molecules as well as in the stabilization and increased biocompatibility of nanostructures[1]. Magnetic nanoparticles (MNPs) can be made cheaply and easily, and coated with different polymeric materials as to increase colloidal stability, morphology and functionality. Their small size results in large surface areas per unit volume, making them ideally suited for adsorptive separations. As MNPs can be derivatised with any of the existing ligands routinely used in chromatography (affinity, hydrophobic, ion-exchange, mixed-mode, etc.), they can be applied in protein purification. In this work we explored the surface modification of MNPs with GA and related biopolymers by different routes[2] and used these materials as a platform for the creation of magnetic bioseparation supports (Figure 1)[3]. The presence of the polymers at the surface of coated MNPs (11-14 nm) was confirmed by FTIR spectra. DLS measurements registered agglomerates with different sizes depending on the biopolymer coating method. The adsorption isotherm of GA at the surface of bare magnetite followed a Langmuir model (maximum capacity of 1 g GA/g MNP). We have further modified the coated MNPs with natural (antibodies) and synthetic (biomimetic) receptors for biological molecules at high densities. These particles have shown to bind specifically to human IgG (with maximum capacities of up to 340 mg target/mg MNP and K_a values of 10^5 M^{-1}), while retaining the magnetization properties. When comparing with agarose (the traditional chromatographic matrix), functionalised particles bound six times more human IgG (in mg of protein per g of support) than agarose modified with the same ligand, which is a notable result when the manufacture costs and ease of preparation of both supports are taken into account. The best elution conditions for protein recovery were also evaluated.

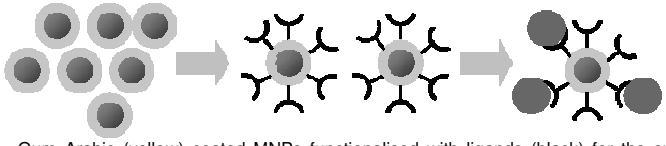

Figure 1 – Gum Arabic (yellow) coated MNPs functionalised with ligands (black) for the specific binding of target proteins (blue).

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In vitro Investigations for Magnetic Lung Drug Targeting: Influence of Particle Size of Magnetite Nanoparticles on the Deflection in Magnetic Fields

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Lung cancer is the most common cause of cancer-related deaths in the western world. Although there are drugs which are effective in vitro, they do not show satisfying results in therapy. One of the problems is to achieve therapeutic concentrations of drugs at the tumor site. The aim of our research is to accumulate effective drug doses in diseased lung regions by means of pulmonary application of superparamagnetic aerosols and high-gradient magnetic fields.

Magnetic nanoparticles (MNP) were synthesized by dissolving $FeCl_2$ and $FeCl_3$ in water. Ammonium hydroxide was added to precipitate the MNP consisting of Fe_3O_4 (magnetite) and citric acid to coat the MNP in order to prevent aggregation and sedimentation. The ferrofluid was filtrated through a 0.1 µm membrane filter to achieve two fractions of different particle sizes. The size was measured by photon correlation spectroscopy. Two different nebulizers were used to atomize the ferrofluid in order to vary the median mass diameter (MMD) of the droplets which was determined by laser diffraction. The eFlow generates aerosols using a vibrating membrane and the Pari Boy is a pneumatic nebulizer. The deflection of magnetic droplets was investigated in the magnetic field of two opposing circular disc permanent magnets (r=25 mm, l=15 mm) with a magnetic remanence of 1450 mT at a distance of 20 or 40 mm between the magnets. The resulting magnetic field gradients were calculated with Mathematica®. About 50 mg iron were sprayed into a square tube (5 x 2 cm) which was placed centrally between the two opposing magnets. The walls to the magnets were covered with paper. The paper with the deposited MNP was decomposed and the iron content was quantified by flame atomic absorption spectrometry.

The hydrodynamic diameter of magnetite in fraction 50 was about 50 nm and in fraction 120 about 120 nm. The aerosol droplet size does not depend on the particle size of MNP. The eFlow generates aerosols with a droplet size of 4 to 6 µm and the Pari Boy about 3 µm. The size decreases with increasing ferrofluid concentration. The strongest magnetic field gradients were generated at the edges of the opposing circular disc magnets. The gradients near the pole surface were up to 120 T/m. At a distance of 2 cm between the two opposing magnets a nearly homogeneous gradient of 15 to 20 T/m was achieved. The gradients decrease with increasing distance.

Atomic absorption spectrometry showed that up to 100% of iron were intercepted onto the paper. The best deposition was achieved by eFlow. This was most likely caused by the lower flow velocity compared to the pneumatic nebulizer and the large droplet size of up to 5.3 µm that is generated by the vibrating membrane. Large droplets contain more magnetite nanoparticles and are deflected easier. But this high deposition is not applicable for the magnetic drug targeting. Aerosol droplets of such a size only reach the upper airways and lead to adverse reactions due to high deposition in mouth and trachea.

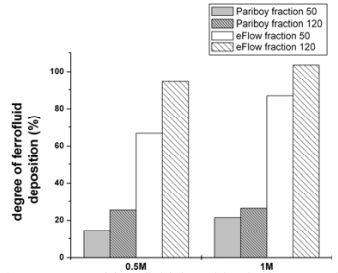

Fig. 1: Degree of ferrofluid deposition in the magnetic field at a distance of 2 cm between the magnets.

Future effort will be directed towards the generation of smaller droplets with diameters between 0.5 µm and 1 µm. In further experiments we want to generate a reproducible aerosol flow velocity by using a vacuum pump and we want to verify the reproducibility of our experimental results.

INNOVATIVE NANOPROBES FOR MAGNETIC IMMUNOASSAYS: ELABORATION AND CHARACTERIZATION

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Magnetic nanoparticles (MNPs) are commonly used for contrast enhancement in magnetic resonance imaging, as magnetic carriers in automated immunoassays instruments, or for cell concentration at the bench. They are being evaluated all over the world for drug delivery or cancer therapy by hyperthermia. More recently have MNPs been arisen as candidates for new labels in **magnetic immunoassays**[1] in lieu of commonly used enzymatic, fluorescent, or radio-isotopic labels. The advantages of magnetic immunoassay are its great sensibility, its rapidity and its easy way of use.

Our objectives are to identify promising candidates for magnetic immunoassays in order to dramatically improve sensitivity and to allow for multiparametric testing [2]. Magnisense has developed a totally unique, newly patented technology called MIAplex®[3] which measures the non-linear magnetization to characterize candidates magnetic "signatures". MNPs are used as nanoprobes for the antibody-antigen-antibody coupling, *Figure 1.A*. With the MIAplex® signature, the tested antigens can be quantified.

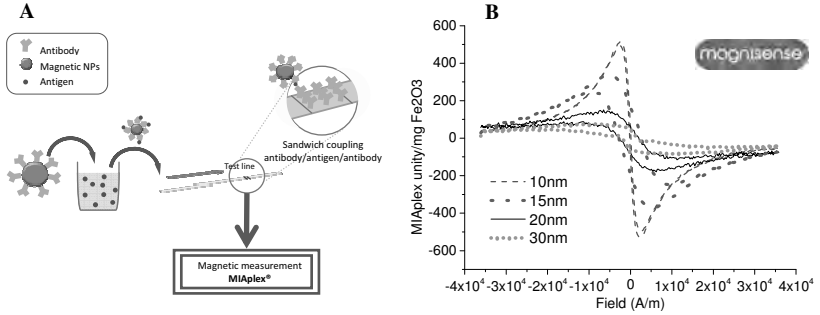

Figure 1: A. Magnetic immunoassay: principle, B. Impact of the γFe_2O_3 particle size on MIAplex® signature

In our study, ferrite nanoparticles: γFe_2O_3, MFe_2O_4, (M = Co^{2+}, Ni^{2+} and Mn^{2+}) are synthesized in water via direct micelles. The surface is functionalized either with various bisphosphonate compounds or with polymers. The impact of the interactions, modulated via composition, size and surface coating, on magnetic behaviour is studied with conventional measuring: Squid, EPR and Mössbauer spectroscopy and with MIAplex®. One example is presented *Figure 1.B*.

This study allows linking the MNPs physico-chemical properties with their magnetic behaviour and to build up a magnetic "signatures" library as to target the promising candidates for the MIAplex® technology. Those new MNPs will be extended to different biological models, leading to multiparametric testing.

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Contribution to the study of Ferrite nanobeads: Synthesis, characterization and investigation of Horizontal Low Gradient magnetophoresis behaviour and reversible aggregation

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Magnetic nanobeads are used in biological and medical research as well as in therapy. Due to the low surface/volume ratio of the nanobeads, and the unclear magnetic behaviour of the particles, commercial magnetic nanobeads have low efficiency for large volume production and potential industrial application[1][2]. These limitations difficult their use as efficient and low cost carriers for bioindustrial purification and extraction processes. Scientific bibliography shows the important labours for the improvement and the understanding of the magnetic behaviour of the magnetic particles and magnetic microspheres[3]. The reversible aggregation of the nanobeads is a key for the recycling of the magnetic particles for a repetitive use in industrial processes.

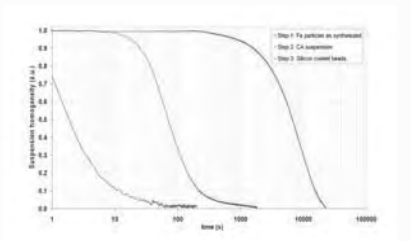

Figure 1: Monitoring of the separation process at different steps of synthesis route, using horizontal low field gradient constant magnetophoretical conditions,

In the present work we investigate the possibilities of the use of Horizontal Low Gradient Magnetic Field (HLGMF) for filtration, control and separation of the synthesized magnetic particles, considering the concentration and other characteristics of the suspension, the size and the type of nanoparticles and focusing on the scale up processes. The reversible aggregation is considered in the different steps on magnetic nanobeads synthesis. For these purpose, we synthesised nanomagnetic beads of Fe2O3-silica core-shell nanobeads by co-precipitation, monodispersion and silica coating. SQUID, STEM, SEM, FTIR, Zeta potential techniques were used to characterize the synthesised nanobeads. An extensive magnetophoresis study was performed at different magnetophoretical conditions. Different reversible aggregation times were observed at different HLGMF, at each step of the synthesis route: Several orders of magnitude differences were observed when comparing CA suspension with the final silicon coated beads. Reversible aggregation times are correlated with the properties of the nanobeads at different steps of the synthesis process.

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Production of Monosized Microspheres Using Flow Focusing

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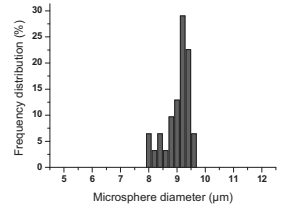

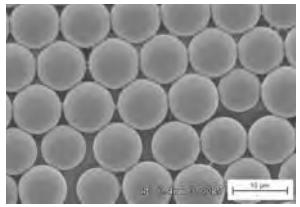

Fig. 1: Scanning electron microscopy image of the microspheres and their size distribution.

Flow focusing is a new technique for the production of monosized microspheres by incorporating the emulsification-evaporation method into a microfluidic channel system. Flow focusing gives better control over particle size, and particles produced have a narrower size distribution compared to the standard solvent extraction method. In flow focusing two immiscible fluids come in contact with each other and pass through a small orifice into a collecting chamber. At the narrowest point, droplets form. The solvent is extracted, and each droplet forms a particle.

In this paper we report the production of monosized microspheres for targeted drug delivery applications. The inner phase (disperse phase) was a polymer mixture consisting of 92% L-PLA, 3% $MePEG_{17}$-*b*-$PCL_{10\text{-}19}$ and 5% bis(picolylamine)-functionalized PLA dissolved in chloroform at a final concentration of 5% (w/v). The outer phase (continuous phase) was a 2% aqueous PVA (poly(vinyl alcohol) solution (13-23 kDa, 86%-89% hydrolyzed). Different volumetric flow rates ratios were investigated. Desired monosized microparticles (9±0.4 μm) were produced at a 1:15 volumetric flow ratio of disperse phase: continuous phase **(Fig. 1)**. Delivery of the particles to the target tissue can be controlled better when the particles have a narrow size distribution. For example, these monosized microspheres were trapped almost completely (99.4%) in the lung of mice after intravenous tail vein injection (**Fig. 2**) and are thus excellent lung perfusion imaging agents.

Fig. 2: Surface-rendered microSPECT/CT image taken 5 min after tail vein injection of 0.2 mg of ^{99m}Tc-radiolabeled microspheres into a healthy mouse. The radioactivity is shown in red.

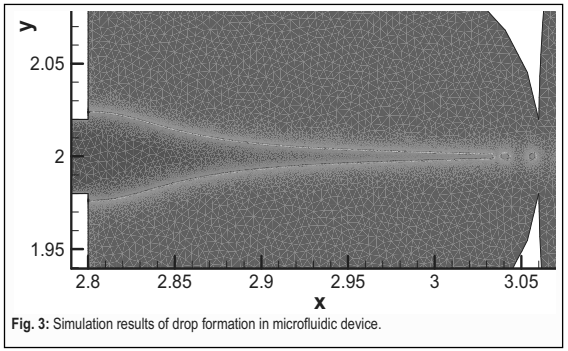

Fig. 3: Simulation results of drop formation in microfluidic device.

In our current work, we are preparing monosized microspheres with this flow focusing method that incorporate magnetite nanoparticles with lipophilic coatings for magnetic drug targeting. Such monosized microspheres will respond very homogenously to a magnetic field, unlike current magnetic microspheres with broad size distributions. Furthermore, we are working on computational simulations of the microsphere production to investigate the effect of the geometry and the operational conditions on particle size and distribution (**Fig. 3**).

Poster 21

Magnetization state in magnetic nanoparticle agglomerates

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Magnetic nanoparticles with size below certain limit exhibit single domain configuration of magnetization with either blocked or superparamagnetic (SPM) behavior of magnetization[1]. When subjected to the alternating magnetic field the magnetization of particles in suspension aligns with this field either through rotation of the whole particle when magnetization is blocked, or through flip of magnetization in the particle for SPM particles. By measuring the frequency response of the magnetic susceptibility of such suspension one can observe Brown or Neel relaxation for blocked or SPM particles[2]. Brown relaxation measurements can be used for detection of functionalization of surface and application in biotechnology were already demonstrated.

In nanoparticles preparation a common method is coprecipitation where nanoparticles form by nucleation and subsequent growth or agglomeration[3]. With time or subsequent steps additional agglomeration can take place and final nanoparticles can be formed from a multitude of smaller crystallites[4]. As the magnetic coupling is possible across short barriers, in such agglomerates both cooperative blocked state and SPM state from individual crystallites are possible. Therefore we analyzed the magnetization state of such agglomerate nanoparticle by measuring the frequency spectrum of magnetic susceptibility. In our study we used magnetite or cobalt ferrite nanoparticles, prepared by coprecipitation and characterized by specific surface area (SSA), electron microscopy (TEM) and dynamic light scattering (DLS). Two types of particles were prepared, with average hydrodynamic diameter of about 14 nm and of about 30 nm from DLS. SSA for both types gives about 12 nm diameter; electron microscopy shows crystallites of about the same size. Homogeneous magnetite nanoparticles larger than about 20 nm should have blocked magnetization and thus exhibit Brown relaxation. However, our measurements show only constant magnetic susceptibility at frequencies up to 40 kHz (above expected Brown relaxation), similar as for the smaller particles. Similar results are obtained also for induced agglomerates where smaller particles were agglomerated by addition of excess of SDS surfactant. In contrast, measurements of susceptibility for the cobalt ferrite nanoparticles with similar crystallites' size and DLS diameter of about 35 nm exhibit Brown relaxation at about 8 kHz. We conclude that in the Co ferrite case the crystallites themselves are already blocked and thus also the agglomerate of such crystallites exhibit blocked magnetization state. This significantly affects applicability of magnetic susceptibility measurements for detection of hydrodynamic diameter as it limits the applicable nanoparticles to single-crystallite nanoparticles.

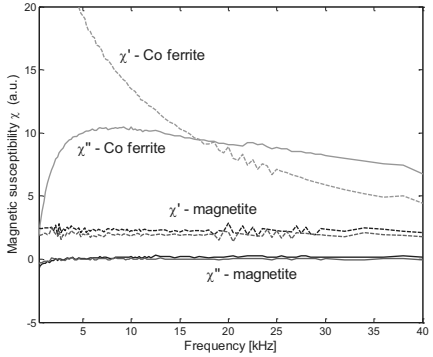

Figure 1:Magnetic susceptibility as a function of frequency for magnetite and cobalt ferrite nanoparticles

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Poster 22

Combined MRI-adaptive Magneto-thermo-polychemotherapy for Improved Cancer Treatment

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The early detection of invasions and metastases by MRI-monitoring (MRM) is an important problem in the detection of malignant tumors. We synthesized and tested dextran-ferrite (DF) for the early detection of invasions and metastases at combined MRI-adaptive magneto-thermo-polychemotherapy (CAT) with slime aspiration to improve cancer treatment. During investigation of invasions by BIOSPEC USR 70/30 (Bruker), we have found that weak signals of protons from small sites of pathogenic cells are neutralized by intensive signals from normal tissues. To solve the problem ferrite nanoparticles can be used as MR-negative contrast agents [1]. Contrast enhanced MR-images of invasions are represented in Fig. In our experiments hypodermic and skin tumors were treated with magnetically controllable drugs. Increase of drug concentration in tumor tissues due to the magnetic field was achieved by use of $SmCo_5$ or NdFeB bandages (0.2-0.3 T induction) according to recommendations [2] and by superconducting electromagnet (7T). Quantification of magnetic nanocarriers of such antitumor drugs *in vitro* and *in vivo* in mice bodies were carried out by "BioMag" device based on non-linear magnetization of nanoparticles [3]. Initially 60 female mice C57Bl/6j with mammary adenocarcinoma Ca 755, 60 female mice with Lewis lungs carcinoma and 60 male mice with melanoma B16 underwent native MR-imaging with T_1-weighted (TR/TE=500/15ms) and T_2-weighted (TR/TE=1900/80ms) spin-echo and T_2-weighted gradient-echo (GRE) (TR/TE=500/15) sequences. Then 1.0 ml DF sol (particles diameter 24–40 nm, dose 10.0 mg Fe/kg) were injected in mice caudal vein and after 2-24 hours second MRM and DF-enhanced T_2-weighted GRE sequences were performed. Signal intensity decrease was recoded and visual analysis was performed. At early stages of oncogenesis thermochemotherapy at +46 ºC for 30 min using DF with the dose 60 mg Fe/kg containing of Cysplatin (CP) or Melphalan (MP) were performed. The DF (Fe_3O_4 weight -83 mg; CP - 20 µg; MP -2 µg; pH 7.4) was injected into multiple tumor sites and was concentrated in the tumor tissue with magnetic bandages. Mammary adenocarcinoma Ca 755 tumor ~45 mm^3 treatment by AC magnetic field (0.88 MHz, 7.3 kA/m, 0.15 kW) led to regression in female mice before metastases up to 40 % and increased up to 280 % life span was achieved, tumor ~300 mm^3 CAT with slime aspiration and the invasions and metastases (Fig.) systemic treatment by Cyclophosphamide led to 200 % increased life span.

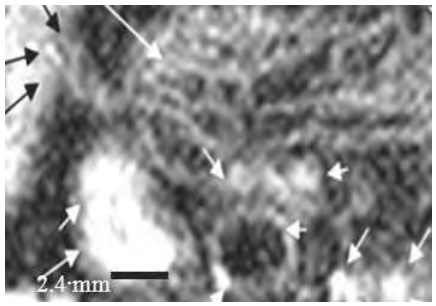

Fig. MRI of invasion and metastases of Ca 755 tubs in normal tissues, which was implanted subcutaneously in the female mice C57Bl/6j with subsequent DF intravenous injections: a place of intertwisting of the tubs in a tumor in a garrot (G) and yield of G from a tumor (black arrows); a place of an inlet, untwisting of G and invasion of the tubs in normal tissues (major white arrow); metastases (short white arrows).

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Investigation of the connection of magnetic active core sizes and hydrodynamic diameters of a magnetically fractionated ferrofluid

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A lot of different properties of magnetic nanoparticles (MNP) play an important role when using these particle systems. Besides the outer shape of the particles, which is examined using microscopic measurements, a lot of questions arise regarding the magnetic properties, e.g. the use with hyperthermia and drug targeting. In this work we address the question which relation between the size of the magnetically active core of magnetic nanoparticles and the size of the overall particle in the solution (the so called hydrodynamic diameter d_{hyd}) exists. On the one hand, we use temperature dependent magnetorelaxation (TMRX[1]) method in the temperature range between 4.2 K and 320 K, which enables direct access to the energy barrier distribution and by using additional hysteresis loop measurements can provide details about the size of the magnetically active cores. On the other hand, to determine the size of the overall particle in the solution, we use the magnetooptical relaxation of ferrofluids (MORFF[2]) method where the stimulation is done magnetically while the reading of the relaxation signal, however, is done by optical means. As a basis for the examinations in this work we use a ferrofluid that was developed for medicinal purposes. This water based ferrofluid has a core of iron oxide and a shell of Carboxymethyldextran (CMD). The iron oxide cores are consisting of a mixture of maghemite Fe_2O_3 (68%) and magnetite Fe_3O_4 (32%). The ferrofluid has been fractionated into seven fractions using a magnetic fractionation method.

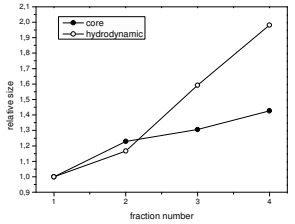

Figure 1: Relative core sizes and relative hydrodynamic sizes of the 4 relevant fractions

The comparison of the obtained magnetically active core diameters and the hydrodynamic diameters show a direct relation. With an increase of the magnetically active core diameters the hydrodynamic diameters increase as well. This behaviour is nearly identical with the correlation between overall core size, that is magnetically active part plus magnetic dead layer, and hydrodynamic size, which can be found in all major literature.

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Liposomal Tracers for Magnetic Particle Imaging (MPI)

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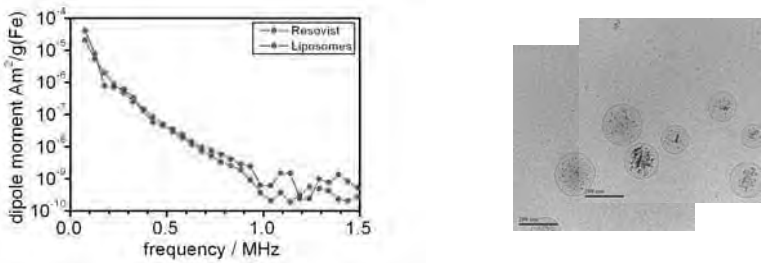
Magnetic Particle Imaging (MPI) is a novel tomographic imaging technique that requires the use of magnetic nanoparticles as tracer materials to generate three-dimensional images.[1] The main advantage of this technique is that the spatial distribution of magnetic nanoparticles is directly detected from a non-linear remagnetization analysis promising a high sensitivity and resolution. MPI relies on the nonlinearity of the magnetization curves of the tracer material and the fact that the particle magnetization saturates at a specific magnetic field strength. Magnetic Particle Spectroscopy (MPS) is the spectrometric variant of MPI, and can be simply seen as zero-dimensional MPI. MPS provides the remagnetization signal without reconstructing images. Therefore, MPS is an efficient way of characterizing the absolute response of magnetic particles when they are exposed to an oscillating magnetic field.



Magnetic particle spectrum (left) of commercial and liposomal Resovist® formulations (right)

The use of MPI for molecular imaging applications is hampered by its currently low sensitivity and the short blood circulation time of the some 10 nm-sized nanoparticle tracers. These shortcomings can, however, potentially be overcome with new concepts for signal amplification based on long-circulating liposomal carriers. Liposomes can carry large payloads of tracer material promising a theoretical carrier-based signal enhancement directly proportional to the number of encapsulated individual particles, provided that the particle remagnetization is unaffected by restraining a large number of magnetic particles into the small intraliposomal space. We have found experimentally that the MPS signal generated by suitable MPI tracer materials, such as Resovist® (Bayer Schering Pharma) nanoparticles, was indeed not affected by the incorporation of these particles into 200 nm-sized liposomal carriers (figure). We studied the potential of these liposomal tracer formulations for applications in molecular imaging and image-guided drug delivery. Our first results and potential applications will be discussed.

Literature

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MAGNETIC FLUIDS OF MAGHEMITE-POLY(VINYLPYRIDINE) NANOPARTICLES AS MULTIFUNCIONAL PLATAFORM FOR THERAGNOSTIC.

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We evaluate the efficiency of a series of maghemite-poly(Vinylpyridine) colloidal magnetic fluids, together with commercial ones, for magnetic inductive hyperthermia and as negative contrast agents (CA) for MRI. Results are explained in terms of the structure and magnetic properties of the samples.
The fluids were prepared using maghemite nanoparticles, grown in a poly(vinylpyridine) (PVP) matrix. The maghemite-PVP nanocomposites were obtained by the mixture of PVP and iron bromide solutions, followed by the precipitation of the nanoparticles induced by the addition of a base. At this point certain amount of Polyethyleneglycol (PEG) is added and as a result the final ferrofluid consists in a very stable dispersion of polymeric beads, functionalized with PEG. The average size of the superpramagnetic iron oxide nanoparticles (SPIONs) was tuned in the 4 to 15nm range by changing the iron concentration and the Fe^{2+}/Fe^{3+} ratio. Dynamic light scattering shows that the colloidal fluids are composed of beads with average sizes ranging from 50 to 100nm approximately. Each bead contains several SPIONs bundled with average sizes about 10% of the hydrodynamic size of the bead.
Samples with larger magnetic cores size have higher specific loss power (SLP) per Fe atom in the 5 – 100kHz frequency range. The sample with SPIONs of 15nm (Sample F) and iron concentration of 2.27g/L was heated up two degrees in just 2min with an ac field of 14mT.
The transverse relaxivity (r_2) is the most important parameter to establish the efficiency of a negative CA. In relaxometry studies a strong dependence of r_2 with the average size of the magnetic cores was found in the frequency range of commercial imagers (8 – 60MHz). In the images obtained in a commercial 8.5MHz MR scanner samples with SPIONs average sizes over 10nm exhibit similar and/or superior contrast performances than Endorem.

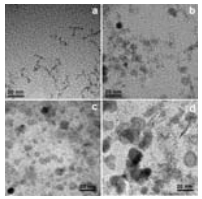

Figure 1: TEM images of samples A(a), C(b), E (c) and F (d).

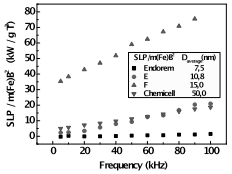

Figure 2. SLP per mass of iron of samples E, F and the commercial magnetic fluids Endorem and Chemicell.

Figure 3. MRI images of samples D, E, F, and Endorem (all 0.02 mg/ml) obtained at 8.5MHz using a High resolution Gradient Echo sequence.

Keywords: Magnetic nanoparticles; Superparamagnetism; Hyperthermia; SLP; Relaxation, CA.

Application of Magnetic Nickel Nanowires to macrophage cells and osteoblast cells: biocompatibility study and clinical future perspectives

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There has been a dramatic increase in the interest in nanomaterials over the past few years. Their beneficial properties, related to their size, shape, composition and ability to be functionalised with various antibodies, has resulted in a remarkable growth in potential biomedical and industrial applications for both nanoparticles and nanowires. Nanowires have several advantages in comparison to nanoparticles, including their aspect ratio which can be easily controlled and combining this with a uniform surface functionalisation, nanowires can be used as flow cytometric fingerprints with better selectivity than nanoparticles and have potential for efficient diagnostic targeting.[1] However, despite these expanding applications there is still very little known of the toxic effects, and specifically whether the cytotoxicity of nanoparticles and nanowires is caused by the material itself or by the shape.

In this study nickel nanowires were fabricated in two wire lengths: a) short and b) long, by electrochemical template synthesis using Anopore inorganic alumina membranes (Anodisc 25, Whatman, U.K.) with an average pore diameter of 200 nm, as reported in our previous work.[2,3] Short and long Ni NWs with average lengths of 4.3 ± 1.0 μm and 24.0 ± 7.0 μm and aspect ratios of 21.5 and 120.0, respectively, were grown for our experiments. Nanowire samples were characterised by SEM, TEM and zeta potential. The uncoated nanowires displayed an uneven surface, possibly due to natural oxide layer passivation and had a surface charge of -8.7 ± 0.8 mV.

Two cell lines were chosen in order to investigate the influence of nickel nanowires' aspect ratio on the functional response of cells. THP-1 macrophage cells were chosen as they naturally represent the first line of response to nanomaterials in the human body. MC3T3 pre-osteoblast cells were chosen as the closest cell line to investigate the secondary biocompatibility effect of the nanomaterials during bone remodelling and formation. High content screening (HCS) and analysis was employed to measure during a time course study up to 24h, the possible toxic effects of nickel nanowires on both cell lines and if different aspect ratios might improve their biocompatibility. HCS results showed that after 24 h short nickel nanowires did not induce the same degree of toxicity for both THP-1 and MC3T3 cells. Furthermore, the concentrations of two proinflammatory cytokines, interleukin-6 (IL-6) and tumour necrosis factor alpha (TNF-α), were also investigated by ELISA assay. In this study it was found that for THP-1 cells short wires did not demonstrate the same degree of IL-6 and TNF-a secretion as long nanowires. Whereas, MC3T3 cells showed signs of toxicity recovery after the first 6 h of exposure.

Finally, in this study we expand on the previously published work and we present the importance of examining each nanomaterial on various cell lines and the necessity for fast and clinically robust high throughput system in order to completely characterise this efficiently ahead of any clinical applications, these ranging from fast diagnostic screening to selective flow cytometric complex analysis.

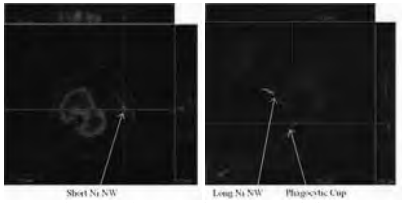

Figure 1: Confocal microscopy images of THP-1 phagocytes and fluorescent (a) Short, 4.3 μm and (b) Long, 24.0 μm nickel nanowires. Nucleus of THP-1 phagocytes stained with Hoechst 33342 dye (DAPI), F-actin stained with Alexa Fluor 488 phalloidin (FITC) and Ni NWs are labelled with RAM IgG (TRITC).

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Cytotoxicity effect of dirhodium(II) citrate loaded magnetic nanoparticles and magnetoliposomes in breast cancer and normal breast cells

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Superparamagnetic iron oxide nanoparticles (SPION) represent an attractive platform as carriers for drug delivery systems [1]. The success of SPION as carrier agents is mainly due to its high surface area, permeability, and retention in tumor tissue [2]. Moreover, the use of these type of nanoparticles for target drug delivery is advantageous in relation to the conventional antitumor agents because they can act specifically in tumor cells [3]. The introduction of new chemotherapy agents, such as dirhodium(II) carboxylates, in clinical trials, has been limited because they induce high toxicity in normal cells. Dirhodium(II) citrate $[Rh_2(H_2cit)_4]$, for instance, presents great potential as antitumor agent, but it induces inespecific toxicity [4]. Thus, association between SPION and $[Rh_2(H_2cit)_4]$ may represent a great alternative for ensure its therapeutic action without affecting normal cells. Our aim was to evaluate the cytotoxicity of $[Rh_2(H_2cit)_4]$ (50 μM) free, loaded to maghemite nanoparticles $[Magh\text{-}Rh_2(H_2cit)_4]$, or encapsulated in magnetoliposomes $[Lip\text{-}Magh\text{-}Rh_2(H_2cit)_4]$, on breast carcinoma cells lines (4T1 and MCF-7) and mama normal cells (MCF-10A). The cytotoxicity these samples was measure by the MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay. **Table 1** shows the cell viability percentage according to the treatment, cell lines, and exposure time. The cytotoxic effect of $[Magh\text{-}Rh_2(H_2cit)_4]$ and $[Lip\text{-}Magh\text{-}Rh_2(H_2cit)_4]$ was more pronounced in breast carcinoma cells than in normal cells, after 24 and 48 hours of treatment. Moreover, cytotoxicity was intense after 72 hours in the 4T1 carcinoma cell line ($p < 0.05$). Cells treated with SPION without $[Rh_2(H_2cit)_4]$ had no alterations in the viability compared to control (data not shown), after 24 and 48 h. Thus, the association of $[Rh_2(H_2cit)_4]$ with nanoparticles resulted in a specific cytotoxic effect to carcinoma cells when compared to normal cells. Therefore, we suggest that $[Magh\text{-}Rh_2(H_2cit)_4]$ and $[Lip\text{-}Magh\text{-}Rh_2(H_2cit)_4]$ have a high potential for application in chemotherapy.

Table 1: Distribution of cell viability percentage according to the treatment, cell line and exposure time.

Treatment (μM)	Cell line	24 h		48 h		72 h	
0 (control)	MCF-7	100,00 ± 1,50	A*; a#	99,94 ± 1,95	A; a	100,00 ± 1,06	A; a
	4T1	100,00 ± 1,21	A; a	100,00 ± 1,46	A; a	100,00 ± 1,34	A; a
	MCF-10A	100,00 ± 3,30	A; a	100,00 ± 1,05	A; a	100,00 ± 0,92	A; a
$[Rh_2(H_2cit)_4]$	MCF-7	94,96 ± 2,44	A; a	97,48 ± 2,84	A; a	81,19 ± 2,30	B; a
	4T1	90,31 ± 1,38	A; a	87,79 ± 2,63	A,B; a	81,42 ± 2,56	B; a
	MCF-10A	97,75 ± 3,77	A; a	97,82 ± 1,40	A; a	84,30 ± 2,55	B; a
$[Magh\text{-}Rh_2(H_2cit)_4]$	MCF-7	66,25 ± 3,32	A; a	82,67 ± 2,92	B; a	78,02 ± 1,44	A,B; a
	4T1	53,79 ± 2,75	A; b	30,47 ± 2,01	B; b	26,13 ± 1,43	B; b
	MCF-10A	82,72 ± 1,70	A; c	74,26 ± 1,50	B; a	49,50 ± 3,00	C; c
$[Lip\text{-}Magh\text{-}Rh_2(H_2cit)_4]$	MCF-7	67,41 ± 0,76	A; a	59,57 ± 0,63	A; a	62,08 ± 1,55	A; a
	4T1	42,62 ± 1,30	A; b	31,53 ± 2,45	B; b	15,91 ± 2,94	C; b
	MCF-10A	104,85 ± 2,55	A; c	75,03 ± 2,56	B; c	59,73 ± 1,69	C; a

The data represent the mean ± SE (mean standard error) of two independent experiments in triplicates. In the treatments with $[Rh_2(H_2cit)_4]$, $[Magh\text{-}Rh_2(H_2cit)_4]$, $[Lip\text{-}Magh\text{-}Rh_2(H_2cit)_4]$ the concentration of $[Rh_2(H_2cit)_4]$ was 50 μM. * Different capital letters denote statistical difference between viability in the different times (rows) for a given cell line (breast cancer cells MCF-7, 4T1 or normal cells MCF-10A) under the same treatment ($p < 0.05$). # Different tiny letters indicate mean statistical difference between the viability of different cell lines (columns) for a given time (24, 48 or 72 hours) ($p < 0.05$).

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Design of a glucose amperometric biosensor by novel arginated Fe_3O_4 nanoparticles

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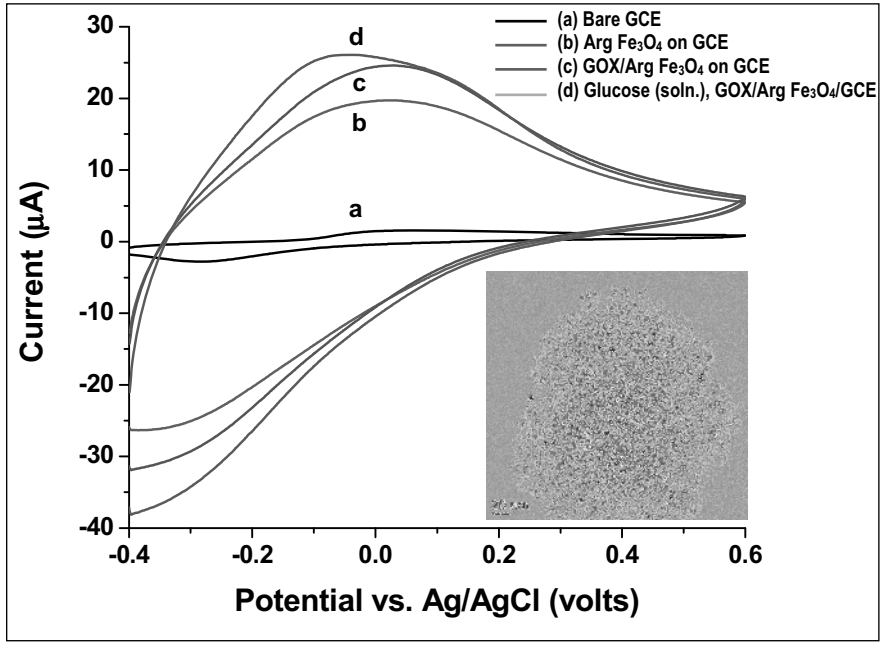
Immobilization of bioactive molecules on the surface of magnetic nanoparticles is of great interest because the magnetic properties of these bioconjugates improve the recognition of biomolecules for biomedical applications. Magnetic nanoparticles were prepared by co-precipitation method and stabilized by biocompatible arginine. The crystallite size of the arginated Fe_3O_4 nanoparticles estimated using X-ray diffraction (XRD) pattern using Scherrer's formula and the particle size estimated by transmission electron microscopy (TEM) has been found to be 6.8 nm and 4.2 nm, respectively. The magnetic nanoparticles were deposited onto glassy carbon electrodes and cyclic voltametry were employed to access the ability of the same for sensing glucose. The amperometric glucose biosensor was fabricated by using arginated Fe_3O_4 and glucose oxidase (GOX). The above fabrication played an effective role of an electron shuttle and allowed the detection of glucose at a low potential of –0.08V (versus Ag/AgCl). The sensor showed a fast response time, low detection limit and high sensitivity of glucose. The anodic and the cathodic peak currents increased linearly with the scan rate indicating surface controlled process. It also demonstrated very high stability which can be attributed to the mild fabrication process and no damage was caused to the enzyme molecule. On the other hand, the magnetic nanoparticles possessed good biocompatibility that could retain the bioactivity of the enzyme molecule immobilized on the electrode. The study of the applicability of the proposed biosensor to real sample analysis is currently underway.

Keywords: Amperometric sensing, glucose, magnetic nanoparticles



Poster 29

Size distribution and magnetic structure of iron-oxide nanoparticles studied by measuring magnetization curves at various temperatures

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The size distribution of an assembly of iron-oxide nanoparticles (SH U555C) is studied by measuring magnetization curves at $T = 40, 80, 160$, and 300 K and then fitted by a uniform model and a core-shell model, both based on the Langevin function for superparamagnetism with a log-normal particle size distribution. The uniform model fits lead to spontaneous magnetization $M_s(T)$ significantly smaller than that for the bulk and to particle sizes that decrease unphysically with decreasing T. The T independence of size distribution and $\mu_0 M_s(300\text{K}) = 0.55$ T are assumed in the core-shell model fits. After further assuming the same shell thickness at $T = 300$ and 160 K, the size distribution is finally obtained, which agrees well with those determined by other techniques. However, the modeling low-field shell thickness increase at low T, which indicates that T and field induced changes occur in the magnetic structure of the particles.

Poster 30

Magnetic manipulation of actin orientation, gliding and polymerization using nano-sized iron oxide particles

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Cytoskeleton contributes the shapes, motility, division and the molecular transportation of cellular components, among many other important physiological functions. The capacity to remotely manipulate cytoskeleton is therefore of great interest. In this study, we explored the possibility of achieving such goal using an external magnetic field.

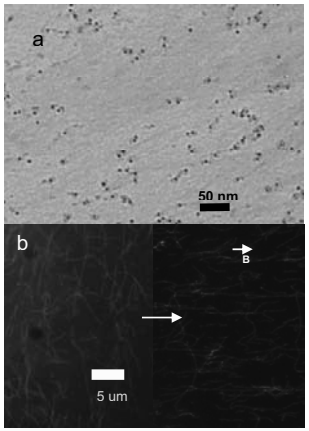

Figure 1: (a)The incorporation of iron oxide particles into actin filaments was examined by transmission electronic microscopy. The 10-nm iron oxide particles were shown as the dark circles bound to the F-actin which is the lighter, filamentous structure shown in the micrograph. (b) Before the sample was placed in the parallel magnets, F-actin was randomly arranged (left); F-actin became aligned with the magnetic field (B) after being placed between the two parallel magnets (right).

In order to produce functional actin filaments that are magnetizable, 5% of biotinylated g-actin, 5% of rhodamine-labeled g-actin was mixed with regular g-actin monomers (90%) for polymerization, followed by adding 10-nm iron oxide particles with streptavidin coating (SHS-10-01, Ocean NanoTech, LLC) at 1:1 ration to the biotinylated g-actin. The incorporation of iron oxide particles into actin filaments was confirmed by electron microscopy (Fig. 1a). The iron oxide particle-incorporated f-actin was subsequently subject to a series of magnetic manipulation experiments where g-actin or f-actin were placed in a uniformed magnetic field.

As a result, actin filaments can be aligned parallel to the external magnetic field in vitro (Fig. 1b). The magnetic field also biased the direction of movement when actin filaments are gliding on myosin-coated surface. Furthermore, when polymerized from g-actin monomers, a bias in polymerization orientation is also observed in the presence of the external magnetic field. The capacity to control three different types of actin behavior renders wider freedom for future applications. If similar effects can be achieved in vivo this might enable manipulating cell behaviors with an applied field, another potential application is to construct actin-based track system, whose specific pattern is modulated using magnetic fields, to program the routes of motor proteins and therefore control the substance delivery carried by the motor proteins.

Poster 31

Preparation method for magnetite and continuous solid solutions of magnetite-maghemite

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Iron oxides are well-known compounds and can be easily prepared in laboratories. They are composed of Fe along with O and/or OH[1]. Fe_3O_4 (magnetite) and γ-Fe_2O_3 (maghemite) are the most important and used magnetic iron oxides. Both compounds are easy to prepare, ferrimagnetic and biocompatible, being therefore very attractive for different applications, ranging from industrial domain to the biomedical one.

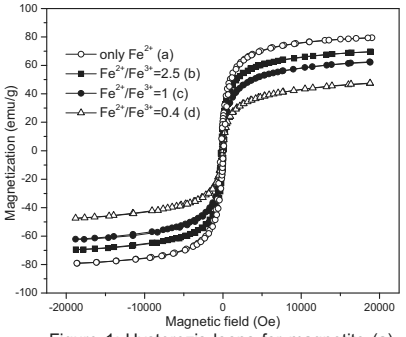

Figure 1: Hysterezis loops for magnetite (a) and continuous solid solutions of magnetite-maghemite (b, c, d).

In order to synthesize magnetite, several methods are available. The most used one is the Massart method based on the chemical co-precipitation of Fe^{2+} and Fe^{3+} salts in alkaline solutions[2]. By using this method, the obtained particles are generally assumed to be magnetite[2]. On the other hand, there are reports that contradict this general assumption, trying to prove that the final main product of the Massart method is maghemite and not magnetite[3].

In order to spread some light on this recently appeared debate, we imagined a new method to prepare magnetite, maghemite and combinations between them. This method is based on a salt-assisted solid-state chemical precipitation reaction.

This new method has some advantages over the other Massart-based chemical methods, being faster, easier, more flexible, and economic, allowing a good control of the final magnetization of the final reaction product. For instance, the Massart method uses Fe^{2+} and Fe^{3+}, while this method uses only Fe^{2+} salt in order to prepare magnetite. Moreover, to obtain magnetite by using Fe^{2+} and Fe^{3+}, one should take into account also the optimum ratio between iron salts, and even for the correct supposed optimum ratio, the method is not very reproducible, many authors reporting different results by using almost the same salts ratio.

For synthesizing magnetite, this method uses Fe^{2+} salt and a precipitation agent, while for maghemite preparation it uses Fe^{3+} salt and the same precipitation agent. All the reagents were used in their solid state. In order to obtain final products with controlled magnetization (fig. 1), the method uses the both iron salts (as in Massart method). Depending on the salts' concentrations, one can obtain final products with magnetizations near either magnetite or maghemite. In fact, the final products are continuous solid solutions of magnetite and maghemite. This last approach could be a solution of the problem, showing that, in fact, by using Massart method, one obtain a solid solution of magnetite-maghemite, with magnetizations near to the magnetite's or maghemite's, depending on the molar ratios between them and reaction conditions, respectively.

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Poster 32

Detection of disease-related DNA through metallic nanowires

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Detection and quantification of biological and chemical species represents a central goal for many areas of biomedicine. The applications of nanomaterials, such as nanowires, nanoparticles, carbon nanotubes, in biomedicine can solve a series of specific problems related to diagnosis or therapy[1].

In the last ten years, nanowires were shown to have a high potential to be used in biomedical applications, especially for diagnosis and magnetic separation purposes. Thus, the nanowires were tested in biosensors[2], bioassay methods for pathogen detection[3] or magnetic manipulation of cells[4].

From the medical analysis standpoint, the most important biomolecules used for diagnosis are antibodies and DNA. The discovery of specific target DNA sequences of medical interest in the incipient phase of a disease such as tumor or viral pathology is correlated with an accurate assessment of patient's prognosis and with an appropriate way to monitor the therapy. Usually, these specific DNA sequences are detected and quantified using molecular techniques (PCR, RFLP, RT-PCR) along with electrophoretic migration in agarose gel. Regarding this issue, an alternative technique to the conventional ones could be the use of a bioassay method based on metallic nanowires that specifically detect and qualitatively identify the amplified target DNA sequences obtained by using specific modified primers.

This study presents our results concerning the use of metallic nanowires to detect and qualitatively identify a specific DNA sequence (i.e. mutation of FLT3 gene) obtained from patients with acute myeloblastic leukemia.

The nanowires were prepared by electrochemical deposition of metal ions into the pores of a membrane template, followed by nanowire release. A gold film, 500 nm thickness, deposited on the backside of an Al_2O_3 membrane served as the working electrode for reduction of metal ions from solution. The dimensional aspect of the nanowires is controlled by (i) the membrane pore diameter, which dictates the particle width, and (ii) the current used for deposition, which sets the particle length.

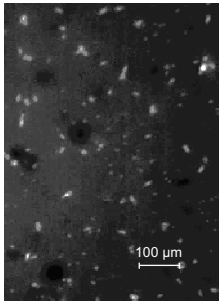

Figure 1: Fluorescent cy5-tagged DNA immobilized on nanowires

The nanowire detection is carried out through a fluorescence-based analysis system (Tissue Gnostics Faxs system) by measuring the fluorescence generated by the florophore-tagged amplicons (i.e. products resulted from PCR amplification process) immobilized on nanowires (fig. 1).

Comparative studies emphasized the usefulness of the nanowires-based bioassay method for such biomedical issues and also underlined the necessity for further analysis and tests in order to certify the efficiency, sensitivity and specificity of this method.

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Poster 33

Fe/Fe_2O_3 and Fe/Fe_3O_4 core-shell nanoparticles for biomedical applications

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Fe-oxide nanoparticles are of considerable interest nowadays because of their unique characteristics, such as superparamagnetism, high saturation fields, and extra anisotropy contributions, which arise from the effects of finite size and large surface area [1]. Usually they are obtained by chemical methods, but more recently some groups reported on their successful preparation by wet high-energy ball-milling [2,3]. In this paper, we will present our recent results on the preparation and characterization of Fe_2O_3/Fe and Fe_3O_4/Fe core-shell nanoparticles, specially designed for biomedical applications, as image contrast agents in magnetic resonance imaging (MRI) and magnetic carriers for drug delivery.

Three types of core-shell nanoparticles have been prepared by high-energy ball milling using WC vials and 22 balls of WC with the diameter of 10 mm (11 balls / vial). 12 g of metallic Fe of average particle size less than 10 µm were mixed with air, Ar and distilled water, respectively, at a rotation speed of 300 rpm. For wet milling the ratio used was of 12 g(Fe):8 ml (H_2O), the amount of distilled water being increased progressively to 50 ml. In the presence of air or Ar, the Fe core was progressively covered with a Fe_2O_3 shell as shown by HR-SEM investigations and XRD results, and the obtained Fe/Fe_2O_3 nanoparticles have diameters of 200-300 nm after 68 h of milling. Wet milling was then used to subsequently reduce the Fe and Fe^{3+} to Fe^{2+}, and the obtained Fe/ Fe_2O_3/Fe_3O_4 nanoparticles have diameters of 40-100 nm after 62 h of milling in Ar or air followed by 6 h of milling in distilled water. Fe/Fe_3O_4 nanoparticles of 20-60 nm were obtained by wet milling of Fe microparticles for 42 h (Fig. 1). For milling times ranging from 12 to 36 h the nanoparticles contain also small traces of Fe_2O_3, whilst for milling times larger than 42 h the whole amount of Fe is transformed in Fe_3O_4, and the resulting magnetite nanoparticles have diameters ranging from 15 to 50 nm. The elemental analysis performed by EDS confirms these results.

The magnetic properties of Fe/Fe-oxide core-shell nanoparticles can be tailored from ferromagnetic Fe/Fe_2O_3 to weak ferromagnetic Fe/Fe_3O_4 and superparamagnetic Fe_3O_4, as shown in Fig. 2. Thus, by choosing the appropriate milling conditions and the starting material we might be able to tune the magnetic properties and make the Fe/Fe-oxide core-shell nanoparticles suitable for different biomedical applications. All these aspects will be discussed in detail and in connection with their use as image contrast agents and magnetic carriers for drug delivery. The main advantage of such core-shell nanoparticles compared with the simple Fe-oxides nanoparticles resides in their easier use and manipulation for specific applications.

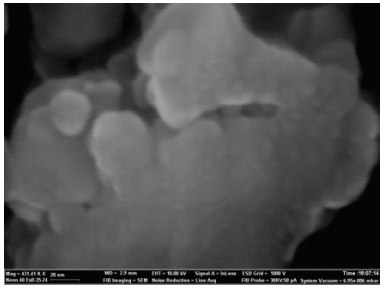

Fig. 1. HR-SEM image of the cross-section of Fe/Fe3O4 core-shell nanoparticles. The bright spots represent the remaining Fe core (6-7 nm in diameter) and the dark matrix is the Fe_3O_4 shell.

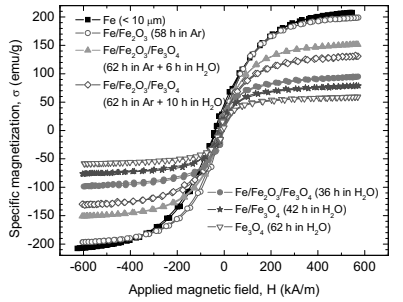

Fig. 2. Magnetic hysteresis loops for Fe/Fe_2O_3, $Fe/Fe_2O_3/Fe_3O_4$, Fe/Fe_3O_4 and $FeO{\cdot}Fe_2O_3$ core-shell nanoparticles obtained by dry and wet milling.

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Poster 34

Interpretation of Mössbauer spectra of magnetic nanoparticles delivered into mouse spleen

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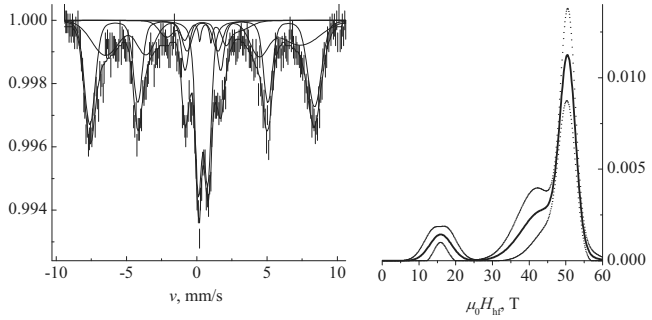
Mössbauer spectroscopy is a well-known means for study of structure, magnetic and thermodynamic properties of magnetic particles. The problem of interpretation of the Mössbauer spectra of the magnetic nanoparticles injected into a spleen of a living organism is due to the difficulty of decomposition of partial spectra of exogenous iron contained in nanoparticles and endogenous iron contained, for example, in ferritin and hemoglobin. The temperature evolution of the spectra shape of initial nanoparticles agrees qualitatively with the standard behaviour of an ensemble of single-domain particles with magnetic anisotropy [1]. Along with a noticeable increase in the linewidths of the spectra of the mouse spleen after injection of nanoparticles, an effective doublet of lines in the central part of the spectra is observed. The doublet parameters are typical for Mössbauer spectra of iron in ferritin-like proteins. An initial analysis of such spectra and their decomposition into partial components is based on the formal approach, in which the continuous distributions of hyperfine field $P(H_{hf})$ at iron nuclei are taken into consideration [2]. A detailed analysis of the evolution of such spectra with temperature and under external magnetic field requires utilization of stochastic models for description of relaxation effects in the system of homogeneously magnetized single domain particles [3].



Mössbauer spectrum of mouse spleen measured at 78 K in 3 months after intravenous injection of magnetic nanoparticles (left) and the corresponding theoretical H_{hf} distribution (right).

This work is supported by the Russian Foundation for Basic Research (project No. 09-02-12195).

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[3] M.A. Chuev. J. Phys.: Condens. Matter 20 (2008) 505201.

Preparation of Magnetic Styrene-Based Polymeric Microspheres: Influence of Incubation Time and Concentration of Magnetic Nanoparticles

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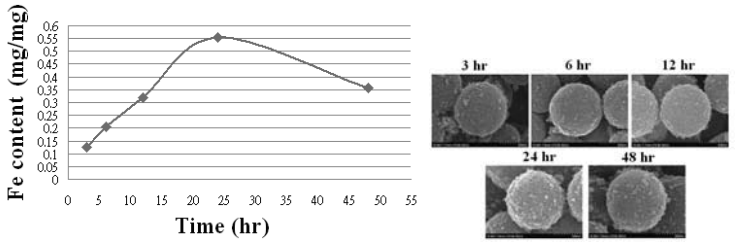
Magnetic polymer microspheres could be prepared by a swelling and penetration process. According to this method, styrene-based polymer particles of micron size were swollen by dipping in aqueous solution of N-methyl-2-pyrrolidone (NMP) and then incubated with superparamagnetic iron oxide nanoparticles (SPIONs). SPIONs were allowed to penetrate into the swollen particles during the incubation. Results indicate that the amount of SPIONs entrapped within microspheres increased with the incubation time and concentration of magnetic nanoparticles incubated with swollen polymer particles. When a concentration of 200 mg/mL SPIONs was used, the saturation magnetizations of the resultant polystyrene (PS), poly(styrene-glycidyl methacrylate) (PS-GMA), and poly(styrene-methacrylic acid) (PS-MAA) magnetic microspheres were determined to be 33.3, 37.3, and 31.9 emu/g, respectively. When PS particles were swollen in NMP solution with an NMP-to-water ratio (v/v) of 0.17 and in the presence of SDS for one day and then incubated with SPIONs for 3 to 48 h, the iron content in PS microspheres increased with incubation time up to 24h, as shown in the following figure. The amount of entrapped SPIONs, however, decreased after that time due to the possible release of SPIONs from microspheres.



Iron content (right) and SEM images (right) of magnetic PS microspheres prepared with different incubated time for the step of incubating swollen PS particles with SPIONs.

Magnetic Polymer Nanocomposite Materials for Targeted Drug Release in Cancer.

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Superparamagnetic particles of iron oxide have been widely exploited for many biomedical applications such as magnetic resonance imaging[1], hyperthermia[2], tissue engineering and most importantly for targeted, site-specific delivery of drugs.[3] Chemotherapeutic treatments are known to be effective in reducing cancerous masses and preventing further secondary cancer development. However, not all tumours respond well to such treatments, usually due to poor site bioavailability or development of multidrug resistance. The solution lies in intelligent drug delivery, where vehicles specifically designed to target a chosen cancerous site could improve the chances of success and the outcome of treatment.

The aim of this project is to develop aqueous dispersions of poly (n-butyl-cyanoacrylate)/iron oxide nanocomposites, in the size range of approximately 150nm, where the surface of the iron oxide nanoparticles (IONP) are modified with different surface charges prior to their combination with the cyanoacrylate. In the reported work, iron oxide nanoparticles were surface modified to be anionic (Citric Acid), cationic (Dodecyltrimethylammonium Bromide-DTAB) and neutral (uncoated IONP). Nanoparticle suspensions were combined with the cyanoacrylate in its monomer and pre-formed polymer forms. The resulting suspensions were characterised by field cycling NMR relaxometry which has been applied to the study of IONP suspensions[4], in addition to light scattering and transmission electron microscopy. In ongoing work, we are investigating the potential of these magnetic colloids for drug delivery applications and as MRI contrast agents.

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Poster 37

Nanoparticles of Molybdenum Chlorophyllin Photosensitizer and Magnetic Citrate-Coated Cobalt Ferrite complex available to Hyperthermia and Photodynamic Therapy clinical trials

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Nanoparticles of Molybdenum Chlorophyllin Photosensitizer and Citrate-coated Cobalt Ferrite complex were synthesized and characterized through of photophysical studies and biological assays for use as synergic nanoparticle drug for Photodynamic Therapy (PDT) [1] and Hyperthermia (HPT) [2] to cancer treatment. Chlorophyllin was obtained from alkaline extraction of Ilex paraguariensis with consecutively metal insertion from hydrolysis with molybdate sodium at 50-60^{o}C for 90 min. Citrate-coated cobalt ferrite magnetic nanoparticles were synthesized and characterized previously as described by Morais P.C.[3]. Fluorescence quantum yield (Φ_f) of Mo-Chl/DMSO was lower than 0.1, with a lifetime of 5.0 ns obtained from laser photon counting technique. The oxygen quantum yield measurement of Mo-Chl was carried out from laser flash-photolysis studies in homogeneous medium saturated with $O_{2(g)}$ (Φ_Δ = 0.50). Cellular viability was also evaluated using gingival fibroblasts cells by a MTT classical assay. These studies with Mo-Chl 5.0 μmol.L^{-1} at different concentrations range of magnetic nanoparticles (range from 10^{12} to 10^{15} particles/mL) showed a cellular viability of approximately 95% for the ideal magnetic material concentration of 1x10^{13} particles/mL. In conclusion, our findings point out toward the useful association between Mo-Chl (natural chlorophyllin photosensitizer) and magnetic nanoparticles, the latter via biocompatible magnetic fluids. The data regarding the tested complex show excellent photophysical parameters, appropriate biological compatibility and favorable photodamage achievements. This means that the complex material system tested in the present study represents a very promising formulation for synergic application of PDT and HPT protocols in in vivo assays, pointing towards its future application in clinical tests of neoplastic tissues. Grants to FAPESP project number 2006-50562-1, CNPq and CAPES (Brazilian agency to scientific research financing)

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Poster 38

Functionalized fluorescent magnetic nanoparticles for biomedical application

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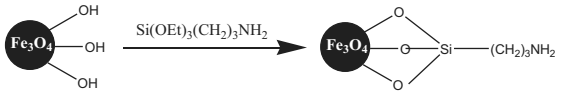
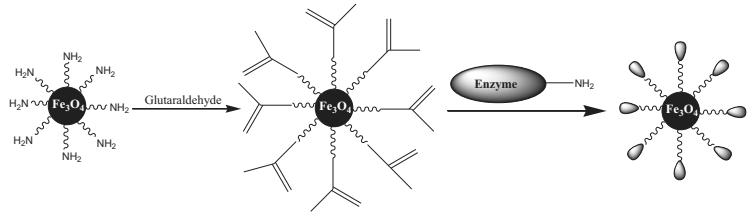
The magnetic nanoparticles are promising materials in various biomedical applications due to their ability to be manipulated with an external magnetic field. Among the multitude of magnetic materials, magnetite (Fe_3O_4) is the most common because of its biocompatibility and safe for in vivo biomedical applications. Immobilization of bioactive molecules on the surface of magnetic nanoparticles is of great interest, because the magnetic properties of this hybrid materials promise to greatly improve the delivery and recovery of biomolecules in biomedical application.

In this work we report the preparation and characterization of functionalized magnetic nanoparticles with an amino group in order to bonding different biomolecules, like glucose-oxidase enzyme. The magnetic nanoparticles were coated with (3-aminopropyl)-triethoxysilane (APTES), the active amino groups ($-NH_2$) facilitate the further functionalization by covalently bounding with other active groups, such as carboxyl (-COOH) (Scheme 1)



Scheme 1. Preparation of amino – modified magnetic nanoparticles

Bioconjugation experiments were performed to immobilize the enzyme glucose oxidase on the surface of amino – modified magnetic nanoparticles. Glutaraldehide was used a a cross-link agent for covalent coupling of glucose – oxidase to amino – modified magnetic nanoparticles, as show in Scheme 2. A florescent marker was attached to realize a dual functionalization og the magnetic nanoparticles.



Scheme 2. Covalent conjugation of glucose-oxidase to amino functionalized magnetic nanoparticles.

Complex physical-chemical and structural characteristics of functionalized magnetic nanoparticles were determined by FTIR, TEM, magnetization measurement and fluorescent spectroscopy.

Targeted magnetic nanoparticles for magnetic fluid hyperthermia.

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Magnetic fluid hyperthermia (MFH) has emerged as a promising technique to eradicate localized cancer tumors, because magnetic nanoparticles have the ability to dissipate energy under an oscillating magnetic field, causing a rise in temperature that proves lethal to cancer cells. Water-dispersible magnetic nanoparticles have been used successfully for *in vitro* MFH; however, these nanoparticles typically possess non-specific binding affinity that correlates with lack of specificity and low internalization rates. In order to increase the specificity and the efficiency of internalization to specific cell-types, we recently prepared epidermal growth factor (EGF) conjugated magnetic nanoparticles to target the epidermal growth factor receptor (EGFR), which is overexpressed in many types of cancer such as breast, colon, pancreas, head, and neck [1].

In this study, the viability of cancer cells that overexpress (MDA-MB-468) and that do not overexpress (MCF-7) EGFR was studied after cells were in contact with targeted (IOcovCMDxEGF) versus non-targeted (IOcovCMDx) magnetic nanoparticles. Targeted nanoparticles caused a significant decrease in cell viability in cells that overexpress EGFR after 18 hours in contact (Figure 1), due to the apoptotic effect of supramolecular levels of EGF on cells that overexpress EGFR. Internalization of targeted nanoparticles was studied as a function of time in both cell lines. Targeted nanoparticles were internalized in both cell lines but with a higher internalization rate in cells that overexpress EGFR. Cells were treated by MFH after incubation with targeted and non-targeted nanoparticles. Cells in contact with targeted nanoparticles showed a higher decrease in cell viability after MFH, as compared to cells that underwent MFH after being in contact with non-targeted nanoparticles (Figure 2).

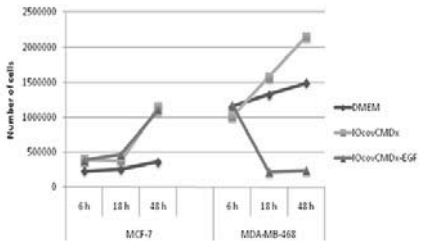

Figure 1. Cell viability of MCF-7 and MDA-MB-468 cells after incubation with DMEM, IOcovCMDx and IOcovCMDx-EGF nanoparticles as a function of time.

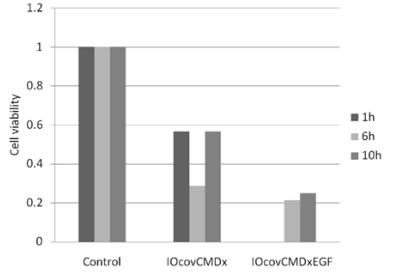

Figure 2. Cell viability of MDA-MB-468 cells after MFH with targeted and non targeted nanoparticles for 1h, 6h and 10h.

[1] M. Creixell, A.P. Herrera, V. Ayala, M. Latorre-Esteves, M. Pérez-Torres, M. Torres-Lugo, C. Rinaldi, Preparation of epidermal growth factor (EGF) conjugated iron oxide nanoparticles and their internalization into colon cancer cells, J. Magn. Magn. Mater, In Press, Corrected Proof (2010).

Manipulating Ferrofluid at a Distance: Magnets Pushing and Dynamic Control

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We show experimental results for manipulation of a fluid of magnetic nanoparticles at a distance. In the first instance we show how a novel arrangements of just two permanent magnets can push ferromagnetic particles (in contradistinction to any single magnet, electric or permanent, which can only pull particles towards itself [1]). This enables a handheld system to act as a non-invasive syringe to inject particles in after topical application on a wound surface or to deliver them to inaccessible regions such as the inner ear. In the second instance we show precision manipulation of a single droplet of ferrofluid by four dynamically controlled electromagnets. Our approach is optimal in the sense that it uses minimum electrical power to achieve manipulation and is an example of precision control of a ferrofluid by magnets acting at a distance.

The magnetic force on ferromagnetic particles is along the gradient of the magnetic field squared ($F = k\,VH^2$), it is always from low to high magnetic field (H) which is why any given magnet will always attract particles to its corners, the regions of highest magnetic field. However, just two magnets can be arranged in such a way that the magnetic field will cancel at a node away from both magnets. Around this node the magnetic field does not cancel, is not zero, and so the forces go outwards from this node, the local magnetic field minimum, to effectively push particles away.

Figure 1: Two opposed magnets create a local magnetic field minimum inside the petri dish, 25 nm ferromagnetic particles freely floating in solution are pushed outwards creating the visible plume.

This type of idea can be extended to dynamically optimally push (and pull) particles to control the motion of a droplet of ferrofluid. Here control is achieved by feedback: the location of the droplet is sensed by a camera, 4 magnets are then optimally actuated to move the droplet from where it is to where it should be. Our optimization algorithm takes into account magnet strength, saturation, and electromagnet charging times, it applies the minimal set of currents to move the droplet, and as the droplet travels over its path the actuations switch smoothly from pushing (by creating local field minima as above) to pulling depending on which set of magnets is closest to the droplet.

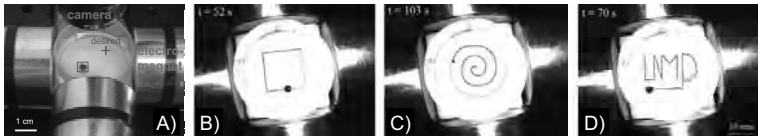

Figure 2: Four controlled electromagnets manipulating two droplet sizes (a trajectory trace is shown)

The two examples above, one static and the other dynamic, are letting us build up methods and capabilities to better direct ferrofluid to desired targets. The next step is to extend the experiments above to focus a distributed ferrofluid (one that is not conveniently held together by surface tension) to deep targets, as we show theoretically and numerically in [2]. For *in-vivo* applications, in the long term, the camera can be replaced by MRI, gamma, or PET imaging and increasing our magnet strength to MRI levels will allow the same control forces at a depth of tens of centimeters.

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2. Shapiro, B., *Towards Dynamic Control of Magnetic Fields to Focus Magnetic Carriers to Targets Deep Inside the Body.* Journal of Magnetism and Magnetic Materials, 2009. **321**(10): p. 1594-1599.

PEI 600 Da conjugated to magnetic nanobeads as a non-viral vector for gene delivery

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Polyethylenimine (PEI) is a wide known cationic polymer used as a non–viral gene delivery vector. Up to date, defining the balance between high transfection efficiency and high toxicity of the polymer remains a crucial problem. Low molecular weight PEI (e.g. PEI 600 Da) is of great interest for its application as a non-toxic vector for non-viral transfection, however its' efficiency remains too low.

The present work was focused on enhancement of transfection efficiency of low molecular weight PEI by its' association with magnetic nanobeads (MNB).

At first, PEI 600 Da/DNA complexes were formed with following association with MNB as shown at the Fig.1. Afterwards these complexes were successfully used for transfection in the following cell lines: COS-7, HEK-293, NIH 3T3. Parameters such as NP and PEI/MNB ratios, DNA dosage were optimized. Transfection efficiency has been evaluated by GFP expression and luciferase assay. Cell viability has been evaluated using MTT cytotoxicity assay.

The results showed 10-times fold enhancement of gene expression after transfection with low molecular weight PEI, conjugated to MNB in comparison with PEI 600 Da alone (Fig.2). MTT cytotoxicity assay showed cell viability more than 80%.

In conclusion, low molecular weight PEI associated with magnetic nanobeads might be an efficient non-viral vector for gene delivery with moderate cytotoxicity, with the following application for magnetically driven transfection.

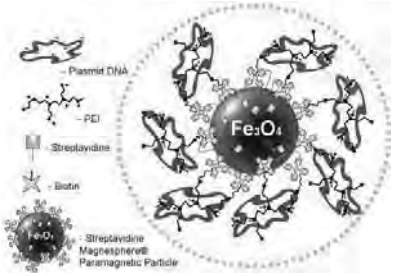

Fig.1 Schematic interpretation of magnetic nanobead-polyethylenimine-DNA complex

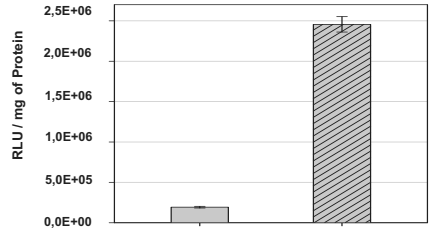

Fig.2 Luciferase expression in COS-7 cell line after transfection

[1] McBain, S.C., H.H.P. Yiu, and J. Dobson, *Magnetic nanoparticles for gene and drug delivery.* International Journal of Nanomedicine, 2008. **3**(2): p. 169-180.
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Fallacy of the Conventional Method for Determination of the Blocking Temperature of Magnetic Nanoparticle Systems

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Magnetic nanoparticles are used in a variety of applications, ranging from well-established niches like ferrofluids for printer inks, to more recent areas in biomedicine like hyperthermia and drug delivery for cancer treatment. One of the primary characteristics of these nanoparticles, used to assess how appropriate a given synthesized nanoparticle is for an application, is the blocking temperature (T_B). This is the transition temperature below which the nanoparticles act like ferromagnets and above which they behave like "super" paramagnets. It is often used to help determine how likely the nanoparticles are to interact and agglomerate, and therefore how colloidally stable the system will be.

The conventional method for measuring the blocking temperature is to cool the nanoparticles in zero field. At the base temperature, usually 4.2 K, a non-zero field (ranging between 400-80,000 A/m [5-1000 Oe]) is applied, and the magnetization is measured during warming and subsequent cooling. The location of a peak in the warming curve is assumed to be the blocking temperature. Unfortunately, this is simply incorrect. The existence of this peak does not depend on the blocking temperature at all, but rather on the magnitude of the field used relative to the coercivity of the nanoparticles in that temperature range. In fact, this peak can be reproduced in ferromagnetic thin films (10-30 nm thick) of cobalt when the field applied is chosen to be near the coercive field of the film in the temperature range of interest. Therefore, this peak can be manipulated to appear at whatever temperature is desired, or not to appear at all.

However, to truly determine what the blocking temperature is, it is necessary to return to the original formulation by Néel in 1949 and its corollary in 1959 by Bean and Livingston. From this, it becomes apparent that the blocking temperature depends primarily on the time scale of the measurements and the size and anisotropy of the nanoparticles. It also requires that the nanoparticles be non-interacting. The latter is a necessary pre-condition to having a superparamagnetic system at all. Therefore, the system must first be diluted in order to render negligible the magnetic interactions between the nanoparticles. Then, depending on available equipment and the physical properties of the sample, several different techniques are available. For monodisperse systems, AC susceptibility, temperature dependent Mossbauer spectroscopy, and the remnant magnetization as a function of temperature will measure the blocking temperature. However, if the system is not monodisperse, as is the case in most real systems, then the polydispersity of the sample will blur out the determination of the blocking temperature using any of the above three methods. In this case, the only truly accurate measurement is a measurement of the hysteresis loop as a function of temperature. Then by plotting the M/T vs. H, the onset of the blocking temperature can be determined, as well as the percentage of the sample that is superparamagnetic at any given temperature.

Poster 43

Functinalized paramagnetic iron oxide nanoparticles (PIONs) for the application of targeted muscle damage therapy

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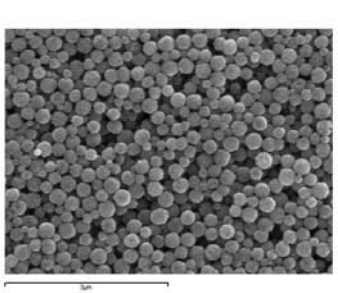
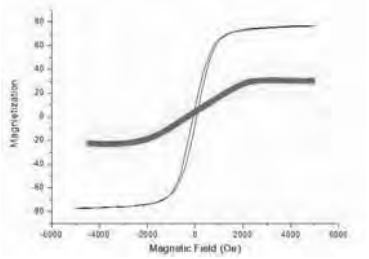
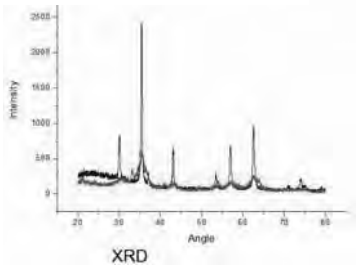
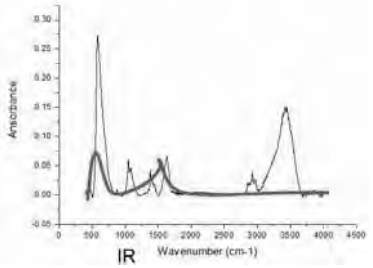
Paramagnetic iron oxide nanoparticles (PIONs) have been synthesized by an aqueous precipitation route. After the PIONs' surface was carboxylated, 1-ethyl-3-(3-dimethyl aminopropyl) carbodiimidehydrochloride (EDC) and N-hydroxysuccinimide (NHS) were used to activate the carboxyls on the surface of PIONs. The activated carboxyls reacted to the amimo groups on the mouse basic fibroblast growth factor (mbFGF) which eventually was coupled to the PIONs' surface targeted for magnetic field navigated muscle damage therapy. The size, magnetism, and chemistry of PIONs were characterized by TEM, VSM , X-ray diffraction and IR, resepectively.









Poster 44

Fractionation of Magnetic Microspheres in a Microfluidic Spiral

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Magnetic microspheres (MMS) typically show a broad size distribution. For clinical magnetic drug targeting, uniform MMS are preferred so that each microsphere acts the same way towards the guiding external magnetic field. Furthermore, the particles must be smaller than red blood cells, but should be large enough to react to the applied magnetic field. Since the preparation of monodisperse MMS can be challenging, size dependent fractionation of MMS of a wide size distribution is a useful tool to obtain MMS with a narrow size distribution.

Here we present a microfluidic chip for continuous MMS fractionation. The fractioning is based on Dean-forces acting on suspended particles in a curved channel. Depending on the flow velocity particles above a certain size move to the inner wall of the bend due to two counter rotating Dean vortices in the channel. The Dean force can thus be thought of as the opposite of centrifugal force. A microfluidic spiral structure was designed and built in PDMS technology in order to take advantage of the Dean effect for particle separation.

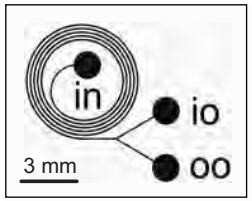

Fig. 1: Schematic of the microfluidic fractionation system with inlet (in), outer outlet (oo), and inner outlet (io).

The chip for MMS fractionation consists of an inlet in the centre of the spiral, the spiral structure, and a 1:1 splitter at the end of the spiral, which separates the flow and directs the two streams toward an inner and an outer outlet. The spiral channel is 100 µm wide and 60 µm high and has 5 windings, with an overall diameter of about 6 mm (**Fig. 1**). MMS suspensions are pumped through the fractionation chip by means of a syringe pump.

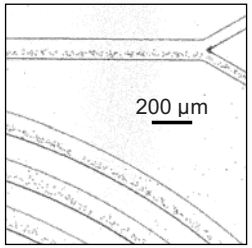

Fig. 2: Microscope image of the spiral with the splitter. Particles accumulate in the inner fraction.

In experimental runs the suitability of the chip was confirmed. In a first experiment the flow velocity was adjusted in a manner that all of the nearly monosized particles of size 10 µm (micromod) could be found in the inner fraction (**Fig. 2**). In the next experiment a mix of 2 µm and 12 µm large particles was fractionated. The outer fraction contained only the 2 µm particles and all of the 12 µm particles could be found in the inner fraction. Following this a specially prepared batch of MMS (chemicell) with a broad size distribution and a mean diameter of 3.5 µm was fractionated into fractions with mean diameters of 2.7 and 4.2 µm.

To conclude, the chip is thus able to separate different particle fractions in an ongoing process (not batch processing). The size fractions can be chosen simply by adjusting the flow velocity of the carrier fluid.

The authors thank *chemicell* GmbH, Berlin, Germany and *micromod* Partikeltechnologie GmbH, Rostock, Germany for MMS samples. The work was funded by the "International Bureau of the Federal Ministry of Education and Research of Germany" and by a fellowship within the "Postdoc-Programme of the German Academic Exchange Service (DAAD)".

Magnetic properties of iron oxide multicore nanoparticles classified by asymmetric flow field-flow fractionation

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Magnetic nanoparticles are very useful for various medical applications whereas for some applications a relative large particle size is advantageous (e.g. hyperthermia or magnetic cell separation). Because of the statistically oriented magnetic axes in the magnetic multicore nanoparticles (MCNP) the resulting magnetic moment without an external field is relatively low which causes a good stability against sedimentation for these relatively large particles (mean core diameter: 50 nm). The possible suitability of MCNP for different biomedical applications was shown before [1].

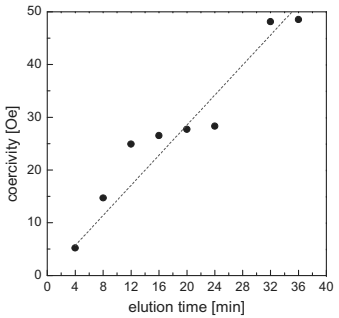

Fig. 1: Coercivity of the particles in the respective fractions obtained at different elution times.

In this contribution we describe the modification of the magnetic properties of the MCNP by size dependent fractionation. Fractions of MCNP with defined but different sizes were obtained by asymmetric flow field-flow fractionation (A4F). In this method a cross flow is applied perpendicularly to the main flow in a separation channel to fractionize the sample in dependence on size. Smaller particles diffuse back into the middle of the main flow faster than larger ones and elute first [2].

A clear increase of the particle size with increasing elution time was confirmed by multi-angle laser light scattering coupled to the A4F system, dynamic light scattering, and Brownian diameters determined by magnetorelaxometry. By this way 20 fractions of different hydrodynamic diameters in the range from 100 to 500 nm were obtained. The hysteresis curves with the typical parameters coercivity and relative remanence were measured by vibrating sample magnetometry. A strong correlation between particle size and magnetic properties could be confirmed. The coercivity which is in direct correlation with the specific heating power varies from 5 Oe to 50 Oe (Fig. 1).

Different fractions of a MCNP containing fluid obtained by A4F were characterized concerning their magnetic properties for the first time.

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How synthetic and bacterial magnetic nanoparticles influence human leukocyte activity

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The examined biological activities of leukocytes belong to the first defensive mechanism of organism to dangerous matter. The interaction of the magnetic nanoparticles with cells depends on various parameters, e.g. cell type, duration of incubation or presence of inhibiting or supporting agents[1]. We have gradually tested the influence of magnetic chemically synthesized magnetite particles coated by sodium oleate and PEG (MP) and bacterial magnetic particles called magnetosomes (M) obtained from magnetotactic bacteria Magnetotacticum Magnetospirillum AMB-1, on the process of phagocytosis and the metabolic activity in leukocyte. Lyzozyme activity is organized into oxygen-independent liquidation mechanisms of engulfed microorganism and peroxidase activity into oxygen-dependent one. The both of this activity belong to nonspecific immunity. We observed the leukocyte cell count in dependence on concentration of nanoparticles during the 18 hours incubation in RPMI-1640 with 10% fetal bovine serum under the 5% CO_2 at 37˚C. Control sample (K) contains $3.4x10^6$ cells. As it is seen in Fig.1, increasing concentration MP and M caused decrease cell number in all samples in comparison to K. The both tested samples M and MP lysed leukocyte cells during incubation. As it can be seen MP with concentration 10 and 20μg/ml lysed almost all leukocytes during 18 hours incubation and their cell viability was in the 14±0.05% range. On the other hand magnetosomes begin to influence leukocyte activity at the concentration of 1 μg/ml and this influence grows with increasing concentration up to 20 μg/ml. The magnetic nanoparticles interact with human viable cells without any further specific groups, attached to the cell surface and are incorporated into vesicles via endocytotic mechanisms. Most of the nanoparticles are retained in the cells within vesicles, mainly phagosomes[1]. With respect to obtained results for metabolic activities of human leukocyte, we can concluded that magnetosomes are more suitable for biological applications than synthetic magnetic spherical nanoparticles which are more aggressive material than magnetosomes and their using is unavailable for this types of the test mainly for the concentration 10 and 20 μg/ml.

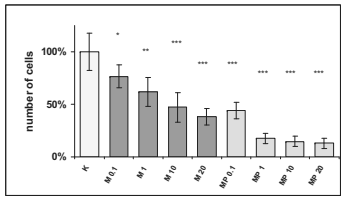

Fig. 1: Number of leukocytes for incubation in CO_2 at 37˚C, n=10

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Acknowledgement:
This work was supported by the Slovak Academy of Sciences (grants VEGA Nos. 2/0077/09, 1/4290/07, 2/0051/09 and Nanofluid, Centre of Excellence) and Development of technology of magnetic fluids for biomedical applications Project No. 26220220005.

Effect of MNP-core size and aggregates of MNP on the signal amplitude for Magnetic Particle Imaging

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Magnetic Particle Imaging (MPI), a new imaging modality in medicine, relies on the measurement of the nonlinear part of the magnetisation curve ($M(H)$) of magnetic nanoparticles (MNP). For a high spacial resolution and a high sensitivity the $M(H)$-curve should be as steep as possible. Thus, larger MNP-moments, i.e. large core diameters, e.g. 30 nm for magnetite [1], are favorable, while small particles (e.g. 5 nm) show a much smaller signal. In order to evaluate this claim, we investigated MNP with small (6 nm) and large (20 nm) mean core sizes, where the latter particles have also a very narrow size distribution (σ=0.1). In addition to available TEM-data (Transmission Electron Microscopy), we used Magnetorelaxometry (MRX) and $M(H)$-data to estimate the size distribution of the effective magnetic particles . The MPI-signal amplitude was calculated using the data of quasistatic $M(H)$-measurements. In this approximation the reversibility of this magnetisation behaviour (no hysteresis at high frequencies) was presumed.

We found that the estimated MPI-signal amplitude of the 20 nm MNPs amounts to 57% of its theoretical maximal value ($M(H)$-step) and is 200 times larger than that of the 6 nm MNP. Next, we investigated Resovist (the actually applied tracer material for MPI-measurement), the magnetisation curve of which ranges in between of mentioned 6 nm and 20 nm MNPs (Figure), and found a normalised signal strength of 30%. This is surprising, because for Resovist the mean size of the single cores is known to be about 5 nm, only. On the other hand, the results of the size distribution, measured by MRX, PCS and finally $M(H)$, complemented by Small Angle X-ray Scattering (SAXS)-data [2] indicate the existence of aggregates. The mean hydrodynamic diameter of about 55 nm is accompanied by an effective, mean magnetic diameter of the aggregates of about 23 nm. A satisfying description of the $M(H)$-curve of Resovist was possible under the assumption of a bimodal size distribution, where a fraction of about 30% of the total particle volume is represented by aggregates. Separating the MPI signals of this two size fractions we found that the MPI-signal amplitude of Resovist is dominated by the aggregate's part. Furthermore, the effects of MNP-concentration and MNP-immobilisation on the MPI-signal were investigated.

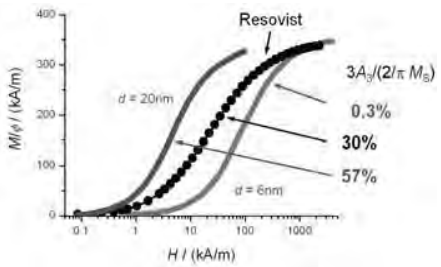

Figure: Magnetisation curves of Resovist, normalised to the magnetite content ϕ, together with two suspensions of magnetite MNPs with a mean core diameter of 6 and 20 nm, respectively. The related, normalised MPI-signal amplitude for a drive field strength of 25 kA/m is indicated.

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Immobilization One Thiadiazole Derivatives in Magnetite Which Shell by Mesoporous Silica & Application in Heavy Metal Removal from Biological Sample

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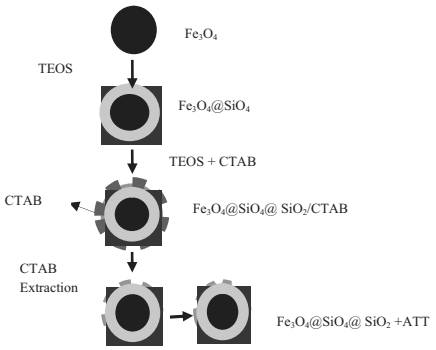
With both nanoparticles and nanocapsules, magnetic behavior is desirable, to allow the use of magnetic fields to direct the delivery vectors and increase their residence time in the vicinity of the target area. However, it must be noted that most metals with strong magnetic behavior, such as iron or cobalt, show toxicity problems when they are present in quantities exceeding those required for accomplishing their biological functions, and therefore, they need to be encapsulated in biocompatible covers to prevent redox reactions when they are free in the blood. Silica and related materials appear to be good candidates for this approach[1]. In the other hand Mesoporous silica microparticles are a class of nanostructured materials with nanometer pores that are currently thought to have potential applications as drug delivery vehicles because of their large surface area, high pore volume, and tunable nanoscale pores. and the degradation product can diffuse through the local tissue, enter the blood or lymph, and are finally excreted through the kidneys in the urine and[2,3,4] .Recently, much attention has been focused on 1,3,4-thiadiazole derivatives due to their broad-spectrum activities such as antitumor, analgesic, anticancer, anti-inflammatory, and antibacterial activities[5]. Also among possible functional groups, the mercapto compounds have received considerable attention as heavy-metal ion trapping agents[6].



Here in this report magnetite was senthesized by coprecipitation method then shelled with a layer of silica and at the next step a layer of mesoporous silica was covered outside the nanoparticles by sol-gel method then 5-amino-1,3,4-thiadiazole-thiol (ATT) immobilized in synthesized nanoparticles with simple procedure. and followed by a series of characterizations, including transmission electron microscopy (TEM), nitrogen physisorption, FT-IR spectrum and elemental analysis. heavy metal uptakes of modified nanoparticles were then examined by atomic absorption spectroscopy. For more investigation Cu was preferred; then amount of adsorption, kinetic and mechanism of adsorption was inquired in water and at the end power of nanoparticles for heavy metal removal from blood was shown.

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Poster 49

GLYCINE NITRATE PROCESS FOR THE ELABORATION OF MANGANITE NANOPARTICLES AS POSSIBLE SELF-REGULATING MEDIATORS FOR HYPERTHERMIA

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One promising route for hyperthermia uses alternative magnetic fields allowing magnetic materials to absorb electromagnetic energy and convert it into heat. In particular, magnetic fluid hyperthermia takes advantage of magnetic nanoobjects dispersion which could be administered by intravenous injection. Among the numerous specifications required, one should retain: (i) the *in vivo* temperature must be controlled (42-46°C) in order to avoid overheating of safe tissue (ii) the magnetic core size should be controlled in order to avoid embolization risks, and (iii) it must be surface-derivatized not only for ensuring their stealthiness in the blood compartment towards macrophages (e.g. pegylation) but also for being labelled with appropriate targeting ligands. Concerning the first requirement, our strategy is to take advantage of the temperature-dependence of the magnetic properties of manganese perovskites $La_{1-x}Sr_xMnO_3$, which Curie temperature may be tuned from –130 to 100°C by varying Mn^{3+}/Mn^{4+} ratio. So, as soon as medium temperature reaches Curie temperature, particles will loose their magnetic order and therefore their heating ability. Such particles would be both heaters and fusers [1].

That is why few papers dealt for instance with magnetic suspensions based on manganese perovskites of the type $La_{1-x}Sr_xMnO_3$ (Lanthanum Strontium Manganese Oxide, LSMO) [2-6]. While the parent compound $LaMnO_3$ is a single-valent (Mn^{3+}) antiferromagnetic insulator [7], replacement of lanthanum by strontium ions causes a gradual decrease of the steric distortions and the structure changes from the orthorhombic to rhombohedral symmetry. This leads to an insulator–metal transition and due to double-exchange interactions to ferromagnetic ordering with a Curie temperature in the range 283-371 K for $0.175 \leq x \leq 0.4$ [8].

These nanoparticles were prepared by several routes: the freeze drying technique [2], sol-gel technique employing citric acid and ethylene glycol [3,4], microwave refluxing technique [5], and conventional solid-state reactions [6]. Nevertheless, each of these techniques necessitate an extra stage of calcination/annealing at a temperature over 700°C in order to get well-cristallized compounds. Therefore, the size distribution of the nanoparticles was large and "connecting bridges" between the individual grains were systematically observed. In order to narrow the size distribution, high-energy planetary ball-milling [2-6], rolling/milling [5] and size sorting [4] steps were combined, making these synthesis routes time- and precursor-consuming.

That is why we investigated another route based on the Glycine Nitrate Process (GNP). Among combustion synthesis routes, GNP was firstly proposed in 1990 by Chick et al. to elaborate chromite and manganite powders [9]. This process uses the energy released during the highly exothermic reaction between a reducing agent (glycine) and an oxidizing one (nitrate) which enhances the particles nucleation to the detriment of their growth.

The main goal of this communication is to validate the suitability of the GNP to prepare easily and efficiently $La_{1-x}Sr_xMnO_3$ nanoparticles filling the requirements for hyperthermia applications: unaggregated nanoparticles with grain size between 15 and 50 nm, capable to heat in AC magnetic fields and to be coated by a silica layer in order to be biocompatible, easily surface-derivatized and stable in aqueous dispersions [10,11].

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Poster 50

Three Dimensional Dynamics of Ferromagnetic Swimmer

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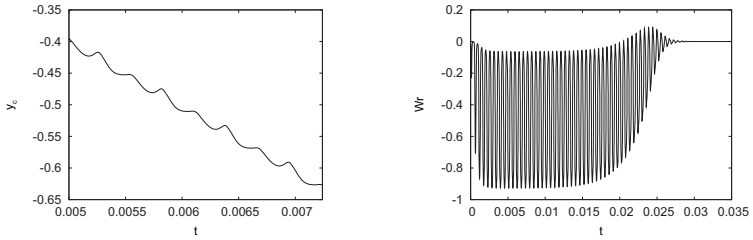
There is growing interest in creation of bioinspired microdevices to carry out different functions in biotechnology and medicine.

We are developing a new route for the creation of such devices, which are powered by the AC magnetic field. They are created by linking with 1000 kb long biotinized DNA fragments commercially available functionalized core-shell ferromagnetic particles. Magnetization curve of the diluted sample of the ferromagnetic particles shows hysteresis with the coercitive force $H_c = 550\ Oe$ and specific remnant magnetization 3 emu/g.

Filaments of the linked ferromagnetic beads in magnetic fields $H < H_c$ are magnetized along their axis and orient in the direction of the magnetic field. Abrupt inversion of the direction of the magnetic field leads to the formation of the loop and its symmetry breaking. Experimentally and numerically it is shown that the loop is unstable with respect to the three dimensional perturbations and after some transition period which depends on the magnitude of the initial perturbation relaxes to the straight configuration. In the unidirectional AC field the filament self-propels in the plane of its initial curvature perpendicularly to the AC field. Its locomotion consists in periodic sequence of power and return strokes similarly to some biflagellate algae. Dependance of the displacement on time for four periods is shown in Figure (left). It is possible to notice period doubling – the same return strokes repeat after two periods of AC field. The motion of the filament in the plane is unstable with respect to the three dimensional perturbations. This instability is characterized by the calculation of the writhe number. For the initial inclination angle of the plane of curvature to the field 10^{-4} and $Cm = 72$ it is shown in Figure (right). It shows that after transition period which depends on the frequency of the AC field the writhe number becomes equal to zero. This corresponds to the transition from the oscillating U-like shape to the oscillating S-like shape oriented perpendicularly to the AC field. Unusual orientation of the ferromagnetic filament perpendicularly to the AC field is caused by its flexibility – neutral with respect to the AC field perpendicular orientation due to the deformation has lower mean energy per period than the straight configuration along the AC field.



Colloidal Nanoparticle Interactions in Liquid Environment Quantified by Cryogenic Electron Microscopy

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Biomedical applications of nanoparticles take place in liquid environment and depend crucially on the colloidal interactions between the nanoparticles.[1] Our aim is to study these interactions on the single-particle level *in situ* in the liquid. An approach that we have introduced to study the interactions between magnetic or semiconducting nanoparticles is cryogenic electron microscopy.[2-6] Dispersive, magnetic dipolar, electric dipolar, and coulombic interactions affect the positions of the thermally moving nanoparticles, leading to different local structures in the liquid. For example, magnetic nanoparticles self-assemble into living polymer structures that grow in an external magnetic field. In zero field, semiconductor quantum dots exhibit similar linear structures, which we ascribe to the presence of a permanent electric dipole moment. The figure below is a recent unpublished illustration of the response of magnetic self-assembly nanostructures to an increase in coulombic repulsion. In all cases, not only do the interactions lead to a rich variety of nanostructures in the liquid dispersion, but conversely, the visualized structures constitute a powerful characterization of the colloidal interactions. Moreover, besides obtaining interesting qualitative information, we are able to analyze the colloidal interactions quantitatively, by determining the precise positions of individual nanoparticles in the cryogenic snapshots and by applying statistical thermodynamic theory.

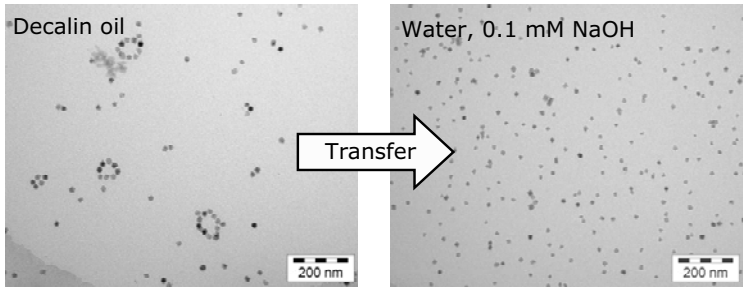

Figure. Magnetic oxide nanoparticles before and after transfer from decalin oil to aqueous medium viewed by cryogenic transmission electron microscopy. For the transfer to water, the surface of the nanoparticles was modified using polyacrylic acid.[7] In the oil, sterical repulsion is shorter ranged than magnetic attraction and magnetic structures such as flux closure rings are observed, whereas in water at low ionic strength, long-ranged electrostatic repulsion dominates and the particles are separate.

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Preparation, Characterization and Testing of Alginate based Magnetic Carriers and others for Arsenic Removal from Water

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Abstract

Arsenic content in water is a severe health problem is several countries. To remove it from water we are developing a research project that applies magnetic separation methods. In order to increase the removal efficiency we are using magnetic aggregates. These magnetic aggregates are iron-based and were all (except for one) prepared by us. In a first stage, only pure magnetic aggregates were used. On a second stage alginate covered iron-based cored aggregates were prepared (see Figure 1). In this paper we report the preparation steps and some of the results of the characterization and absorption tests we have performed (see Figure 2) in order to conclude about its structure and efficiency in what concerns arsenic removal. We also compare the results obtained between the magnetic aggregates that contain an alginate cover with the ones that do not.

Figure 1 - Alginate covered iron-based core magnetic aggregates.

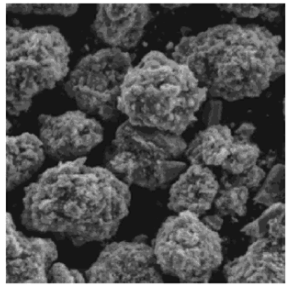
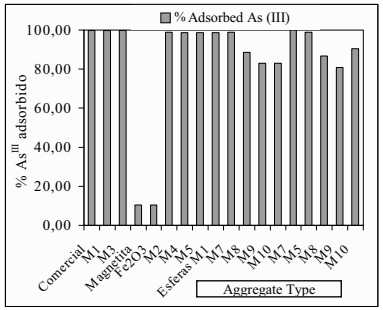


Figure 2 - Arsenic adsorption in the several prepared aggregates.



Preparation and characterization of Selol-loaded magnetic nanocapsules for hyperthermia cancer therapy

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Magnetic nanocapsules (MNC), specifically those capable of tag a particular tumor, offer the potential to deliver site-selectively, and even cell-specifically, heat to the site or microscopic environment of a tumor [1,2]. They can be engineered for using in cancer therapy to produce damaging heat while in the presence of external AC magnetic fields. Magnetic polylactic-co-glycolic (PLGA) nanocapsules loaded with Selol (a Selenium-based anti-cancer drug [3]) were successfully prepared by the nanoprecipitation method [4]. Maghemite (γ-Fe_2O_3) nanoparticles were incorporated into the nanocapsule using a highly-stable ionic magnetic fluid sample [5]. The obtained nanocapsules present no agglomeration while revealing a narrow monomodal size distribution, with average diameter in the range of 250-300 nm and size dispersity index around 0.3. Preliminary data demonstrated that Selol-loaded magnetic nanocapsules represent a novel and promising magnetic drug delivery system incorporating nanotechnology and hyperthermia. Such combination is highly desirable aiming reduction of side effects and toxicity and prolongation of the pharmaceutical drug efficacy. Therefore, the results herein reported represent an important breakthrough for developing studies based on *in vitro* and *in vivo* protocols and future clinical trials in hyperthermia cancer therapy.

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INFLUENCE OF COBALT CONTENT ON THE HYPERTHERMAL EFFICIENCY OF FERRITE NANOPARTICLES

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In the last decade magnetic nanoparticles had a large impact in biomedicine, especially towards promising areas like contrast agents for magnetic resonance imaging and hyperthermia therapy (MFH) [1,2]. The most studied materials are iron oxides (magnetite and maghemite), thanks to their biocompatibility. However, there is an increasing interest to investigate other kind of materials, like mixed ferrites, metals and core-shell structures, in order to improve the hypertermic efficiency. Among the different types of magnetic particles, cobalt ferrites seem to be excellent candidates in terms of hyperthermic performance, while their use for actual treatment is hampered by the high toxicity of cobalt. One of our newest activities in this widespread scenario is the attempt to obtain cobalt ferrite-based hyperthermia vehicles that exhibit reduced toxicity, while retaining their unique magnetic properties.

In this contribution we present the synthesis, the investigation of the static and dynamic magnetic properties and the hyperthermic efficiency of highly monodisperse ferrite particles with average size of few nanometers containing different amounts of cobalt ($Co_xFe_{3-x}O_4$, $0.1<x<0.7$), obtained by thermal decomposition of metal-organic precursors (metal acetylacetonate) into hot solvents in the presence of a coordinating surfactant. Calorimetric measurements of Specific Absorption Rate (SAR) showed that there is a limit in size (>6 nm) to obtain a significant heating, independently on the Co content. For larger particles we found that, even for low cobalt content, which makes them less toxic with respect to stoichiometric cobalt ferrite, these particles exhibit a very high hyperthermic efficiency.
The careful correlation between magnetic and hyperthermic properties of these materials allowed us to conclude that such unusual behaviour is probably to be ascribed to the distribution of divalent ions among tetrahedral and octahedral sites in the spinel structure of the ferrites changing with the Co content. This hypothesis is under further investigation by structural studies with specific techniques, like EXAFS and Magneto-Optic Spectrocopy.

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Poster 55

Size-dependent magnetic relaxation in magnetite nanoparticles: lessons for Magnetic Particle Imaging

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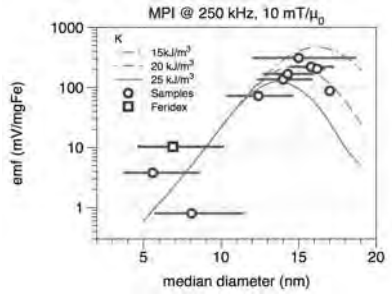
Magnetic Particle Imaging (MPI) is a promising method of visualizing magnetic nanoparticles *in vivo* that shows significant potential for biomedical imaging[1]. In MPI, the amount and location of magnetic nanoparticles (MNPs) are determined non-invasively, using a combination of RF and DC magnetic fields. MPI exploits the nonlinear magnetization of superparamagnetic MNPs to enable detection and spatially resolved imaging, and since MPI signal is generated from nanoparticle magnetization alone, it is free from spurious background signal. With so many recent innovations in nanoparticle synthesis, it is possible to produce MNPs in a wide variety of sizes, shapes and compositions; in order to engineer optimized MNPs for MPI, we must develop a clear understanding of MNP magnetization dynamics[2]. MNPs exhibit complex, size-dependent behavior in RF fields. In this work, we use our previously developed model[3] of dynamic MNP magnetization to experimentally optimize magnetite MNPs. The low-RF regime used in MPI corresponds closely to the magnetic relaxation times of magnetite MNPs with diameters between 10-20 nm; interestingly, these are the sizes most suitable for MPI. Within this range, our model predicts size-dependent magnetic relaxation that leads to significant enhancement in MPI signal.



We have synthesized magnetite MNPs in the target size range, measured their MPI signal at 250 kHz and observed size-dependent behavior that agrees with our model and demonstrates that proper optimization can yield signal gains of 30x over commercially available samples (figure compares MPI signals from monodisperse magnetite MNPs with Feridex)[4]. In this paper we present additional measurements at 500 kHz showing the generality of our optimization method. We also include detailed measurements of the size-, and concentration-dependent magnetic anisotropy. Finally, we draw some general conclusions about how MPI designers can tailor imaging systems and match them to particle characteristics to best exploit unique MNP magnetization dynamics.

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Poster 56

Oleate coated magnetic cores based on magnetite, Zn ferrite and Co ferrite nanoparticles – preparation, physical characterization and biological impact on *Helianthus annuus* photosynthesis

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Magnetic nanoparticle stabilization in liquid media can be accomplished by convenient choosing of coating molecular shell. In the case of oily magnetic suspension the oleic acid was found to be the best surfactant – the corresponding nanoparticle dispersions being the most stable within a wide range of carrier oily liquids. We studied the efficiency of oleate ions - from sodium oleate – as coating shell in the case of some aqueous magnetic suspensions, prepared starting from magnetite, Zn ferrite and Co ferrite powders intended for further biotechnological applications. In all cases the magnetic nanoparticles were co-precipitated from mixtures of ferric chloride and bi-valent metal (Fe, Zn, Co) sulfate in their respective stoichiometry - in the presence of 25% NaOH. For about 4 g of precipitated nanoparticles, 2 g sodium oleate in 20 ml of deionized water was added - continuous stirring and high temperature (over 80 ^{0}C) being assured during both preparation stages - for 60 minutes. Physical characterization was carried out by applying X-ray diffraction and Vibrating Sample Microscopy. XRD patterns show that all samples are well crystallized with a cubic structure, with no significant line broadening or detectable signal from of any other crystalline or amorphous phase. The influence of magnetic nanoparticle suspensions (diluted in the range: 20-100 microl/l, corresponding to magnetic nanoparticle levels of 10^{-14}-10^{-15}/cm^3) on sunflower seedlings during their early ontogenetic stages was studied considering photosynthesis pigment levels in the green tissue samples. Chlorophylls and carotenes in selective extraction solvent were assayed by spectrophotometric method using Lichtenthaler's formulae. Photosynthesis efficiency was assessed by means of the ration chlorophyll A/chlorophyll B and (chlorophyll A+ chlorophyll B)/ (chlorophyllA + chlorophyll B + carotenes)(1). Average values and standard deviations corresponding to five replies were considered for graphical comparative analysis in the case of the three magnetic colloidal suspensions. The results suggest the good penetrability of magnetic colloidal nanoparticles within the plant tissues being concordant with the biocompatible feature of magnetite and the relative toxic effect of ZnO (2).

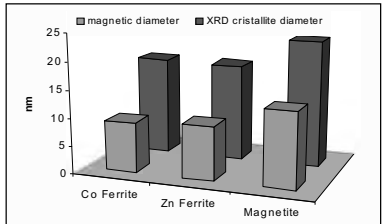

Fig. 1 Physical diameter average estimated by VSM and XRD

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Comparative cytogenetic study on magnetic nanoparticle toxicity in plants

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The response of grassy plant seedlings to magnetic nanoparticle suspension was studied at the level of chromosomal aberrations by applying squash method and Carr reactive dying for optical microscopy. Two magnetic nanoparticle suspensions were used both stabilized with sodium oleate and dispersed in deionized water: first one based on Fe_3O_4 and the second consisting in $ZnFe_2O_3$ powder dispersion, the supply concentrations being the same: 20-40-60-80-100 microl/l. The plant species chosen for the experiment was *Helianthus annuus* L; (sunflower), an agricultural plant with 34 chromosomes. Magnetic nanoparticles were prepared by chemical co-precipitation as shown in [1]. Mitotic activity in the seedling root meristemes was analyzed for every mitosis phase: prophase, metaphase, anaphase and telophase the mitotic index being also quantified (by scoring the dividing cells to the mitotic resting ones). The nanotoxicity of magnetic colloidal suspension was assessed by means of chromosomal aberration percentage. Mitotic index was found diminished in both cases, the differences between the effects of the two magnetic nanoparticle structures being graphically analyzed and interpreted. The main types of chromosomal aberrations identified using immersion objective microscope were: interchromatidian bridges, retard and expulsed chromosomes, chromosomal fragments and micronuclei, no specific chromosomal aberration type being observed for one magnetic suspension or the other. It seems that higher nanotoxicity was found in the case of Zn ferrite powder. As known not all DNA lesions are susceptible of resulting in genetic mutations since molecular repair mechanisms could erase part of them while, on another hand, there are genetic mutations with positive implication in agricultural plant growth. So that not only the toxic side of the magnetic nanoparticles impact needs to be considered but also the benefits resulting from possible biotechnological tool developed by using magnetic nanoparticle supply.

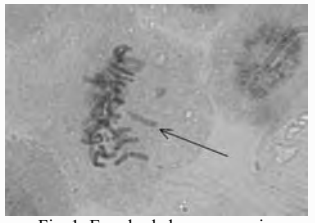
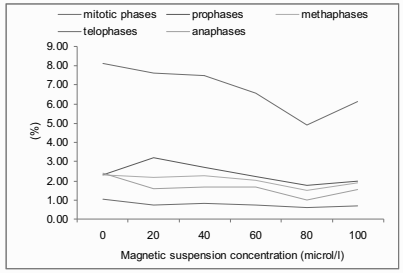


Fig. 1. Expulsed chromosome in metaphase corresponding to 100 microl/l Zn ferrite colloidal suspension



Fig, 2. Cell in different division phases

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Phase transfer of Magnetic Iron-Oxide Nano-Particles using phosphate based ligands

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Superparamagnetic particles of iron oxide have been widely exploited for many biomedical applications such as magnetic resonance imaging[1], hyperthermia[2] and targeted, site-specific delivery of drugs.[3] Recent improvements in the synthesis have yielded highly crystalline and mono-disperse iron oxide nanoparticles[4]. One shortcoming of this method is the particles have very poor bio-compatibility. Attempts have been made to phase transfer these particles. This has yielded mixed results with the particles often loosing dispersion through aggregation of primary particles. Further on from this, due to the nature of the particle coating, the range of phase transfer agents is limited leaving little room for customisation of particle surface for bio-availability.

The aim of this project is to develop a means of phase transferring non-polar suspensions both primary iron oxide nanoparticles, in the size range of 10nm and clusters of iron oxide yielding surface active sites upon which a wide variety of polymerisation can occur. In the reported work, fatty acid stabilised iron oxide nanoparticles were phase transferred using a ligand exchange method. The ligands incorporated phosphate groups. Aqueous suspensions of these particles were analysed using light scattering, zeta potential, ATR-IR and field cycling NMR relaxometry. These particles were subsequently polymerised using a "grafting from" approach and analysed using the above methods and GPC. This method allows for the transfer of highly crystalline iron oxide nanoparticles and subsequent customisation of the surface through direct polymerisation.

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Poster 59

Synthesis Conditions, Interactions and Their Effect on the Anisotropy

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Interactions in magnetic nanoparticle systems are widely prevelant at biologically relevant concentrations, but have been generally ignored in the analysis of magnetic nanoparticle systems. This includes not just a comparitive analysis between different nanoparticle syntheses, but also in the effectiveness of magnetic nanoparticle systems for biological applications like hyperthermia, drug delivery, and contrast agents in magnetic resonance imaging. Here, we have studied iron oxide nanoparticles formed under varying synthesis conditions, to examine the correlation between synthesis and interactions, and how this impacts the measured anisotropy and heating of each system.

Citric acid coated iron oxide nanoparticles were synthesized using a one-step and two-step coprecipitation method at different temperatures. The nanoparticles were determined to be less than 10 nm in size, from x-ray diffraction, with a lattice constant close to that of bulk maghemite (γ-Fe_2O_3). The stability of the nanoparticles in aqueous media was compared using absorbance measurements over a 12 hour period. For stable systems over a reasonable time frame (>12 hours), the nanoparticles were then characterized further magnetically – including the collective anisotropy. This was followed by determining the magnetic heating characteristics in an alternating magnetic field and specific absorption rates. The temperature of synthesis and mode of functionalizing the particles affected both their physical and magnetic properties. Higher temperatures led to increased specific absorption rates for both methods (typically by a factor of about 1.6), but more stable hydrophilic nanoparticles were obtained in the one-step method.

Furthermore, as determined by vector magnetometry, we have shown that the collective anisotropy of a system correlates directly with the interactions between the nanoparticles, not the concentration of nanoparticles present. The implication is that although the concentration defines the average area available to a nanoparticle, it is the interactions that define the stable spacing. This results in a disproportionate enhancement of the heating capability of these interacting nanoparticle systems when compared by amount of material present. This has implications in any biomedical application, where it becomes important to not only maintain these interactions under biological conditions (e.g. pH), but also for the design and understanding of nanoparticle systems.

Poster 60

Highly magnetic nanocomposite actuator for artificial muscle applications

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The combination of force and flexibility enables virtually all body movements in living organisms. Presently used technical machines in contrast, are based on rigid, linear or circular geometries. As a possible alternative, magnetic elastomers can be realized through dispersion of micro- or nanoparticles in polymer matrices and have attracted significant interest as soft actuators in artificial organs, implants, and devices for controlled drug delivery. At present, magnetic particle loss and limited actuator strength have restricted the use of such materials to niche applications.

This contribution reports on the direct incorporation of metal nanoparticles into the backbone of a hydrogel and application as an ultra-flexible, yet strong magnetic actuator [1]. Flame synthesized carbon coated cobalt nanoparticles [2] with vinyl anchor functionality were directly incorporated into the polymerization of 2-hydroxyethyl methacrylate (HEMA). To overcome the problem of inhomogeneously dispersed nanoparticles in the polymer, the carbon shell of the particles was covalently functionalized [3] with an organic component similar to the monomer. Later favored the in-situ polymerization of the magnetic particles with the monomer and resulted in the formation of a highly magnetic, mechanical stable and homogenously dispersed polymer. Since pure metals have a far higher saturation magnetization and higher density than oxides, the resulting increased force/volume ratio afforded significantly stronger magnetic actuators with high mechanical stability, elasticity, and shape memory effect. Depending on the final shape, different polymer blocks (60 wt% nanoparticles with respect to monomer) could be produced offering an alternative approach to flexible and magnetic actuators towards artificial muscles.

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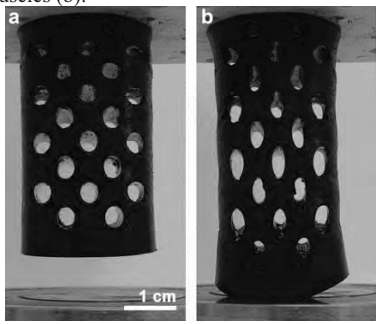
Figure 1: Photo of as prepared soft magnetic hydrogel (a). By applying an external magnetic field the polymer can contract or elongate making it appropriate in application such as for magnetic actuator or even artificial muscles (b).



Activ-Adembeads : A new ready-to-use tool for sensitive magnetic beads-based immunoassays and biosensors

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The use of superparamagnetic beads as label is evident in the detection of analytes in complex samples using microfluidic or biosensors devices. Ademtech has designed calibrated magnetic particles presenting a unique size distribution. Due to its particular patented technology, Ademtech has customized specific surface chemistry meeting the requirements of IVD and Life science sample preparation.

Activ-Adembeads were especially developed for efficient protein immobilisation. These magnetic beads offer an innovating activated surface to interact with proteins, providing assay standardisation without pre-activation step, nor coupling reagents. This new coupling strategy was efficiently carried out with monoclonal and polyclonal antibodies, enzymes or small proteins without loss of functionality. Thus, assay sensitivity can be improved using less reagent required and faster reactions times.

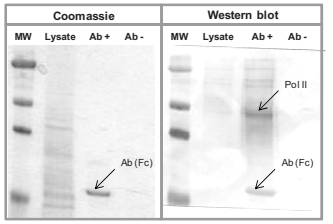

Fig 1. Immunoprecipitation of RNA Pol II from HeLa cells.

On one hand, conjugated Activ-Adembeads have proved their performance in immunoprecipitation applications (Fig1) providing high binding capacity while keeping non-specific interaction low.

On the other hand, Activ-Adembeads can be used in sensitive magnetic beads-based biosensors. Immobilisation of gliadin was successfully performed. Immobilisation yield is higher than 90% (determined by Bradford assay) and ELISA using HRP-conjugated secondary antibody has highlighted efficient orientation of gliadin. Gliadin-conjugated magnetic beads can be used in a competitive assay using electrochemical immunosensor (Fig2).

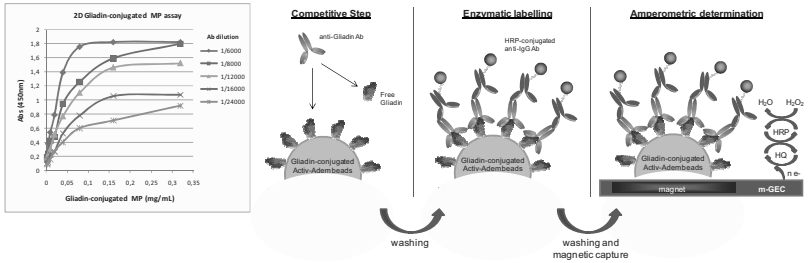

Fig 2. Principle of magnetic beads-based Electrochemical immunosensor for the diagnosis of gliadin.

Therefore, Activ-Adembeads offer great promise for a simple, cost-effective, and user-friendly analytical tool that can be used in immunoassay and biosensors.

DMSA-COATED MAGNETIC FLUID CLEARANCE BY MOUSE LIVER

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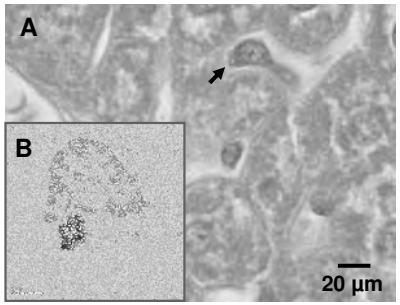

Figure 01: MNPs inside Kupffer cell

Magnetic Fluids (MFs) have emerged as a promising material basis for new possibilities in the biomedical field. In order to better understand the *in vivo* effects of MFs, the present study reports the biodistribution and the biocompatibility in mice liver of a magnetite nanoparticle (MNP) surface-coated with meso-2,3-dimercaptosuccinic acid (DMSA), and its clearance by this organ. The test was performed from one to 120 days after intravenous injection of 100 µl of MF containing about 4.3 x 10^{15} paticles/cm^3. In order to detect the MNPs, all different organs and samples of blood, bone narrow, and feces were homogenized in distilled water following a normative dilution (0.5g of tissue to 0.15 ml of water) and 10 µl of each homogenized were used to obtain the magnetic resonance spectra with sensibility of 10^{11} to 10^{18} particles/cm^3. Mice´s livers were processed for light microscopy (fixation with Davidson at 4 ^oC for 24 h, embedded in Histosec® resin) and electronic microscopy (fixation with Karnovisky at room temperature for 4 h, embedded in Spurr resin). The magnetic resonance analysis showed an initial preference deposition in the lung, followed by the redistribution to the liver and its elimination with the feces. The light and electronic microscopy revealed a biocompatible nanoparticle, since the liver did not show morphological alterations, although a weak inflammatory reaction was observed. MNPs were observed inside kupffer (Fig. 01) and also between hepatocytes, suggesting the elimination by the bile canaliculi as a bile component. In a time-dependent manner, MNPs were mainly detected in the portal space inside the bile ducts and in the intersticial connective, confirming the billiar elimination via of the MFs. The results above suggest that DMSA-MF could be a nanotool for specific biomedical studies and therapies with its clearance been mainly played by the liver.

Multiplug magnetic beads trapping in a capillary with a magnets "necklace"

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Magnetic beads (MBs) have now proven to be a powerful tool in both research and biomedical applications. They are available in a wide range of sizes and their surface can be modified with molecules having biological specificities and functions[1]. In microfluidics, where the goals are faster reaction/analysis time and reduced sample consumption, MBs offer many advantages. First of all, compared to an open microchannel, a packed bed of beads increases the specific surface available for molecule adsorption/binding. The diffusion pathway is significantly reduced, improving interactions between molecules. Moreover, in comparison to classical beads, they can be easily manipulated by magnets[2].

The present investigation presents a multiplug trapping system for MBs in a capillary. The capillary is inserted through a chain of cylindrical permanent magnets alternating with cylindrical non-magnetic spacers. The magnets and spacers are simply placed on the capillary like pearls on a string. The magnets are indeed drilled along their magnetization axis, parallel to the capillary and can be placed either in attraction or in repulsion. This system has the advantage of a very simple assembly. Moreover the axial symmetry and the proximity of the magnets and capillary produce high magnetic forces, giving the opportunity to work at higher flow velocities than with other setups classically made up by two magnets spaced by 1 mm with their magnetization perpendicular to the capillary. Finally only the length of the capillary theoretically limits the length of the magnets chain, and it is possible to adapt the number of magnets to the desired number of plugs, increasing in a controllable manner the surface available for protein adsorption in the case of immunoassays or immunoextraction. Using numerical simulation we have mapped the magnetic force for different geometries. This force was then introduced into a convection-diffusion model to understand the formation of the multiplugs and the influence of the flow velocity on their size and position. Finally as proofs of concept, the accumulation of MBs was visualized by microscopy in a capillary placed between rectangular magnets having a magnetization parallel to the capillary, and preliminary experiments were carried out in a capillary with the designed magnets geometry.

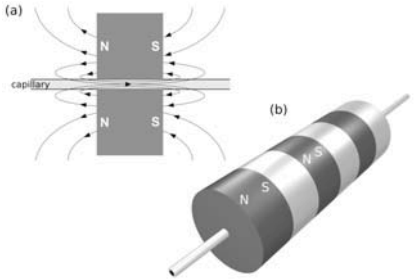

Figure 1: (a) Scheme of the magnetic field lines in a drilled magnet with magnetization parallel to the capillary (b) Magnet "pearl necklace" on a capillary with the magnets in dark grey and the spacers in grey

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Quantitative FMR measurements of Magnetic Immunoassays

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Magnetic immunoassays procedures involve the use of magnetic nanoparticles with the external surface provided with antibodies which have affinity to specific target molecules. Aggregates of these nanoparticles can be formed depending on the magnetic properties, temperature and size distribution. Moreover, these particles may exhibit a strong paramagnetic behavior associated to these parameters [1]. Thus, they have a free behavior of paramagnetic particles in solution without attracting each other. In this work, we report the possibility of using ferromagnetic resonance (FMR) to quantify the magnetic nanoparticles in the commercial CD25 human antibody that recognizes the human CD25 antigen [2]. The size distribution of the magnetic nanoparticles in CD25 obtained by dynamic light scattering (using a 10 mW He-Ne 633 nm laser, ZetaSizer-Malvern 3000HSA) shows an average size of 118 nm and polydispersity of 0.4 as shown in Fig. 1. FMR measures were performed in a JEOL FA200 X-Band spectrometer using only 3 μL solution samples in a standard cylindrical cavity. To quantify the magnetic nanoparticles the FMR spectra of solutions of CD25 in Milli-Q water in different proportions from 100:1 (100%) to 1:99 (0.01 %) were acquired. Fig. 2 shows the detected FMR spectra of the magnetic nanoparticles. Notice that we are able to detect FMR signals of the magnetic nanoparticles in solution 1:99. Using the data presented in Fig. 2 a calibration curve of FMR signal vs % of CD25 in Milli-Q water was made. In Fig. 3 we can observe the linear response of the amplitude peak to peak (App) of the ESR signals to the magnetic nanoparticles concentrations. Unfortunately, we do not have the original concentration value of the magnetic nanoparticles in CD25. Assuming a common initial concentration of 50 mg/ml [3], the results in Fig. 2 and 3 show that we were able to detect magnetic nanoparticle mass between 15 (solution 1:100) to 150 (99:1) nanograms. Improvements can be made in the instrumentation by using a loop gap resonator that can handle smaller volumes than the standard cylindrical cavity with larger sensitivity. The possibility of using FMR has been discussed by other authors [4] and can be an interesting alternative to other methods as SQUID magnetometry, which requires expensive liquid helium.

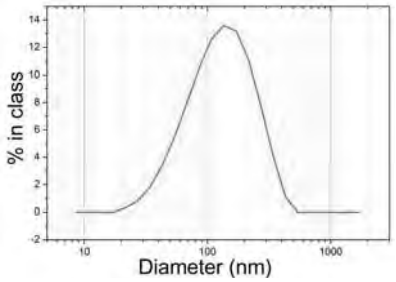

Fig. 2: Size distribution of the magnetic nanoparticles obtained by dynamic light scattering.

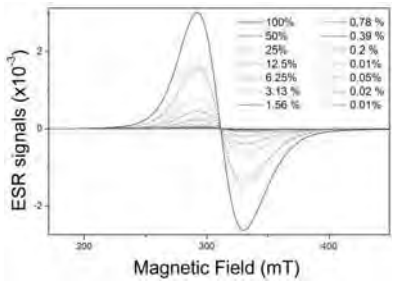

Fig. 1: FMR signals of magnetic nanoparticles in CD25.

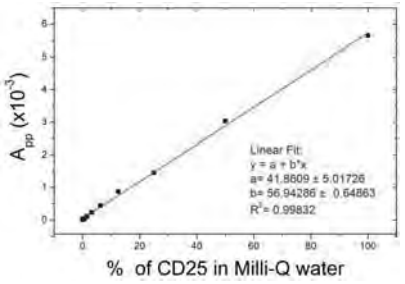

Fig. 3: Linear response of the FMR signal to different concentrations of magnetic nanoparticles in CD25.

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Poster 65

MFM characterization of magnetic nanoparticles for yeast cell labeling

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Magnetically-driven pharmaceutical forms are widely used in the treatment and diagnostics of various diseases. These forms have numerous advantages, such as their ability to be targeted to pathological sites with the permanent magnetic filed as well as their ability to perform gradual release of a drug component using alternating magnetic field. The most common examples of such nanocarriers are the magnetically-driven liposomes and other nanostructures with the magnetic particles entrapped in the inner space. To perform efficient targeting, drug delivery and drug release such nanostructures should have strictly defined magnetic properties. To evaluate these properties it is necessary to know the exact number of magnetic nanoparticles incorporated within the single nanocarrier. It is also useful to have the information about the depth of penetration for each nanoparticle and the formation of various nanoparticle agglomerates in the nanocarrier.
MFM is the efficient method for the visualization of the distribution and the arrangement of magnetic nanoparticles within the nanocarrier. Data obtained from the MFM images can also be used for the evaluation of the depth at which the nanoparticles are encapsulated. In order to evaluate the capabilities of the MFM method for the characterization of the nanoparticles in the single nanocarrier we used the yeast cell culture *Saccharomyces cerevisiae* labeled with the magnetite nanoparticles. Solver Pro-M scanning probe microscope (NT-MDT) was used for the MFM characterization of the nanoparticles in the labeled yeast cells. Obtained MFM images (fig. 1) were processed with the Nova software (NT-MDT). For each nanoparticle we measured the force of its magnetic interaction with the probe and the distance between the probe and the nanoparticle. On the basis of the obtained data we evaluated the depth of penetration for each nanoparticle into the cell wall and the number of entrapped nanoparticles. Similar approach can be successfully used for the characterization of the nanoparticles in the magnetically-driven liposomes and other magnetically-driven pharmaceutical forms.

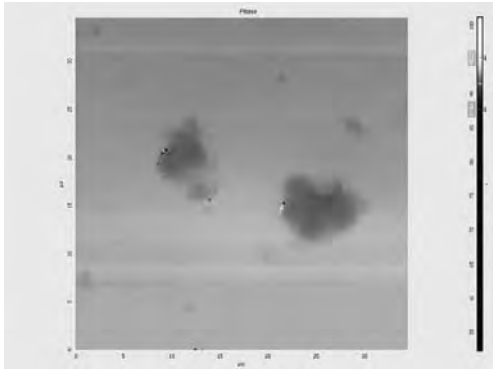

Figure 1: MFM imaging of the magnetic nanoparticles on the surface of the labeled yeast cells

Poster 66

A synthesis to magnetic metal nanoparticles and dual-functional microspheres

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Functional silica-encapsulated nanomagnets, *e.g.*, have been studied for their biomedical and environmental application, because they reveal several benefits such as biocompatibility, functionality, stability against degradation, and a hydrophilic character. Various sol-gel based strategies have been used for generating silica-encapsulated iron oxide particles.[1] However, reports on silica-encapsulated magnetic metal particles like Co, Fe, or Ni are scarce, even though many advantages are expected (*e.g.*, large saturation magnetization, enhanced magnetophoretic mobility).

We take advantage of amino-functionalized siloxanes not only to directly control particle nucleation and growth by coordinating to the metal surface but also to provide reactive siloxane groups on the particle surface as a functional interface for further deposition of oxides, such as SiO_2 and TiO_2.[2] This procedure permits the synthesis of Co and Fe nanoparticles of various sizes by thermolysis of $Co_2(CO)_8$ or $Fe(CO)_5$ in solution, respectively, and the preparation of magnetic microspheres. After surface passivation with low doses of oxygen (*smooth oxidation*), the nanoparticles show a good resistance to oxidation. The reaction mechanism was investigated by UV-visible and FTIR spectrometry; the size, structure, and magnetic properties of the particles were characterized by TEM, EDX, XPS, Mössbauer spectroscopy, XRD, AES-ICP, and magnetic measurements.

The applied bifunctional siloxane compound not only controls particle formation by complexation of cobalt but also serves as a coupling agent for SiO_2 and TiO_2 deposition, resulting in functional magnetic microspheres (*e.g.*, catalytic/magnetic microspheres). Magnetic Co@SiO_2 microspheres are obtained by adding and heating TEOS in water/ethanol in the presence of the APTES-functionalized Co nanoparticles. These Co@SiO_2 microspheres can be further functionalized, e.g. by molecular Rh complexes to form magnetically recycable hydroformylation catalysts or by TiO_2 deposition to form photocatalytic systems for the treatment of biological or organic pollutants in water.

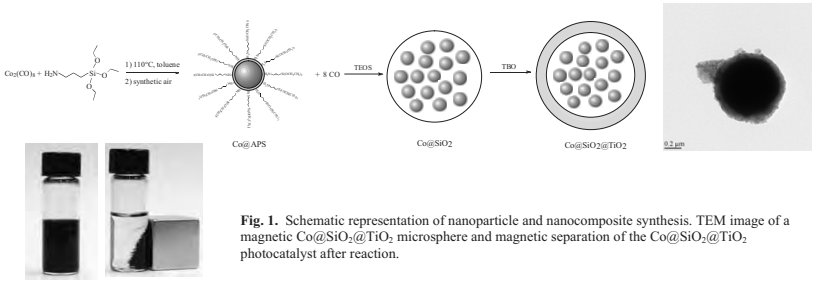

Fig. 1. Schematic representation of nanoparticle and nanocomposite synthesis. TEM image of a magnetic Co@SiO_2@TiO_2 microsphere and magnetic separation of the Co@SiO_2@TiO_2 photocatalyst after reaction.

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Encapsulation of rifampicin and nanosized magnetic particles within biocompatible polymeric nanocapsules

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Key words: magnetic nanoparticle, magnetic fluid, rifampicin, alginate, chitosan

Nowadays there is an increasing interest in the investigation of nanostructured material systems, such as polymeric nanoparticles, aiming its application as drug carriers. Besides incorporating the therapeutic agent within the hosting template addition of magnetic nanoparticles may open up new opportunities[1,2]. Rifampicin is an efficient antibiotic for tuberculosis treatment and its vectorization to the lungs can be very useful, increasing the levels of action at the targeted site while reducing the side effects. This study describes the incorporation of rifampicin and magnetite within polymeric nanostructured alginate/chitosan. The nanosized magnetic particles used in this work were prepared as described in the literature[3]. Encapsulation of rifampicin and magnetite within the hosting template was successfully performed using the ionotropic gelification of polyanion method followed by polycationic crosslinking through an adapted protocol described in the literature[4, 5]. The same procedure was used to prepare samples with and without magnetic nanoparticles. The magnetic nanocomposite samples were investigated by transmission electron microscopy (Figure 1), X-ray diffraction, atomic absorption spectrometry, infrared spectroscopy, nanoparticle size analysis and drug association efficiency. The drug association efficiency was 32% in the nanocapsules without magnetic nanoparticles and 80% with magnetic nanoparticles. The results indicated that alginate-chitosan nanocapsules incorporating magnetic nanoparticles is a very promising material system for drug encapsulation.

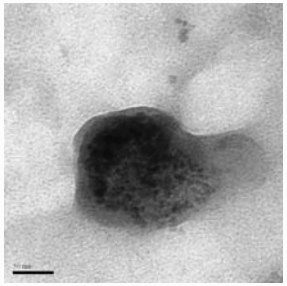

Figure 1: alginate/chitosan nanocapsule loaded with magnetic nanoparticle and rifampicin.

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Temperature-Responsive Magnetite/PEO-PPO-PEO Block Copolymer Nanoparticles for Controlled Drug Targeting Delivery

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Stimuli-responsive nanomaterials offer exciting new opportunities with respect to numerous applications.[1] particularly in biomedical fields such as controlled drug-delivery systems.[2]

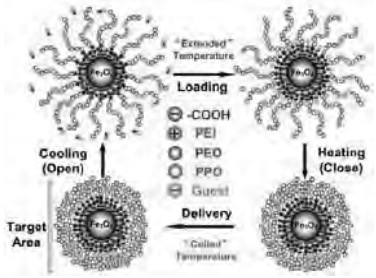

Figure 1. Schematic representation of temperature-responsive MagPluronic nanoparticles working as a targeted drug-delivery system with controlled payload and release.

Temperature-responsive magnetite/polymer nanoparticles were developed from iron oxide Nanoparticles and poly(ethyleneimine)-modified poly(ethylene oxide)-poly(propylene oxide)-poly(ethylene oxide) (PEO-PPOPEO) block copolymer. A typical product has an 20 nm magnetite core and an 40 nm hydrodynamic diameter with a narrow size distribution and is superparamagnetic with large saturation magnetization (51.34 emu/g) at room temperature. The most attractive feature of the nanoparticles is their temperature-responsive volume-transition property. DLS results indicated that their average hydrodynamic diameter underwent a sharp decrease from 45 to 25 nm while evaluating the temperature from 20 to 35^{o}C. The temperature-dependent evolution of the C-O stretching band in the FTIR spectra of the aqueous nanoparticles solution revealed that thermo-induced self-assembly of the immobilized block copolymers occurred on the magnetite solid surfaces, which is accompanied by a conformational change from a fully extended state to a highly coiled state of the copolymer. Consequently, the copolymer shell could act as a temperature-controlled "gate" for the transit of guest substance. The uptake and release of both hydrophobic and hydrophilic model drugs were well controlled by switching the transient opening and closing of the polymer shell at different temperatures. A sustained release of about 3 days was achieved in simulated human body conditions.

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MAGNETIC ZEOLITE AS CARRIER OF PROTEINS

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Recently, different fields of research have been focused on a group of crystalline aluminosilicates known as zeolites. The main property of these minerals is their capacity to exchange ions and to adsorb selectively some reactive species. When zeolite incorporates a magnetic coating, additionally is able to respond effectively against an external magnetic field. Magnetic carriers have been widely used in many applications, including biological cells separation, wastewater treatment, and mineral ore processing, among others. For other hand, Polymers biodegradable and biocompatible have been widely used in drug delivery systems for their physicochemical properties.

The aim of this project was synthesize a carrier for protein with magnetical properties and high efficiency of encapsulation based in magnetic zeolite coated with biocompatible polymers.

The magnetic zeolite was prepared by the wet impregnation with excess solvent method. The magnetic zeolite was loaded with albumin bovine by absorption batch system. Albumin bovine loaded magnetic zeolite and coated with biocompatible polymers were synthesized by the double-emulsion/solvent evaporation method.

The samples were characterized by X-ray diffraction (XRD), zeta potential (pZ), scanning electron microscopy (SEM), and vibrating sample magnetometer (VSM).

The results showed an innovative system with high efficiency of encapsulation (>80% ratio 1:10 protein:magnetic zeolite) of protein and magnetic properties similar to uncoated magnetic zeolite.

ACKNOWLEDGMENT

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Activity of cholesterol oxidase immobilized onto magnetic nanoparticles

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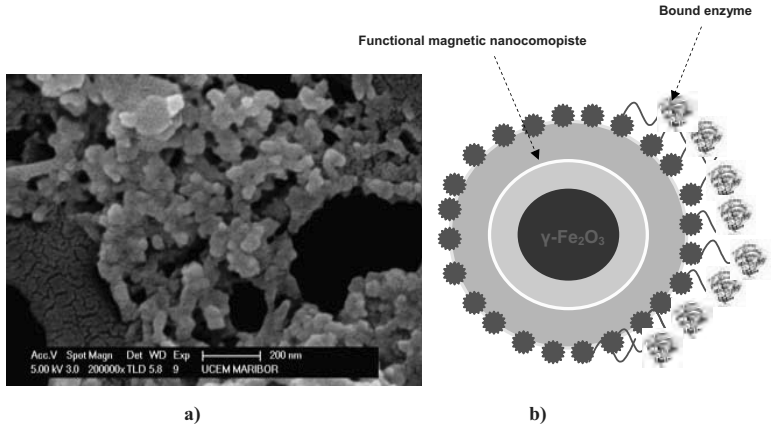
A concise guide of the synthesis of maghemite magnetic nanoparticles, γ-Fe_2O_3, for immobilization of the biocatalyst is reported. Magnetic nanoparticles, that are gaining an exceptionally increased attention as potential enzyme support in the recent years, were synthesized by the coprecipitation technique of ferrous and ferric ions in alkaline medium at harsh stirring and high temperature, respectively. Surface functionalization of magnetic nanoparticles was carried out stepwise and divided into two major steps. Primary functional layer of silica that enhanced the stability of magnetic nanoparticles was synthesized under strictly regulated reaction conditions from sodium silicate or Na_2SiO_3. Next, the secondary functional layer formed with organic molecules of amino silane or 3-(2-aminoethylamino)-propyl-dimethoxymethylsilane in order to achieve higher functionality and reactivity of the magnetic nanoparticles surface was synthesized in acidic reaction medium. Furthermore, the prepared magnetic nanocomposites were tested for the covalent attachment of cholesterol oxidase (ChOx), an enzyme of considerable commercial interest, respectively. The enzyme binding was confirmed by FT-IR and SEM analysis showed uniformly dispersed functional magnetic nanoparticles, which ranged in size from 22.5 to 50.8 nm, surrounded by amorphous silica. The bound ChOx appears an important enzyme in the treatment and analysis protocols of cholesterol concentration.



a) **b)**

Figure. a) Scanning Electron micrograph of functional magnetic nanoparticles; **b)** Schematic representation of the nanocomposite with the bound enzyme.

Poster 71

The role of the hydrophilic coverage on magnetic core in MRI contrast enhancement at different field strengths

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Preparation of stable water based magnetic fluids (MF) is of renewed interest nowadays. Different coatings on particles have been developed to prevent their aggregation. The biocompatible magnetic iron oxide nanoparticles are not toxic at all. The progress in the MRI focuses on the enhancement of contrast between the images of different tissues. The hydrophilic coverage of magnetic nanoparticles provides a particular advantage, since that allows the water molecules to move close enough to the magnetic cores, thus these particles create higher relaxivity parameters of the protons, and so the stabilized MFs can increase the contrast enhancing effect in MRI in this way [1]. By now there are some contrast agents in the clinical application, which contain iron oxide nanoparticles (for example, Resovist, Endorem, Lumirem) [2].

In the present work, magnetite nanoparticles (MNPs) were stabilized with citric acid (CA) and polyacrylic acid (PAA) in order to be dispersed them in water [3]. Our aim was to detect differences among the contrasting performance by measuring T1 and T2 relaxations of the tested magnetic fluids at different field strengths using H-NMR (0.47 T; 9.4 T) and MRI (1.5 T) devices and to compare them to Resovist. Considering the recent trend of MRI development, it is important to predict the behavior of the nanoparticles under different magnetic fields. The effect of MNPs on the r2 relaxivity is characteristic under the different field strengths (Figure 1.). The highest values were obtained for the naked magnetite in the 1.5 T MRI device. The PAA covered magnetite particles induce smaller effect, and those coated with CA have less contrasting efficiency. The r2 relaxivities of our synthetic MNPs have a maximum at 1.5 T with increasing magnetic field strength, where the most of the MRI works in worldwide. The r2 relaxivities of the Resovist increased with increasing field strength. The r1 relaxivities of the magnetic fluids tested here decreased drastically, if the field strength was increased.

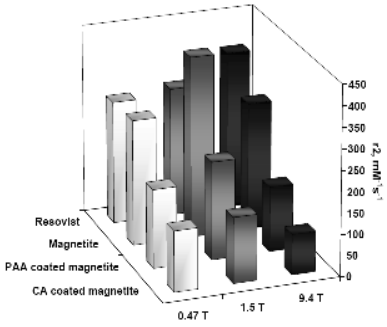

Figure 1. The r2 relaxivity parameters of the different MFs containing MNPs without coating (Magnetite) and coated with dextrane (Resovist), polyacrilic (PAA) and citric acid (CA), measured at room temperature and magnetic fields 0.47, 1.5 and 9.4 T.

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Poster 72

The AMBR assay: A novel biosensor based on magnetic torque

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Rapid, sensitive, inexpensive and portable diagnostic devices are a valuable tool in helping medical personnel deliver better patient care. We present here a proof-of-principle experiment for the Asynchronous Magnetic Bead Rotation (AMBR) assay which we believe could fulfill this clinical need. The AMBR assay is modeled after a traditional sandwich assay, in which the analyte of interest is captured between a solid phase and a label. In the AMBR assay, the solid phase is a 7 μm nonmagnetic sphere, and the label is a 1 μm superparamagnetic bead. In the experiments presented here, the solid phase and labels are coated with streptavidin, and the analyte is a biotin-coated 40 nm particle. Biotin and streptavidin are two proteins that have very high affinity for each other ($K_d \approx 10^{-15}$ M) and for this reason are a popular choice for the initial development of biosensors. After the analytes bind to solid phase sphere, the magnetic label beads are introduced, which then bind specifically to the analytes on the surface of the solid phase, creating a sandwich complex. The sandwich complex is then transferred to a rotating magnetic field, where the rotational frequency of the complex is a function of the number of attached magnetic beads, as illustrated in Figure 1. The novelty of this project lies in using two spherical objects to create a sandwich assay, and quantifying the rotational frequency of the sandwich complexes to determine the concentration of analyte present, which to the best of our knowledge has never been done.

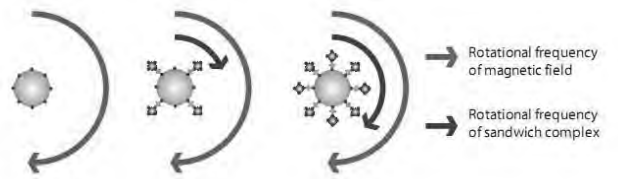

Figure 1—Schematic of concept. The solid phase is represented by the gray spheres, the analyte by the green particles, and the magnetic label beads by the orange beads. The relative rotational frequencies of the magnetic field and sandwich complex is shown.

Superparamagnetic beads experience constant torque in a rotating magnetic field, and thus the rotational frequency of the complexes is a function of the number of attached magnetic label beads, which in turn is a function of the analyte concentration.

We present results that show that the rotation of a sandwich complex is a function of the number of attached superparamagnetic label beads; the concentration of analyte particles over two orders of magnitude; and that the rotation of a sandwich complex is stable over a sixty minute observation period. While still in the initial development stages, we believe that the AMBR assay shows potential as a rapid, simple and inexpensive biosensor.

Poster 73

Efficient Targeting of Magnetic Nanoparticle Complexes in the Cardiovascular System

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Abstract

Gene- and cell-based therapies have great potential for the treatment of human diseases. A major obstacle in this course of action is the positioning and accumulation of genetic material at the target site. A possible approach for this purpose is to label the DNA material or cells with *magnetic nanoparticles* (MNP) and target them via static or time-dependent magnetic fields. Especially in the cardiovascular system, where large flow rates prevail, this is the most promising technique.
The aim of our work is to optimise the targeting process with the help of numerical field calculations. The trajectories of the magnetic nanoparticles have to be calculated with respect to the physiological boundary conditions. Subsequently, the amount of particles which can be retained by the external magnetic field source at the target location can be estimated. Therefore the numerical calculations of the magnetic properties of the external field source and particles are combined with the hydrodynamcs of the blood flow and pressure as well as the structural mechanics of the target tissue. During all those consecutive steps we construct several prototypes of field generators which will be tested in experiments. According to these results we build static as well as time-dependent adequate magnetic field sources and optimise them and the underlying physical model in an iterative approach.
We want to develop an adequate model for the physiological boundary conditions of the cardiovascular system and aspire a magnetic field that will be able to exactly transport and position magnetic nanoparticle labeled nucleic acids, viral particles and cells *in vitro*, *in vivo* and *ex vivo*.

Poster 74

Fluxgate magnetorelaxometry of superparamagnetic nanoparticles for hydrogel characterization

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Hydrogels are under investigation as new drug delivery systems for bioactive molecules because they have the ability to incorporate a huge amount of water or buffer. The duration of the long time drug release can be controlled by the network density of the hydrogels. Therefore, they offer optimal conditions for long in vivo lifetimes and release of e.g. proteins [1]. In [2] we introduced a new nondestructive characterization method for hydrogels based on the magnetic relaxation behavior of superparamagnetic nanoparticles (MNP) and investigated the formation process of hydrogels. It is also possible to investigate the release behavior of hydrogels formed by UV light photocross-linking of hydroxyethyl methacrylate hydroxyethylstarch (HESHEMA) in deionized water together with the MNPs. The MNPs are protected by a gum arabic shell. The photoinitiator and the UV light of the photocross-linking process do not influence the ferrofluid [2]. To characterize the release behavior, hydrogel samples with three different network densities were prepared in triplicate. The samples were measured with our fluxgate MRX system in unshielded environment [3]. To degrade the network of the hydrogel, samples were covered with α-amylase solution and incubated at 37 °C in a thermal shaker.

To quantify the release behavior, two reference samples were prepared. For completely immobilized MNPs, a HESHEMA sample containing MNPs was freeze-dried. For completely mobile MNPs an unpolymerized HESHEMA solution with MNPs and α-amylase solution was prepared. To demonstrate the degradation of the hydrogel, the fraction of mobile MNPs is plotted versus time in the figure. Depending on the network densities of the hydrogels the amount of released mobile MNPs varies with time. The experiments show that MNPs with a gum arabic shell are stable during the photocross-linking process as well as in α-amylase solution. Therefore, they are qualified for characterizing the release behavior of photocross-linked hydrogels.

This work was financially supported by the DFG via SFB 578.

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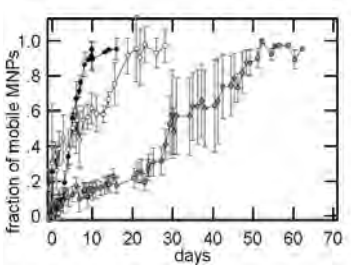

Release behavior of hydrogels with three different network densities.

Poster 75

Therapeutic blood purification using functionalized core/shell nanomagnets

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The direct removal of disease causing factors from blood would be most attractive in a number of clinical situations (e.g. intoxications, severe inflammatory response syndrome and sepsis)[1]. At present, such direct removal is routinely performed for small molecules (urea, potassium, creatinine) by (hemo-) dialysis, filtration and adsorption. These processes depend on diffusion through membrane barriers, where the pore size and structure currently limit hemodialysis to low-molecular compounds (filter cut off at ~50 kDa)[2]. The majority of harmful substances, e.g. endo-/exotoxins in sepsis, antibodies and immune-complexes in immunologic disorders are large biomolecules and are therefore poorly accessible to dialysis [2, 3]. As a result, patients require whole plasma exchange (with full loss of plasma and risk of transfusion reactions upon application of plasma) or purification through an adsorbent (e.g. charcoal).
In this work, the direct injection of stable nanomagnets into whole blood for the efficient, magnetic extraction-based extracorporeal removal of low and high molecular compounds is investigated. We demonstrate the successful extraction of metal ions (Pb^{2+}), steroid drugs (digoxin, cardiac drug) and proteins (interleukin-6, inflammatory mediator) whilst monitoring blood integrity using a series of clinically important parameters[4].

Carbon coated metal nanomagnets equipped with heavy metal complexants, digoxin antibody fragments and entire human interleukin-6 antibodies were added to fresh human whole blood. During gentle swinging, the nanomagnets scanned the liquid driven by Brownian motion (particle diffusion) and captured the target compounds. After removal of the toxin-loaded nanomagnets by magnetic separation, the blood was analyzed for remaining toxin or inflammation markers, iron metabolism and blood integrity (e.g. no clotting). Lead, digoxin and interleukin-6 levels were significantly decreased after the blood purification procedure. The extraction using nanomagnets was in a clear dose-effect-dependence and could be accurately titrated: e.g. digoxin levels could be decreased from toxic (~ 6 nmol/L) to therapeutic concentration levels (< 2.6 nmol/L). Treatment with nanomagnets did not affect the integrity of blood and all levels remained in the clinical norm range. Combined with existing therapies, these results may have major implications for the treatment of severe intoxications (digoxin, barbiturates), sepsis (specific filtering of cytokines or toxins), metabolic disorders (thyreotoxicosis, hyperfibrinogenemia and hyperlipoproteinemia) and auto-immune diseases (removal of pathogenic auto-antibodies or immune-complexes).

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Poster 76

Scalable Magnetic Designs to Achieve Comparable Capture Rates and Capture Efficiency across Multiple Vessel Diameters

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Magnetic separators are used during a variety of magnetic carrier-based purification and bead coating processes such as protein separations, nucleic acid isolations, and immunodiagnostics. The separators typically contain strong magnets oriented in a geometry set to enhance the magnetic field projected into the container vessel to ensure adequate magnetic bead capture. The purification and bead coating processes are often developed on a small, experimental scale before transfer to a functional process scale for use in industrial and manufacturing settings. In addition, batch size varies as dictated by material need, sales projections, and manufacturing schedules. Accordingly, the strong magnets used for magnetic carrier separation must be scaled to prevent process variation between batch size due to inadequate wash efficiency and discrepant capture rates. Magnetic separator scalability may be defined as the consistent capture of magnetic particles as measured by capture rate and capture efficiency (99.9%) independent of reaction vessel diameter. Both physical and magnetic factors effect particle capture and include vessel size, viscous drag force, particle size, and particle magnetic susceptibility, as well as magnet configuration, magnet thickness, and strength grade. Magnetic scaling is discussed herein with an emphasis on theoretical magnetic scale modeling in which the magnet thickness and magnetic parameter are scaled to produce a constant capture rate at varying vessel diameter. Experimental outcomes are described for three scaled versions of the circular magnetic separator (shown in the Figure) using magnetic microparticles.

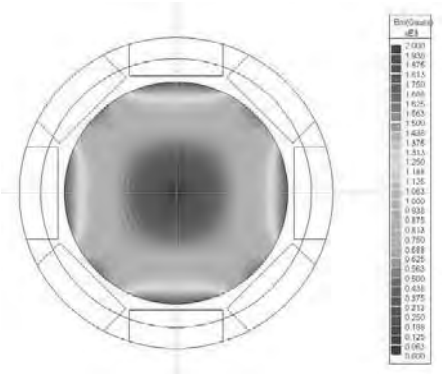

Figure. Circular magnetic separator with the high gradient quadrature magnetic field.

Poster 77

Observation and Numerical Simulations of the Self-Organization Phenomena of Feeble Magnetic Particles due to the Induced Magnetic Dipole Interactions

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One of the characteristic features of the utilization of high magnetic fields is that the dynamical effect can be given even on the feeble magnetic materials without any direct contact with the matter. The magnetic force is acting on the materials when they were introduced in non-uniform magnetic fields. Using this magnetic force, contactless control of material position or structures can be realized. Magnetic field control is applicable to a wide range of materials because the magnetism is a property shared by all materials. Therefore, the usage of magnetic fields is expected to be a useful way to control various processes. To optimize the effect of magnetic force on some processes, the behavior of feeble magnetic materials under high magnetic fields should be understood well. From this stand point, we carried out the optical observation of the behavior of feeble magnetic materials under high magnetic fields using the CCD camera or the confocal scanning laser microscope. Furthermore, to deepen the understandings of their behavior, numerical simulations were carried at the same time.

It was observed that feeble magnetic particles form a chainlike structure under high magnetic fields. In this study, the observation and numerical simulation of this phenomenon was carried out. The schematic figure of the configuration of the simulation and the experiment is shown in Fig. 1. The magnetic field was applied parallel to the sample plane and perpendicularly to the gravity. Glass spheres of 800 μm in diameter and manganese dichloride aqueous solution (40wt%) were used as sample particles and the surroundings, respectively. Figure 2 shows each one shot of the observation results and the numerical simulation. When glass spheres moved due to the magnetic field gradient, they form the chain-like structure based on the interaction between the induce magnetic dipoles. Detail of this research will be reported in this presentation.

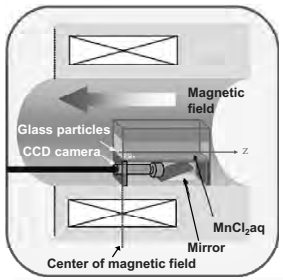
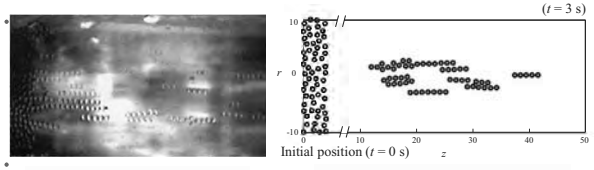


Fig. 1. Schematic illustration of the condition for the simulation and the



(a) Experiment. (b) Numerical simulation.

Fig. 2. Results of the observation and simulation of the behavior of glass particles under magnetic field

Acknowledgement: This work is supported in part by Core Research for Evolutional Science and Technology (CREST) Program "Creation of a Novel Technology towards Diagnosis and Treatment based on Understanding of Molecular Pathogenesis of Psychiatric and Neurological Disorders" of Japan Science and Technology Agency (JST).

Poster 78

Modified magnetic poly(2-hydroxyethyl methacrylate-*co*-glycidyl methacrylate) microspheres for bacterial DNA isolation

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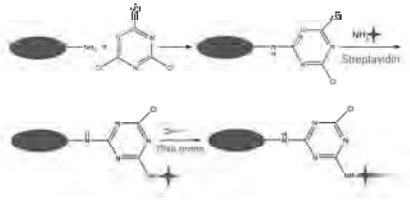
Superparamagnetic iron oxide nanoparticles have been synthesized by an aqueous precipitation route and stabilized with oleic acid. They served as cores in the dispersion copolymerization of 2-hydroxyethyl methacrylate (HEMA) and glycidyl methacrylate (GMA) in toluene/2-methylpropan-1-ol medium. Resulting nonporous magnetic poly(2-hydroxyethyl methacrylate-*co*-glycidyl methacrylate) /P(HEMA-GMA)/ microspheres were *ca.* 3 μm in size and monodisperse. High-quality DNA was isolated using suitably functionalized magnetic microspheres.

1) Carboxyl groups were introduced on the surface of microspheres by oxidation with potassium permanganate and the microspheres were used for whole bacterial DNA isolation. In the presence of PEG 6,000 and NaCl, the DNA changed its structure and was reversibly adsorbed on the surface of microspheres. The procedure was tested for DNA isolation from pure bacterial cultures and from complex food samples. The presence of isolated DNA was proved by PCR.

2) Partial ammonolysis of oxirane groups of P(HEMA-GMA) microspheres introduced reactive amino groups. This allowed functionalizing of the microspheres with streptavidin using the cyanuric chloride linkage (Scheme 1). The amount of streptavidin attached to the microspheres was quantified. In the next step, the streptavidin-containing microspheres were functionalized with biotinylated DNA probe. The attachment of the DNA probe to microspheres was tested and compared with commercial microspheres. Specific and fast binding of complementary DNA prepared from pure bacterial cultures by DNA/DNA hybridization was verified by PCR. A high P(HEMA-GMA) particle stability, close control over the size, narrow size distribution and high flexibility for further functionalization of these microspheres opens up new ways for further investigation in the field of food control.



Scheme 1. Biofunctionalization of P(HEMA-GMA)-NH_2 microspheres with streptavidin and DNA probe.

The financial support of the Ministry of Education, Youth and Sports of the Czech Republic, grant No. 2B06053, and of the Grant Agency of the Czech Republic, grant No. P503/10/0664, is gratefully acknowledged.

Poster 79

High-sensitivity immunomagnetic reduction assay on tumor biomarkers using high-T_c superconducting quantum interference device

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The trend in diagnosing tumors is high-sensitivity in-vitro assays on biomakers related to growth or migration of rumors. In clinics, vascular endothelial growth factor (VEGF) is a popular biomarker related to tumor development. To achieve early-stage diagnosis, a high-sensitivity assay technology to detect VEGF at concentrations of several to tens of pg/ml is to be explored. In this work, Fe_3O_4 magnetic nanoparticles coated with dextran (MagQu Co., Ltd.) and bio-functionalized antibodies against VEGF are prepared to specifically label VEGF in rat serum. The reduction in mixed-frequency magnetic susceptibility χ_{ac} of reagent due to the association among magnetic nanoparticles and VEGF is detected with using a high-T_c superconducting-quantum-interference-device magnetosusceptometer (XacPro-S104, MagQu Co., Ltd.). Such assay method is referred to immunomagnetic reduction, IMR. The reduction in χ_{ac} of reagent is measured as a function of the concentration of VEGF. This function follows logistic function. Furthermore,

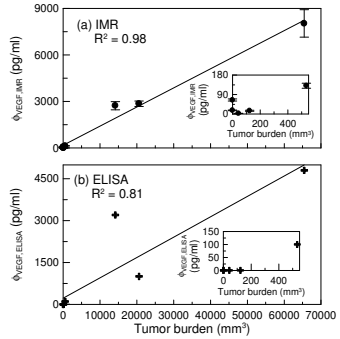

Figure. Detected VEGF concentration in rat serums using (a) IMR (denoted as $\phi_{VEGF,IMR}$) and (b) ELISA (denoted as $\phi_{VEGF,ELISA}$) versus tumor burden of the examined rats with liver tumors. The tumor-burden dependent VEGF concentrations for rats with little tumor burden (< 550 mm^3) are enlarged in the insets.

the low-detection limit was found to be 1 pg/ml, which is sensitive enough for early-stage detection on tumor development. Then, several serum samples from rats with liver tumors are used for the analysis of VEGF concentration $\phi_{VEGF,IMR}$. The tumor burden of each rat is also detected. The results show a linear relationship between $\phi_{VEGF,IMR}$ and tumor burden. The correlation coefficient for the linearity is 0.98. This implies that VEGF is a promising indication for estimating liver tumor burden. On the other hand, the conventional method enzyme-linked immunosorbant assay (ELISA) is used to determine the VEGF concentration in rat serum. The detected VEGF concentration in rat serum using ELISA is denoted as $\phi_{VEGF,ELISA}$. The correlation coefficient of the linearity between $\phi_{VEGF,ELISA}$ and tumor burden was found to be 0.81, which is lower than that of IMR. This evidences that IMR is superior to ELISA.

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Poster 80

Large scale magnetic separation of *Solanum tuberosum* lectin from potato starch industry waste water

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During the potato starch production, large amount of processing water is produced. This waste water contains various biologically active compounds, originally present in potato tubers, among them patatin, inhibitors of proteolytic enzymes and lectin. Potato lectin is a hydroxyproline-rich glycoprotein belonging to the lectin family exhibiting specificity for 1,4-β-linked oligosaccharides of *N*-acetylglucosamine, such as those present in chitin, the second most abundant biopolymer in nature, and in chitosan, partially deacetylated chitin.

Simple and rapid procedure for large-scale, one-step partial purification of potato tuber lectin from potato starch industry waste water has been developed. Low cost, easy to prepare magnetic chitosan microparticles cross-linked with glutaraldehyde were used for purification experiments; such type of magnetic affinity adsorbent enables both efficient adsorption of the lectin and simple elution of the adsorbed lectin by the decrease of pH.

Fig. 1. Flow-through magnetic separation system. 1 – mechanical overhead stirrer; 2 – container; 3 –valve; 4 - screw clamp; 5 – tubing; 6 – bottle; 7 - Sepmag Q magnetic separator; 8 – bottle for collection of magnetic particles depleted solution

Flow-trough magnetic separation system, based on the use of a commercially available magnetic separator Sepmag Q with inserted 500 ml stoppered bottle was used. Separation experiments were performed with 5000 ml of potato starch waste water, containing 100 ml (settled volume) of magnetic chitosan microparticles. The target lectin was desorbed from chitosan by one-step elution with glycine/HCl buffer, pH 2.2. 40% of lectin hemaglutination activity was recovered during the isolation process. The specific activity of the purified potato tuber lectin increased ca. 27 times after the affinity purification step. Magnetic chitosan microparticles could be used at least three times (after simple regeneration step) without the change of the separation process. Ion exchange chromatography was used to check the purity of the separated lectin; substantially purified product was obtained.

In most cases lectin separations were performed in small-scale, using column chromatography of filtered or centrifuged samples. Described large-scale separation technique eliminates the need for prior particles removal and enables isolation of potato lectin directly from processing water. Similar type of separation could also be used for isolation of other biologically active compounds (e.g., proteinase inhibitors) from the same source.

pH-dependence of Biodegradable Silica Nanotubes toward Oral Delivery of Drugs and MR Imaging Contrast Vectors: in situ Etching and Chelating of Gd^{3+} from $Gd(OH)_3$ Nanorods

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In this study, we report a pH dependence of degradable silica nanotubes derived from $Gd(OH)_3$ nanorods. The silica nanotubes were resistant to dissolution in the extreme acidic conditions of pH 1, but quickly degraded at pH 8. The nanotubes were further developed with Gd-DOTA complexes grafted on to the surface acting as MR imaging contrast agents as well as drugs carriers. The released Gd^{3+} ions resulting from the etching of $Gd(OH)_3$ nanorods were chelated by the pre-modified DOTA yielding Gd-DOTA complexes grafted on silica nanotubes. The Gd-DOTA grafted silica nanotubes loaded with doxorubicin displayed enhanced T_1 imaging contrast and anticancer activity.

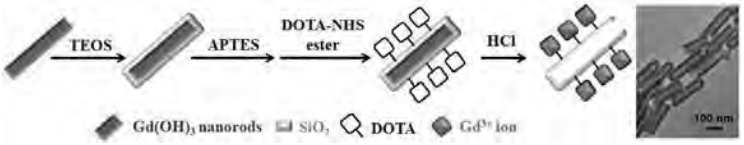

Figure 1. The fabrication processes of silica and Gd-DOTA grafted silica nanotubes and the TEM image of Gd-DOTA grafted silica nanotubes.

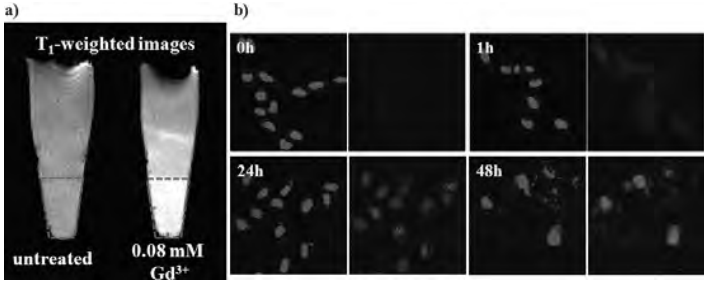

Figure 2. a) In vitro T_1-weighted MR images of HeLa cells only (left) and HeLa cells incubated with Gd-DOTA grafted silica nanotubes for 2h. b) Confocal microscopic images of HeLa cells treated with DOX-loaded Gd-DOTA grafted silica nanotubes (9 nm) at 1, 24, and 48h. The blue color indicates the DAPI stained nuclei.

Intrinsic peroxidase-like activity of Bacterial magnetic nanoparticles

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Figure 1: bacterial magnetic nanoparticles show intrinsic peroxidase-like activity. The top row samples catalysed the reaction of the peroxidase substrate 3,3,5,5-tetramethylbenzidine (TMB), the bottom row of samples catalysed the reaction of the other peroxidase substrate o-phenylenediamine (OPD). The samples left to right show decreasing magnetic nanoparticles concentrations.

Magnetic nanoparticles (MNPs) are generally considered to be biologically and chemically inert in separation techniques and analysis. However, some recent reports prove that MNPs could function like an artificial peroxidase.

Although progress in nanosynthesis has succeeded in making nanoscale particles from iron oxide under precise quality control. But the research about natural magnetic nanoparticles, magnetosomes, is still a current interest. Magnetosomes are specialized organelles synthesized by magnetotactic bacteria (MTB). The magnetosomes comprise nano-sized crystals of a magnetic iron mineral and membrane- enveloped. Magnetosome crystals have species-specific morphologies, sizes, and arrangements and are more uniform in size and shape in comparison to artificial MNPs. The magnetosome membrane ensures superior dispersibility of the particles and can provide an excellent target for modification and functionalization of the particles.The unique properties of magnetosomes attract broad interdisciplinary interest and might be exploited for a variety of applications.

So in the present work, we investigate magnetosomes that also possess an intrinsic enzyme mimetic activity similar to that found in natural peroxidases(Fig. 1). Magnetosomes were able to catalyse the reaction of the horseradish peroxidase (HRP)-substrate 3,3,5,5-tetramethylbenzidine (TMB) producing a blue colour, and o-phenylenediamine (OPD) to give an orange colour in the presence of H_2O_2, but not able to catalyse oxidation of the acid phosphatase(AP)-substrate p-nitrophenyl phosphate(pNpp). That is consistent with the commonly used enzyme HRP. So we characterized this catalytic activity by varying the pH, temperature, H_2O_2 concentration, and so on. Based on this finding, we have developed a novel immunoassay using the intrinsic dual functionality of the bacterial magnetic nanoparticles as a peroxidase and magnetic separator. In this assay, magnetosomes were directly immobilized the antibody or protein of interest on the surface which do not need to be modified with different compounds to make them biocompatible so as MNPs and simultaneously provided three functions: capture, separation and detection.

The stability, biocompatibility, easy to produce and versatility of magnetosomes make them a powerful tool for a wide range of potential applications in medicine, biotechnology, environmental chemistry and geobiology.

Truncated octahedral magnetite nanoparticles: size-controlled synthesis, characterization, and potential application in MR imaging

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We report the first example of the size-controlled synthesis of truncated octahedral Fe_3O_4 nanoparticles with sizes varying from 5 to 22 nm. The saturation magnetization increased linearly as the particle size increased, with values up to 94 emu/g, which is comparable to bulk magnetite (92 emu/g). The magnetic behavior of Fe_3O_4 nanoparticles exhibited a transition from superparamagnetism to ferromagnetism when particles reached 22 nm in size. XRD, electron diffraction including fast Fourier transform filtering analysis, and Ar ion beam etching were conducted, indicating the presence of metallic iron in the 22 nm-sized Fe_3O_4. The magnetic behavior of 22 nm-sized Fe_3O_4 nanoparticles displayed a Verwey transition. The resulting Fe_3O_4 can be readily engineered by different surface modification strategies using cetyltrimethylammonium bromide (CTAB), poly(styrene-alt-maleic acid) (PSMA), and sol-gel condensation yielding a mesoporous silica shell of Fe_3O_4@SiO_2. After surface coating, the high magnetization of 22 nm-sized Fe_3O_4 nanoparticles was further demonstrated, showing great potential in MR imaging. For example, Fe_3O_4 coated with PSMA has an r_2 relaxivity coefficient up to 227.7 $mM^{-1}s^{-1}$, which is much larger than the current commercial T_2 contrast agents, as measured by a 3T MR system.

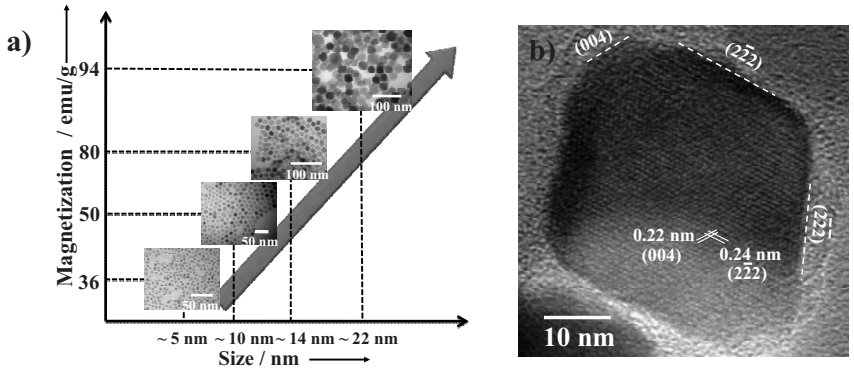

Figure 1. a) The magnetization of Fe_3O_4 nanoparticles corresponded to 5-22 nm nanoparticle sizes. b) HRTEM image of a truncated octahedral Fe_3O_4 nanoparticle (22 nm) taken along with [110] zone axis.

Synthesis, Functionalization, and Magnetic Characterization of Magnetite Nanoparticles for Applications in Nano-medicine

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Superparamagnetic nanoparticles have great potential for biomedical applications, but controlling their properties has proven extremely challenging. Particle size is one of the most critical aspects and has proven one of the most difficult to control, systematically alter, and precisely reproduce. We have designed methods to control particle size through slow addition of precursors at a precisely controlled temperature.

Applications of magnetic nanoparticles in medicine require surface functionalization whether to provide specificity to a tissue, to prolong circulation time, or merely to provide water solubility. This is another area of extreme interest to us. We have been developing functionalization protocols to generate stable dispersions of nanoparticles in water that are insenstitive to temperature, pH, and ionic strength. The overall goal is to have single particles with an organic shell that creates a thermodynamically stable dispersion over a wide range of aqueous conditions. We have primarily explored the end-tethering of mixtures of PEG molecules.

Finally, detailed characterization of these materials have been performed throughout their synthesis and modification. Chemical characterization has included nuclear magnetic resonance spectroscopy, infrared spectroscopy, and UV-visible spectroscopy. Size has been analyzed using transmission electron microscopy, and light scattering. Detailed magnetic characterization has included determining superparamagnetic blocking temperature (both DC and AC), saturation magnetization, Néel relaxation dynamics, and initial susceptibility.

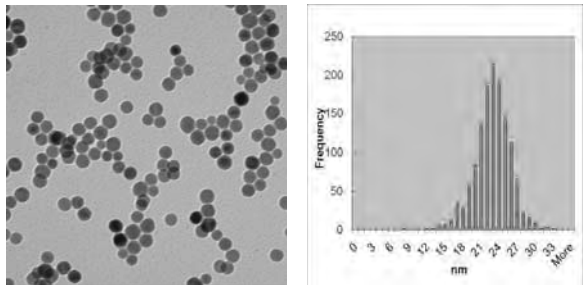

Figure 1. Transmission electron micrograph and size analysis results for magnetite nanoparticles synthesized as part of this study.

Taken as a whole this has provided us with an extraordinary amount of information about these particles and has allowed us to design and fabrication particles suited for various applications. Applications of primary interest to us include biodetection using magnetic remanance[1, 2] and magnetic biopsy needle applications[2, 3].

This work was performed, in part, at the Center for Integrated Nanotechnologies, a U.S. Department of Energy, Office of Basic Energy Sciences user facility. Sandia National Laboratories is a multi-program laboratory operated by Sandia Corporation, a Lockheed-Martin Company, for the U. S. Department of Energy under Contract No. DE-AC04-94AL85000. We acknowledge financial support from the National Institutes of Health.

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Loading Erythrocytes with Magnetite Nanoparticles via Osmotic Pressure Induced Cell Membrane Pores

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Magnetic nanoparticles have been developed for use as magnetic resonance imaging (MRI) contrast agents. Their superparamagnetic properties have the advantage of high magnetic susceptibilities which result in large proton relaxivities when the particles are suspended in aqueous media. Magnetic particles are cleared from the blood stream via ingestion by macrophages. In order to extend the lifetime of the particles in the blood stream, attempts have been made to encapsulate magnetic particles within red blood cells (RBCs) so that macrophage clearance is slowed. A key strategy for loading RBCs with magnetic particles is to incubate them in the presence of the particles under hypo-osmolar conditions. The RBCs can be re-sealed by bringing the osmolarity of the medium back up to physiological values [1-2].

Human RBCs were incubated with iron oxide nanoparticles with a broad range of sizes (ranging from 10 to 630 nm) at different osmolarities ranging from 100 to 290 mOsm. Concentrations of nanoparticles trapped within the cells were measured using transmission electron microscopy and iron-mapping by electron energy loss spectroscopy as shown in Figure 1 and 2. An osmolarity of 200 mOsm was found to be the optimal condition for loading of the cells. At this osmolarity, it was shown that the concentration of particles within the cells equilibrates with that of the incubating medium. At 200 mOsm, the maximum size aggregate of particles that enters the cells is approximately 120 nm.

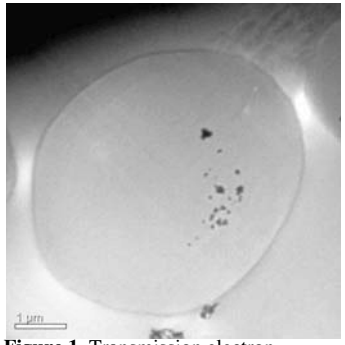

Figure 1. Transmission electron micrograph of unstained human red blood cell after incubation with magnetite nanoparticles at 200 mOsm.

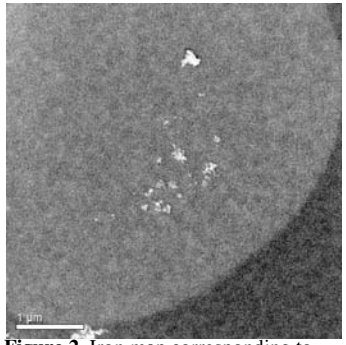

Figure 2. Iron map corresponding to region imaged in Figure 1.

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Temperature triggered drug release, *in vivo* biodistribution and biocompatibility studies of poly(*N*-isopropylacrylamide)-chitosan based magnetic nanohydrogels

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A temperature optimized poly(NIPAAm)-CS based magnetic nanohydrogel, MNHG-1-Fe2 having lower critical solution temperature (LCST) close to 42°C has been synthesized by free radical polymerization and thoroughly characterized. The composite showing spherical morphology (confirmed from SEM & AFM) contains 2wt% CS and ~25 wt% Fe_3O_4 nanoparticles (NPs). The structural characterizations of the sample were studied by FTIR, X-ray diffraction pattern and ^{13}C CP-MAS NMR spectra analysis. The encapsulation of NPs (~10-12 nm) into the composite (~200 nm) was confirmed by TEM and further corroborated with MFM (magnetic force microscopy) image. Its LCST was determined from change in transmittance versus temperature plot obtained from UV-visible spectrophotometer which was further supported by change in enthalpy measurement of the sample from DSC analysis. Further its magnetic properties were studied by VSM and heat generating capacity was assessed under RF field (10 kA/m, 425 kHz). The magnetic field and water bath induced temperature triggered release of anticancer drug doxorubicin from the composite at 37° and 45 °C demonstrated its potential applicability for hyperthermia treatment of cancer. The *in vitro* cytocompability was confirmed on mouse fibroblast L929 cell lines and a dose dependent *in vivo* biodistribution and biocompatibility study of the sample on Swiss mice model weighed between 20-22 g was carried out. There were no significant changes in haematological and various biological parameters viz; complete blood count (CBC), serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), blood urea nitrogen (BUN) and serum creatinine (Creat) up to 7 days following intravenous administration of dose I (650 mg/kg body wt.) and dose II (325 mg/ kg body wt.) of the sample. Further, to evaluate the presence of iron oxide nanoparticles accumulation in different vital organs; lung, liver, kidney, spleen and brain, the optical micrographs of Prussian blue stained paraffin sections of respective tissues were analyzed. Observation of no pathological and haematological change in H & E stained tissues (shown in figure) confirmed the excellent biocompatibility of magnetic nanohydrogel.

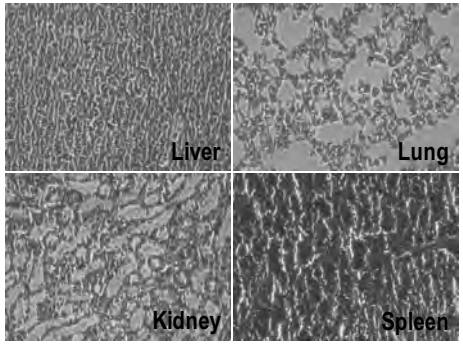

Figure: Histological sections of H& E stained liver, lung, kidney & spleen collected on 7th day after magnetic nanohydrogel injection at 10x magnification

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Preparation and Characterization of Magnetic Polymer Microspheres for the Separation of Radioactive Strontium

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The super magnetic particles consisting of Fe_3O_4 with S=69 emu/g have been synthesised by co-precipitated and the characteristics of modified nano-particles with polymethacrylate (PMA) and PSt are analysed. The surface of a particle combining with functional group ADB18C6 (4-amino-dibenzo-18-crown-6, fixed molecular concentration is about 0.4 mmol/g), which has been shown a specific affinity to strontium, has been produced successfully. The behaviors for sorting out strontium of this particle with the size in 6μm were studied and the effect of pH volume, ion density, strontium concentration, and the ratios of particles and working solution was focus on. The results show here, that this modified magnetic particle can select strontium under acid condition with saturated sorption volume 34.6 and 40.7 mg/g. The sorption characteristic fits Langmuir model well. Further, we set up the procedure to adsorb, separate, and desorb strontium in mimic high radioactive solution.
This work was supported by the National Natural Science Foundation of China (Grant No. 20477058)

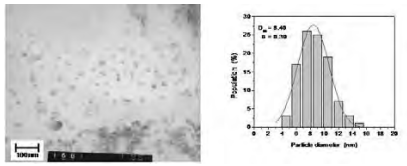

Fig.1. TEM of the oleic acid coated magnetite particles sample and the log-normal size distribution (D_m=8.40nm, σ=0.30)

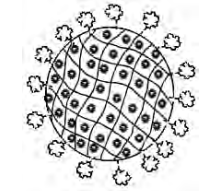

Fig.2. schematic diagram of the functional magnetic nano carries

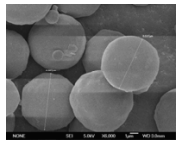

Fig.3. SEM of the spherical carriers of ADB18C6/PMA-Fe_3O_4

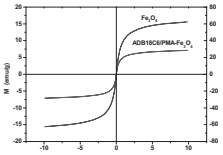

Fig.4. magnetization curve of Fe_3O_4 and polymer microspheres

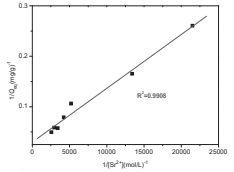

Fig.5. ADB18C6/PMA-Fe_3O_4 adsorb model of Langmur

Optimization of heating performance assisted by dynamic coercivity analysis for magnetic hyperthermia

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Magnetic hyperthermia has attracted increasing attention as a potential cancer therapy due to localized heating effect. Recent observations of large hysteresis loss of magnetic nanoparticles has revealed a promising direction for research in this field [1][2]. One challenge to implement the hysteresis loss technique is the limited performance due to incomplete excitation of the nanoparticles. In order to address this issue the properties of the nanoparticle must be characterized at high frequency and magnetic field parameters should be optimized. However, the intrinsic difficulty in obtaining high frequency magnetic properties has restricted designs for a system that utilizes nanoparticles excited with AC magnetic fields. In this study, we propose and present experimental results for a method to analyze the dynamic coercivity for magnetic nanoparticles.

Commercially available iron oxide (Fe_3O_4 NPs) and gas phase synthesized Fe_3Si NPs[3] served as representatives of superparamagnetic and ferromagnetic nanoparticles, respectively. The nanoparticles were characterized by transmission electron microscope (TEM)(Fig. 1a, 1b). The mean size of iron oxide and Fe_3Si NPs are 6.7nm and 17.0nm, with approximately 18% and 11% standard deviation, respectively. The magnetic energy barriers E_a=KV were found to be 0.15eV and 0.635eV for iron oxide and Fe_3Si NPs, respectively by measuring temperature dependence of H_c and fitting the relationship of H_c versus $T^{2/3}$. Also, coercivity at absolute zero H_0 was extrapolated from the data. The saturation magnetization of Fe_3Si NPs was found to be 961 emu/cm^3.

The dynamic coercivity at high frequency was analyzed using E_a and H_0 values quoted above. Nanoparticles are assumed to be randomly oriented with negligible interactions. By setting the characteristic time in Sharrock's equation as the inverse of field sweep rate, the dynamic coercivity was calculated. At 311kHz, iron oxide nanoparticles show a dynamic coercivity of 75.8Oe while Fe-Si nanoparticles show a value of 177.8Oe (Fig. 1c, 1d dots). Because the coercivity increases rapidly as a function of frequency, the field strength should be optimized accordingly (Fig. 1c, 1d solid lines). It is very desirable to set peak field strength greater than dynamic coercivity to ensure magnetic saturation and consequent hysteresis loss, which is demonstrated by the area of minor loops. The large dynamic coercivity and high-moment of Fe-Si indicate more hysteresis loss. Therefore, it is advantageous to design a proper system that utilizes this kind of high-moment nanoparticles for use in hyperthermia applications.

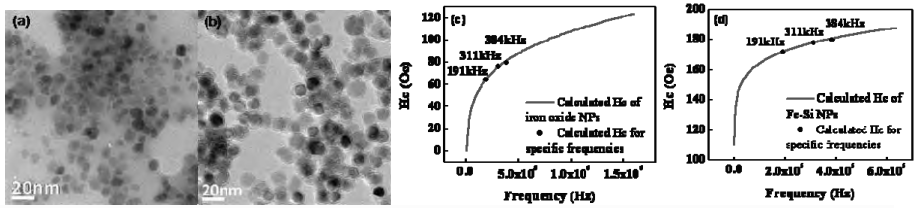

Fig.1 TEM images of (a) iron oxide NPs (b) Fe-Si NPs; Calculated Hc versus frequency of (c) iron oxide NPs (d) Fe-Si NPs

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Heating ability of magnetite labeled single-walled nanotubes

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Carbon nanotubes (CNT) consist of carbon atoms arranged in a series of condensed benzene rings rolled-up into a tubular structure. CNT can be classified in two general categories, based on their structure: single-walled (SWNT), which consist of one layer of cylinder graphene and multi-walled (MWNT), which contain several concentric graphene sheets. CNT have nanometric dimensions: SWNT have diameters from 0.4 to 2.0 nm and lengths in the range of 20–1000 nm, while MWNT are bigger objects with diameters in the range of 1.4–100 nm and lengths from 1 to several μm.

CNT have very interesting physicochemical properties such as: ordered structure with high aspect ratio, ultra light weight, high mechanical strength, high electrical conductivity, high thermal conductivity, metallic or semi-metallic behavior and high surface area. The combination of these characteristics makes CNT a unique material with the potential for diverse applications, including biomedical. Single-walled carbon nanotubes (SWNT) emit heat when they absorb energy from near-infrared (NIR) light. Chakravarty [1] demonstrated the specific binding of antibody-coupled SWNT to tumor cells in vitro, followed by their highly specific ablation with NIR light. Hyperthermia also preferentially increases the permeability of tumor vasculature compared with normal vasculature, which can enhance the delivery of drugs into tumors. Hyperthermia has been clinically used in the management of solid tumors because it can synergistically enhance tumor cytotoxicity when combined with chemotherapy or radiotherapy. Kam [2] in in vitro studies showed selective cancer cell killing obtained by hyperthermia due to the thermal conductivity of SWNT internalized into those cells.

SWNT and SWNT functionalized with carboxyl group were commercially available from Cheap Tubes Inc. Magnetite labeled SWNTs were prepared by precipitation method in nitrogen atmosphere at 60°C. The heating ability of SWNTs is measured by specific absorption rate (*SAR*). Hyperthermic measurements (Fig.1) were performed at frequency f = 750 kHz vs. the AC-field amplitude in the range of 0–3 kAm^{-1}. The *SAR* values as a function of external magnetic field show suitability of the studied SWNT in the biomedical applications. Obtained dependence $(dT/dt)_{t=0} = (H/a)^n$ shows a significant amount of releasing energy from the alternating magnetic field into heat by hysteresis losses during reversal of magnetization, because the coefficient value n ~ 2.8.

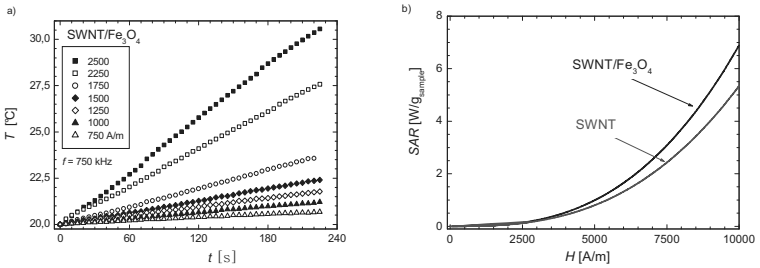

Fig.1. a) Temperature increase produced by magnetite labeled SWNT for different values of an alternating magnetic field, b) *SAR* values vs. magnetic field strength.

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Acknowledgement:
The studies were supported by Polish Ministry of Science and Higher Education grant No. N202 097 32/2406, Slovak Academy of Sciences VEGA 2/0077/09, Nanofluid, Centre of Excellence, APVV 0509-07 and Development of technology of magnetic fluids for biomedical applications Project No. 26220220005.

Closed-Loop Temperature Control of Cobalt Ferrite Ferrofluids Using Continuous Modes Controllers

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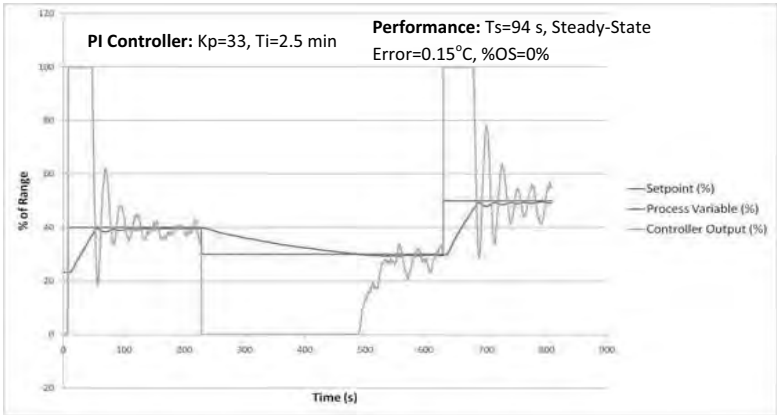
Precise temperature control of a cobalt ferrite ferrofluid (2.5% w/w in heptane) was achieved using continuous mode controllers and a custom-made, variable frequency, magnetic field generator. Propotional-Integral (PI) and Proportional-Integral-Derivative (PID) controllers were implemented using LabVIEW and used to control the amplitude of the magnetic field applied to the ferrofluid. A fluoro-optic thermometer (Luxtron) was used to record temperature. The applied magnetic field had a maximum value of 7 kA/m (100% controller output), and a frequency of 507 kHz. The temperature (process variable) range was 0-100°C (0-100%). When properly tuned, the PI and PID controllers yielded similar results, achieving zero percent overshoot (%OS), a settling time (T_s) of 94 s, and a steady-state error of 0.15°C, for a setpoint of 40°C. The implementation of accurate and fast temperature controllers allows for the consideration of transient responses in fields of study in which magnetic nanoparticles are employed as heat sources.



Poster 91

ELECTROCHEMICAL BIOSENSOR FOR DETECTION OF DNA HYBRIDIZATION USING INDICATOR-BASED AND INDICATOR-FREE MAGNETIC ASSAYS

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The development of advanced biological sensor systems could impact significantly the areas of genomics, proteomics, biomedical diagnostics and drug discovery. Recent advances in biosensors based on nucleic acid have led to the development of genosensor technology for gene sequence analysis and for nucleic-acid ligand binding studies (1-3).
The electrochemical sensor technology based on nanowires, nanotubes and other nanomaterials have recently received considerable attention (5-8). There has been a great interest for development of the electrochemical nucleic acid sensor systems in combination with magnetic separation (9-12). In our study, an indicator-based and indicator-free magnetic assays connected with a disposable graphite electrode (PGE) were successfully developed for the electrochemical detection of DNA hybridization. The changes at the oxidation signals of indicator, echinomycin (ECHI) and electroactive DNA bases; guanine and adenine were monitored in the presence of DNA hybridization by using differential pulse voltammetry (DPV) technique. The selectivity of these magnetic assays for DNA hybridization was also explored in the presence of single base mismatch and noncomplementary DNA sequences. There have not been yet any reports about both indicator-based and indicator-free magnetic assays connected with disposable sensor system by measuring the oxidation signals of ECHI, guanine and adenine in the same measurement scale.

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Poster 92

High gradient magnetic fractionation for the detection of malaria transmitting gametocytes

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The prevalence of gametocytes in human populations is an indicator for the potential of malaria transmission to be sustained. The number of gametocytes in peripheral blood of malaria patients can be very low and the fraction of infectious human hosts is often underestimated. Generally, only molecular methods based on reverse transcriptase polymerase chain reaction (RTPCR) can detect gametocyte densities below the sensitivity range of light microscopy. However sub-microscopic gametocyte densities are still infectious to mosquitoes in an estimated 50% of all cases.

We have recently shown that high gradient magnetic fractionation (HGMS) can concentrate gametocytes from blood samples to enhance their detection by light microscopy. HGMS using commercially available HGMS columns resulted in preparations allowing the detection of gametocyte densities well below the limit of detection of standard light microscopy and with similar sensitivity and higher convenience than RT-PCR.

For a routine detection of gametocytes from patients in the field where large numbers of samples have to be processed, commercially available HGMS equipment is priced at a prohibitive cost. We have therefore developed an alternative method to manufacture HGMS columns and magnets. The columns can easily be manufactured at the study site using basic laboratory disposable materials, industrial steel shot and polyurethane glue. The material cost for a column is estimated to be below one US dollar. A single laboratory worker can easily produce up to 100 columns per day.

We show that our HGMS columns perform similarly well to commercially available columns when used for gametocyte detection with no loss in convenience or time as compared to commercially available equipment. Further optimization of our custom equipment is likely to be even more suitable for this specific application in malaria research.

Diamagnetic trapping of cells above micro-magnets

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Miniaturization of biology-dedicated microsystems, called labs-on-a-chip, provides a better sensitivity for molecular or cellular detection and reduces the volume of solvent and the quantity of handled active principles. However, the resulting small number of molecules or cells and the high surface-to-volume ratio can lead to significant contamination and stiction problems, respectively. Such phenomena raise major issues for samples in the micro- to picolitre (µl-pl) range. For these reasons, contactless handling methods are widely investigated these days.

Diamagnetic levitation is one of the rare natural repulsive forces capable of compensating gravity. Although this kind of repulsion is negligible at our scale, at the microscale (< ~1 mm) it becomes significant and diamagnetic levitation of micro-objects above micro-magnets can be achieved. Widely investigated at the macroscale with superconducting coils [1] or bulk magnets [2], diamagnetic levitation has been little explored at the microscale, mainly because of the difficulty to obtain strong non-uniform magnetic fields. Recent advances made in the micro-patterning of hard magnetic films [3] hold much potential for MEMS compatible applications based on diamagnetic contactless manipulations.

The contactless confinement (Fig. 1.A-B) of Jurkat cells (T lymphocytes) in a paramagnetic medium was successfully achieved above NdFeB micro-magnets (Fig 1.C). Experiments were made with an extremely low concentration of contrast agent (GdDO3A) (C=5-10mM - $\delta\chi$ ~ 4-3 µSI). Cytotoxicity testing (Live/Dead cell viability assays) shows that such low concentrations have no impact on cell viability. However, after a few dozens of minutes diamagnetic trapping is lost and the cells sediment on the edge of the magnets. Such a phenomenon could be explained by the internalisation of the paramagnetic salt by endocytosis [4]. This study opens broad and attractive alternatives for contactless cell arraying and sorting based on their size, magnetic susceptibility and endocytosis capabilities. Such methods are being investigated further, and could be applied for sorting cancer, stem and blood cells.

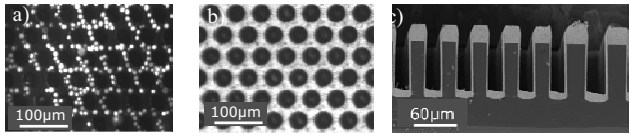

Fig 1 : , a) Micropositioning of Jurkat cells in a paramagnetic medium above this magnetic film, b) Plane-view optical image of a hard magnetic NdFeB film containing an array of micro-holes c) SEM image of the cross-section of NdFeB micromagnets.

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Synthesis and characterization of magnetite-containing particles with dextran/chitosan coating

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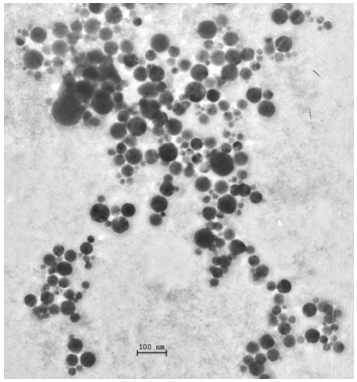
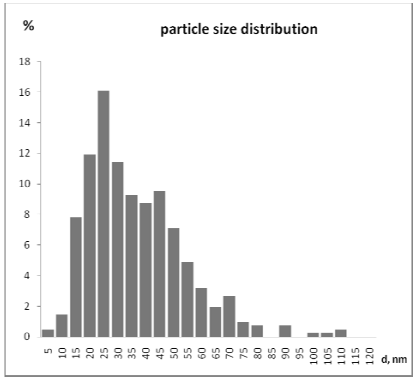
Magnetic particles (MP) are widely used for separation of cell populations. Most common MP for biological applications are composed of magnetite coated with SiO_2 or polymer shells (polystyrene, PMMA, etc.) and monoclonal antibodies immobilized on the surface [1].

In this work, we focused on the synthesis of MP coated with biopolymer composites. The magnetite@dextran/chitosan MP were synthesized in a single-step process. Magnetic carriers with different shape and morphology were obtained depending on the ratio of the biopolymers.

Magnetite was obtained by coprecipitation of iron oxides in the presence of dextran or mixture of dextran and chitosan. The biopolymer/magnetite ratio was 5:2 – this ratio was reported to provide the size of particles less then 100 nm [2]. The MP obtained in the presence of dextran without chitosan were spherical, sized 5 - 80 nm, 82 % were 20 - 50 nm (figure). Addition of chitosan (less then 20% of total biopolymer content) resulted in formation of magnetite-containing particles up to 150 nm.





Unlike dextran-coated, the dextran/chitosan-coated MP provided covalent immobilization of measurable amounts of bovine serum albumine (BSA) in the presence of glutaraldehyde.

In a model cell culture (rat multipotent stromal cells) it was shown that the unspecific binding of the dextran-coated MP to cells was minimal in the presence of serum, with no effect on cell viability and proliferation.

Support by BRFFR (Project X08M-140) is acknowledged.

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Internalization and cytotoxicity effects of different iron oxide magnetic nanoparticle coatings on tumour cells

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Magnetic nanoparticles (MNPs) are promising tools for highly selective tumour therapies by means of local heating [e.g. 1, 2]. Beside a high specific heating power (SHP) [3], a high intracellular MNP loading is essential for the destruction of tumour cells [4]. Therefore and in order to enhance the therapeutic effect, it is important to know which characteristics MNPs must have to increase MNP cell loading. In the present study, we focused on 20 different MNPs samples with comparable iron oxide cores (magnetite/maghemite, 10.6 ± 0.4 nm) but different coatings to figure out the internalization efficiency into a human adenocarcinoma cell line (BT-474) and also the corresponding cytotoxicity subjected to the coating material. Coatings were composed mainly of carbohydrates, fatty acids or starch with or without functional groups (carboxyl, hydroxyl, amino or phosphate groups). For experiments, 0.27 mg MNPs per cm^2 were given to a confluent BT-474 culture for 24 h (37°C, 5% CO_2, 95% relative humidity). After removing unbound magnetic material, cell viability was analysed a) microscopically using a life-dead test based on Calcein AM and propidium iodide staining (alive vs. dead cells) b) microscopically after nucleus staining with HOECHST dye and c) enzymatically with MTS assay. Furthermore, internalized MNP concentrations were determined semi-quantitatively using light microscopy and quantitatively by means of atomic absorption spectrometry. Surprisingly, we found a very heterogeneous behaviour of the different MNP coatings on cell internalization and viability. That means, we found MNPs taken up highly, but with concomitant low, moderate or high cytotoxicity, respectively or that were taken up in a minor concentration leading again to low, moderate or high toxicity, respectively (examples see in figure 1). Accrodingly, results of the different methods were in a good correlation between each other. For further *in vivo* magnetic heating investigations, our results showed arrestingly that for every new MNP formulation dedicated cytotoxicity studies have to be done estimating cellular effects, especially with regard to i.v. MNP injections where healthy cell systems should not be affected. Additionally, the knowledge of MNP internalization amounts in tumour cells is also of special interest to appraise heat generation and therefore therapeutic effects due to magnetic heating treatments.

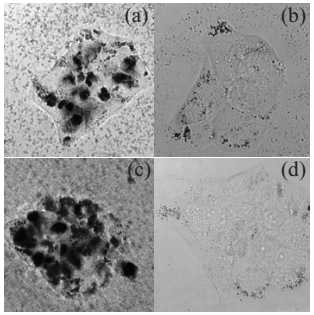

Figure 1: Laser scanning microscopy (transmission mode) of BT-474 cells incubated with MNPs from chemicell GmbH, Berlin a) fluidMAG-Heparin (matrix: heparin): high internalization, moderate toxicity, b) fluidMAG-ARA (matrix: polysaccharide, functional group: glucuronic acid): low internalization, low toxicity, c) fluidMAG-PS (matrix: poly(sodium 4-styrene-sulfonate, functional group: sodium sulfonate): high internalization, high toxicity and d) fluidMAG-DX (matrix: dextran, functional group: hydroxyl): low internalization, low toxicity.

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Systematic protocol for optimizing monodispersed magnetite nanoparticles for combined applications in biomedical imaging and therapy

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In the past decade, applications of magnetite (Fe_3O_4) nanoparticles have been rather limited. The magnetic functionality of the core is primarily used for contrast enhancement in Magnetic Resonance Imaging (MRI), while more advanced functions involving drug delivery and therapy are usually dealt by components of the surrounding shell. Promising applications such as magnetic fluid hyperthermia (MFH) and magnetic particle imaging (MPI) have been demonstrated only at the proof-of-concept level[1]. Several models have shown that in order to carry these concepts to the clinical stage, highly monodispersed nanoparticles must be used. MFH and MPI employ the relaxation dynamics of nanoparticles for heat dissipation or signal generation, respectively. AC-fields are applied at a fixed frequency (typically < 1MHz) and hence the particles should be tailored to precisely resonate at this frequency; hence, monodisperse particles (10 < dia < 20nm) will provide the strongest signal output. Using a dedicated hyperthermia device we observe a sharp peak in power dissipation as a function of particle diameter; at 376 kHz (H_o=17mT) the peak is at 16 nm (Figure 1(a)). Similarly, signals 30 times larger than commercial samples have also been confirmed for monodisperse magnetite in MPI measurements[1]. Even though highly monodispersed magnetite nanoparticles are synthesized via organic routes they are limited in producing water-stable dispersions, a key requirement for any bio-medical application. We have developed a robust protocol for transferring monodispersed magnetite nanoparticles, synthesized via organic route, to aqueous phase. Two amphiphilic polymers, Pluronic-F127 (PF127) and PEG-ylated-poly(maleic anhydride-alt-1-octadecene) (PMAO-PEG), were used for the phase transfer. Both polymers result in highly stable water-dispersions, however, PMAO-PEG coated particles agglomerate in biological media (Figure 1(b,c)). Stability in biological media is absolutely essential for successful translation to *in vivo* stage, hence PF127 coated particles are promising. It is also a fact that agglomeration is inevitable when particles are transported intracellularly in vesicles or bound to surface receptors. Thus, PMAO-PEG particles in biological media offer opportunity to obtain insight on effects of agglomeration on relaxation mechanism of particles. Figure 1(d) shows that 13 and 14 nm particles are negligibly affected suggesting predominance of Neel relaxation, while 16 nm particles show a 30% drop in SLP suggesting contribution from Brownian relaxation. In summary, we demonstrate a systematic and reliable protocol for synthesizing and optimizing non-toxic[2] monodispersed, magnetite nanoparticles with the vision of combining MFH and MPI in biomedicine.

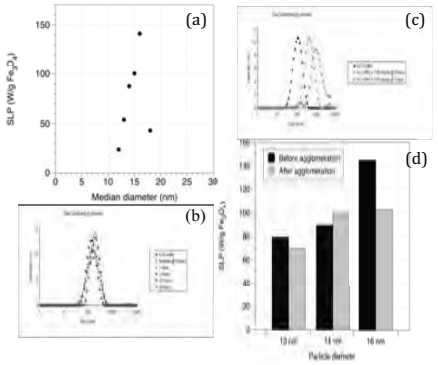

Figure 1. (a) Heating capacity (SLP: specific loss power; Watts/g Fe_3O_4) as a function of particle diameter, (b,c) DLS measurements in water and biological media for PF127 and PMAO-PEG coated nanoparticles, respectively, (d) effects of agglomeration on heating capacity

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[2] M. Gonzales et al, *Contrast Media and Molecular Imaging* (in press)

Polysiloxane Diblock Copolymer Coated Iron Nanoparticles as Excellent MRI Contrast Enhancement Agents

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The magnetic nanoparticles have emerged as a potential multifunctional clinical tool that can provide cancer cell detection when used as MRI contrast agents as well as therapy by targeted cancer cell delivery of agents (antibodies, drugs, and small molecule inhibitors) or local hyperthermia generation by heating the nanoparticles using an alternating magnetic field [1]. Iron oxide nanoparticles are particularly attractive for in vivo MRI applications mainly because of their saturation magnetization which is higher than that of Gd-chelates which are used routinely in diagnostic imaging. In principle, higher magnetization may increase the MRI contrast with the applied magnetic field even more due to the stronger interferences to relaxation times of water that is the source of MRI signal. . In this study, we report the use of metallic iron-based nanoparticles (FeNPs) for MRI application. FeNPs were prepared by thermally decomposing organometalic compounds of iron at high temperature in the presence of oleic acid (OA) and oleylamine (OY) surfactants. A control on average particle size was obtained by varying the injection temperature of the iron precursor. The FeNPs synthesized are composed of an iron core and iron oxide shell (Fig.1). The as-made particles are not water dispersible because of the hydrophobic surfactant coatings. FeNPs were then coated and stabilized in the aqueous solvent using the newly developed polysiloxane PEO-b-PγMPS diblock copolymers [2]. Particles are well suspended in water and retain their core-shell morphology after coating with the copolymer (Fig.1). Relaxometery measurement of the transverse relaxation time T_2 of the FeNPs solution at 3 tesla confirms that the FeNPs are an excellent T_2 contrast agent for MRI (Fig. 2). In comparison to the conventionally used IOPNs, FeNPs offer a much stronger T_2 shortening effect than that of IONPs with the same core size due to their better magnetic properties.

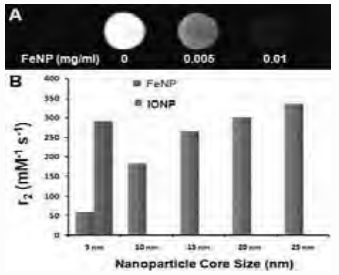

Fig 2 FeNPs with different Fe concentrations lead to signal drops in T2 weighted MRI (A). FeNP exhibits higher r2 (i.e., 1/T2) relaxivity when compared to the IONP with the same core size (B).

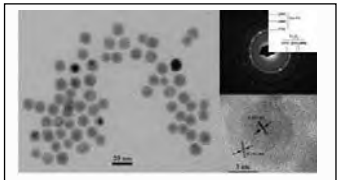

Fig 1. Fe/Fe-O nanoparticles with the core/shell morphology ; the corresponding electron diffraction pattern is shown.

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Magnetic Nano-Micelles for Simultaneously Triggered Drug Delivery and Hyperthermia.

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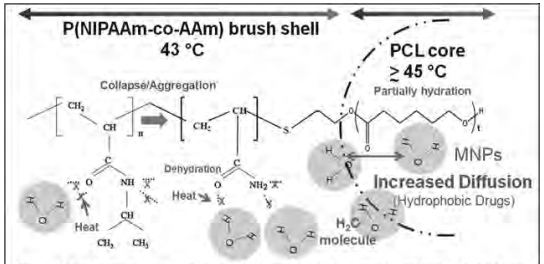
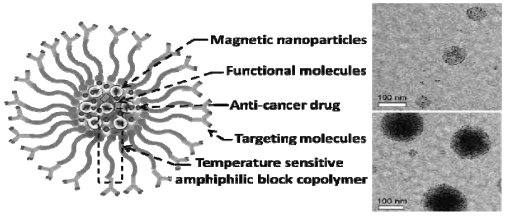


Figure 1. A) Schematic, and TEM images (1:1 (top) and 1:3 (bottom) wt ratio of MNPs:polymer) of multifunctional nanomicelles using engineered magnetic nanoparticles in a temperature sensitive carrier for a simultaneous hyperthermia and chemotherapy, targeting, and imaging. B) Schematic of dehydration of the outer shell above 43-45 °C, and hydration of the PCL core above 45 °C.

Stimuli-responsive macromolecular nano-architectures capable of conformational and chemical changes upon application of an external signal have attracted great interest because of extensive opportunities for *in vivo* applications. Various bio-applicable materials, such as magnetic (MNP) or Au nanoparticles, carbon nanotubes, quantum dots, and pharmaceuticals can be self-assembled within the core of hybrid micelle structures. Therefore, micelles loaded with stimuli-responsive polymers can be engineered for multiple functionalities within a single smart structure. Application of such functional core-hybrid materials includes thermal ablation (hyperthermia), controlled drug-delivery and release, sensing, and diagnostic imaging.

We demonstrated that heating, and drug release triggered by heating, can be performed simultaneously in MNP-loaded micelles (Fig. 1a). As a first step, tailored ~11 nm iron oxide nanoparticles were synthesized for generation of the high heating power. Next, thermo-responsive block copolymer ((PNIPAAm-co-AAm)-b-PCL) nanomicelles were synthesized. These supramolecular structures exhibit characteristic shell and core responses (controlled by the two phase transition temperatures, UCST and LCST) which are favorable for controlled drug release. The nano-micelles loaded with MNPs were demonstrated to be stable in the optimal ratio of MNPs/polymer. Dehydration of the outer shell (Fig. 1b) around the LCST of 43 °C, and hydration of the PCL core at the UCST of 45-50 °C, were observed *via* micro-Raman spectroscopy study. The temperature-dependent hydration states control drug release via reversible changes in the hydrodynamic size/volume of the MNP-loaded micelles. The large volume change observed for doxorubicin (DOX) - MNP-micelles (~75 %) can be leveraged to achieve varying DOX releases rate via temperature cycling between 37 and 43°C. DOX-MNP-micelles in the temperature range from 37 to 43 °C release drug molecules due to both outer thermoresponsive motion, and an increased diffusion rate within the softened PCL core. MNP-loaded micelles have been shown to provide thermally triggered drug release, demonstrating the potential for this approach to be used as a synergetic cancer treatment. Also, these magnetic nano-micelles were applied for MRI contrast enhancement. The versatility of the proposed nanoplatform allows its expansion via multifunctional hybrid core materials (MNPs/gold/quantum dots/carbon nanotubes), as well as antibody conjugation for cancer targeting.



Work at Argonne and its Center for Nanoscale Materials and Electron Microscopy Center is supported by the U.S. Department of Energy Office of Science, Basic Energy Sciences under Contract No. DE-AC02-06CH11357. Technical support from the Nanophotonics and NanoBio Interfaces group at the CNM is gratefully acknowledged.

Abstract : The use of Magnosphere™ MS300/Tosyl paramagnetic beads in HCV and EBV assay formats.

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Paramagnetic beads have been popular in the field of clinical diagnostics as a solid support because of their advantages in automation, ease of handling, and rapid separation. To obtain a sensitive assay using paramagnetic beads, it is very important to avoid non-specific binding from cell lysate proteins and serum.

JSR Magnosphere ™ beads consist of a core shell structure with three layers as can be seen in Figure 1. The JSR Magnosphere ™ surface is coated with an even layer of JSR proprietary polymer. This gives the beads their characteristic low non-specific binding and ensures efficient shielding of the magnetite.

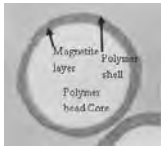

Figure 1. TEM Cross section JSR paramagnetic beads. Magnosphere™ beads are core shell structured.

The non specific binding of the JSR Magnosphere ™ MS 300/Tosyl beads with a diameter of 3 µm have been tested in a HCV (Hepatitis-C Virus) and EBV (Epstein-Barr Virus) assay run on a fully automated, chemiluminescent immunoassay system.

The particular HCV antigen is a recombinant protein sensitive to heat stress if coated on beads. The HCV antigen was coated on both JSR and a competitor's beads.

The performance of the beads was tested on 3 sample groups: HCV positive samples; a small population of HCV negative serum samples and a small number of HCV seroconversion samples. The coated beads were subject to heat stress by incubation at 37°C for 3 and 6 days respectively. The reactivity of the coated beads after heat stress at 37°C for 3 and 6 days was equal for both JSR and competitor beads; both of them seem quite stable until 3 days at 37°C. It was seen that at the 6th day the response for both type of beads is lower. The reactivity of especially the seroconversion samples fall to intolerable values for the competitor beads, while the signal with the JSR beads is still easily recognized.

The JSR Magnosphere™ MS300/Tosyl beads were also evaluated in an EBV assay on a number of non-EBV "cross-reacting" samples. It was observed that the JSR beads showed a much lower level of "cross-reacting" signal than the competitor's beads.

In these particular assays the JSR Magnosphere™ MS300/Tosyl beads have proved to be interesting in terms of ability to stabilize a potentially temperature labile protein and in terms of low non-specific binding from "cross-reacting" samples.

Thermal fluctuation effects in magnetophoresis of superparamagnetic microbeads

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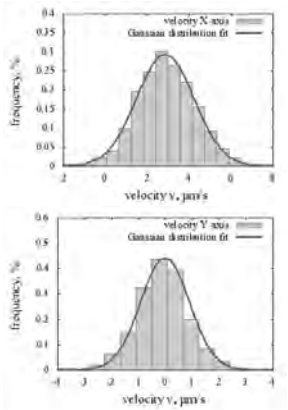
Magnetic nanoparticles embedded in a suitable matrix are widely used in biomedicine in form of superparamagnetic beads. Here by using micro-PIV technique (Dantec) we investigate the properties of these microbeads by observation their thermal fluctuations at magnetophoresis in the nonuniform magnetic field of the permanent magnet. Our method is based on measuring the conditional probability $P(\Delta x, \Delta y; \Delta t)$ of the particle displacement $(\Delta x, \Delta y)$ in the plane of observation under the action of constant force $(m\partial B/\partial x, 0)$ (α - hydrodynamic drag coefficient):

$$P(\Delta x, \Delta y; \Delta t) = \frac{1}{(2\sqrt{\pi D \Delta t})^2} \exp\left(-((\Delta x - \alpha^{-1} m \partial B/\partial x \Delta t)^2 + \Delta y^2)/4D\Delta t\right) \qquad (1)$$



which by $\Delta x = v_x \Delta t; \Delta y = v_y \Delta t$ we transform to the distribution function for the average velocity in the time interval Δt. Series of images separated by time interval Δt for diluted ensemble of microbeads are registred by micro-PIV and processed by ImageJ to get the data of the particle positions. Average velocity distributions are calculated and fitted by the Gaussian (1). The magnetic moment of the particles is determined from the magnetization curves of the diluted sample given by vibrating sample magnetometer. The results at particular value $\partial B/\partial x = 4.3\ T/m$ for 1.31 μm beads are displayed in form of histograms for v_x and v_y. The data for different beads and field gradients are summarized in Table 1.

Table 1: Summary of results

Axis	Magnetic field gradient, $\partial B/\partial x$ (T/m)	Diffusion const., D $(\mu m^2/s)$	Estimated D_E $(\mu m^2/s)$	Mean velocity, v $(\mu m/s)$	Estimated v_E $(\mu m/s)$
Streptavidin Masterbeads from Ademtech, 500 nm					
x	0.0	1.09	0.86	-	-
y		1.04		-	-
x	3.6	3.61		5.3	2.2
y		1.52		-	-
Streptavidin coated magnetic particles from Spherotech, 1.31 μm					
x	0.0	0.45	0.33	-	-
y		0.46		-	-
x	3.6	0.38		2.2	5.4
y		0.23		-	-
x	4.3	0.47		2.9	6.9
y		0.20		-	-

On the Question of Magnetism

Martin Koch (Feldkraft), Joachim Wiest (Cellasys)

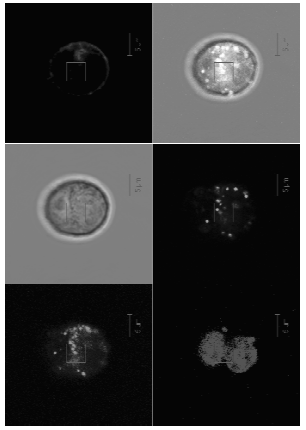
Keywords:
Saturation of beads, Ferromagnetism, Linearity, Nonlinearity, Finestructure, Magnetic Permittivity, Oscillations, Wave- and Klitzing-Resistance.

1. Abstract

Saturation defines a disproportional large magnetization of magnetic material, with a crystalline structure. This effect gets relevant, when small ferromagnetic structures, e.g. magnetic beads, are operated in magnetic field beams, produced by relative large field-devices near by (See example). Saturation splits the linear- from the non-linear- relation of the magnetic flux- and force-field. A split axiom is defined, which may generate a better understanding of electromagnetism, opening up for precise applications due to exact modelling.
A new approach: The combination of Confocal Laser, Magnetic Beads and Moving Dynamic (DM) Fields.

Example 1: 100 nm large iron-oxide beads inside an (dividing) Ins1 cell.

NOVO-PROJECT // LUND University //STETTER-ELEKTRONIK /FK
Experiment (31-12-2009 time 01:07 pm) with 100nm Beads /micromod CLD
Cell-Type: Insulin secreting cells (Ins-1)
Beads Lysosomes Membrane Nucleus



Images produced by a 4-channel laser microscope type Zeiss LSM at the CRC/Malmø.

Title: Magnetic EDTA: Coupling heavy metal chelators to metal nanomagnets for rapid removal of cadmium, lead and copper from contaminated water

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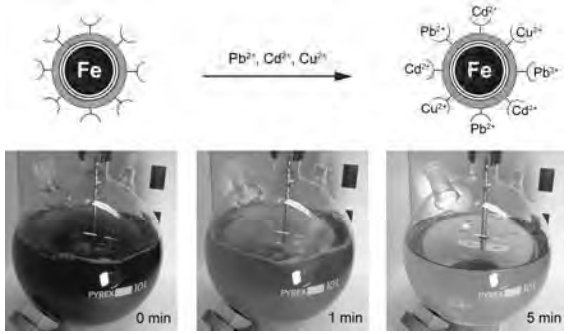
Nanomagnets combine high specific surface area (small particles) and ease of separation (directed movement of the particles in liquids). Until recently, the use of iron oxide particles had opened fascinating applications of magnetic separation, but limited stability prohibited the use of iron oxide based nanomagnets in combination with complexing agents in acidic media which would immediately dissolve the particles. Carbon-encapsulated metal nanomagnets most recently opened access to chemically stable magnets (Grass and Stark 2006; Grass, Athanassiou et al. 2007) with strongly improved bulk magnetization (stronger magnetic forces result in faster removal). The carbon layers further provide an anchoring point for functional groups on the particles surface, accessible by diazonium chemistry.
Attachment of EDTA-like chelators to carbon coated metal nanomagnets results in a magnetic reagent for the rapid removal of heavy metals especially lead, cadmium and copper from solutions or contaminated waste water streams by three orders of magnitude to concentrations as low as µg/L.

Figure 1. Magnetic separation test in 10 l water with 1 g carbon-coated cobalt particles. Left figure, start of magnetic separation as a permanent magnet was placed in the lower left corner. Middle figure was taken after 1 minute. After 2 minutes the stirrer was stopped and the right figure shows the end of the separation. More than 97% of the particles were removed.



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Poster 103

SYNTHESIS, COLLOID AND SURFACE CHEMISTRY OF METAL OXIDE NANOPARTICLES

Vladimir Kolesnichenko, Galina Goloverda, Sarita Sitaula, Brittany Allison, Chisom Ezenwoye, Cameron Williams, and Ronnieka Fleming

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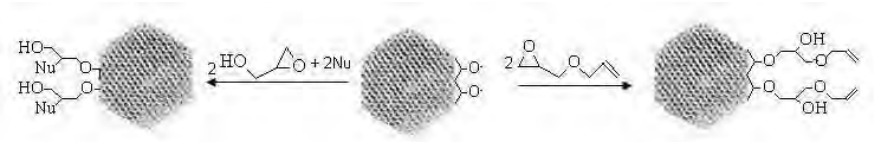
Aspiring to design and synthesize novel magnetic metal oxide particles ligated with biocompatible hydrophilic molecules, we (a) studied condensation of aqueous iron(II) and iron(III) ions at a presence of polydentate hydroxycarboxylic acids and (b) studied condensation of epoxide ring-containing precursors seeded by metal oxides and metal hydroxycarboxylato-complexes.

Polydentate hydroxycarboxylic acids such as citric, tartaric, hydroxycitric, and sugar acids, associate with metal ions and metal oxide surfaces due to their chelating and bridging coordination. This inhibits agglomeration of metal oxide particles and affects kinetics and mechanism of metal ion condensation and crystal growth. Therefore these ligands can help with synthesis of monodispersed variable-sized metal oxide particles that are ready for surface chemistry modification. This study was done by new injection method, by varying metal to ligand stiochiometry and by varying the ligand structure. Aqueous colloids were characterized by Dynamic Light Scattering and zeta-potential measurements; nanopowders were characterized by X-ray diffractometry, TEM, combustion analysis and FT-IR spectrometry.

Obtained colloidal oxide (Al_2O_3 and Fe_3O_4) particles were used in surface chemistry experiments as seeds for condensation of the epoxide ring-containing precursors. Condensation reactions leading to oligoether-coated nanoparticulate adducts, can be carried out at the Lewis acid sites of the bare oxide surface (left side of the scheme below) or at the deprotonated OH-groups of the coordinated hydroxycarboxylic acids (right side of the scheme below). Isolated compounds were characterized by ^{1}H and ^{13}C NMR, FT-IR and mass-spectrometry.

New nanoparticulate adducts can be bioconjugated and used as magnetic imaging agents for MRI, cell tracking, as well as for drug delivery and hyperthermia.



Poster 104

Development of Magnetic Operated Preparations for Boron Neutron Capture of Tumour Therapy

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The most widespread for neutron capture of tumor therapy (NCT) have become compounds with ^{10}B (BNCT)[1]. Earlier[2] we showed the possibility of using Fe-B composite and ferro-carbon composite with absorbed borax as carriers of boron. However, only two boron containing compounds, one of them being L-p-boronphenilalanin (L-BPA), are used in clinical practice. Due to that the aim of present research is to develop methods of obtaining L-BPA magnetic operated preparations and to study their desorption in a model biological liquid.

We have worked out L-BPA immobilization methods on microparticles of iron-carbon composites and metallic iron. L-BPA immobilization on iron-carbon composites was carried out by physical absorption from water solutions, on iron particles – by dextran coating with its following conjugation. The highest sorption capacity of 160.0 mg/g L-BPA was reached for dextran-modified iron particles vs 78.0 mg/g for iron-carbon composites. Spectrophotometric study of the reaction kinetics interaction of L-BPA with dextran allowed to elucidate a mechanism of its conjugation (fig.1). The dynamics of L-BPA desorption was studied by incubation of magnetic preparations with fresh aliquots of 0.6% albumin with the following registration of supernatant UV-spectra. The maximum L-BPA desorption for iron-carbon patterns of composites and for dextran-modified iron particles took place within 5 minutes of incubation. However, L-BPA desorption from dextran-modified iron particles lasts longer than that for ferro-carbon composites (fig.2). It has also been shown that the release of L-BPA from magnetic preparations in the model solution occurs as complexes with albumin.

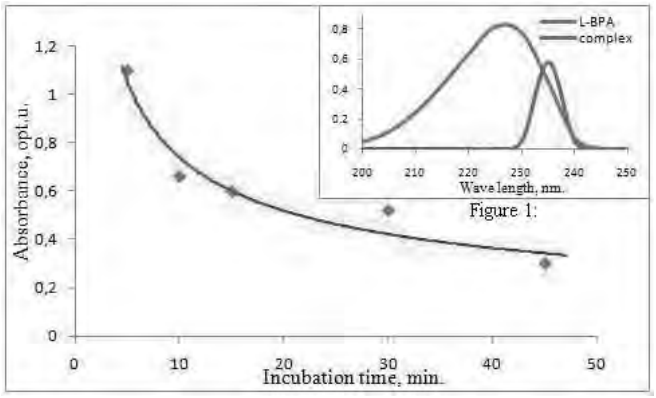

Figure 2:

Figure 1:

Fig.1. Conjugation of L-BPA with dextran (T 37^0 C, pH 7.4)
Fig.2. L-BPA desorption (λ 250 nm) from dextran-modified Fe-particles (T 37^0 C, pH 7.4)

Analysis of the results has shown that the quantity of the immobilized L-BPA in the magnetic operated preparations that we have got, is enough to create therapeutic concentration of boron atoms in tumour for BNCT. Nevertheless it is required that we should continue our investigations in order to choose the optimum ferro-composite types for working out magnetic operated preparations of L-BPA for more effective BNCT of tumours on their basis.

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Silica-coated fluorescent magnetic nanoparticles marked with monoclonal antibodies for breast-cancer targeting

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Over the past several years, we have witnessed an explosive development of nanomedicine platforms in drug delivery and molecular imaging applications. Nanoscopic therapeutic systems, which combine therapeutic agents, molecular targeting, and diagnostic imaging capabilities are emerging as the next generation of multifunctional nanomedicine to improve the therapeutic outcome of drug therapy.
Breast cancer is the most frequent female cancer in western countries. Early detection is a clinical challenge which may significantly improve the treatment and survival of breast cancer patients.
In this work, different types of covalent binding of monoclonal antibodies (mAbs) to silica-coated fluorescent maghemite nanoparticles were investigated.
The magnetic nanoparticles were synthesized with co-precipitation from aqueous solutions of Fe^{2+} and Fe^{3+} ions with concentrated aqueous ammonia. The synthesized nanoparticles were dispersed in an aqueous medium using citric acid as a surfactant. A thin layer of silica was coated onto the nanoparticles using hydrolysis and the condensation of tetraethyl orthosilicate (TEOS) and the deposition of the formed silica on their surfaces.
The amino functionalization of the silica-coated nanoparticles was provided by grafting (3-aminopropyl) triethoxysilane (APS) onto their surfaces. The surface concentration of the surface amino groups was determined using conductometric titrations. To enable the tracking of the nanoparticles using optical microscopy, fluorescein isothiocyanate (FITC) was attached to the nanoparticles' surface.

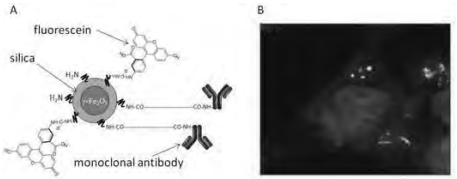

Figure 1: Schematic presentation of functionalized magnetic nanoparticles (A) and image of functionalized nanoparticles in process of internalization (confocal microscopy) (B)

Finally, monoclonal antibodies (mAbs)[1] were covalently bound to the fluorescently labelled nanoparticles in three different procedures (Fig. 1). First, a water-soluble crosslinking agent for covalent binding between the amino group at the nanoparticles' surfaces and the amino group of the mAb was used. The second procedure was based on using a PEG-ilated crosslinking agent for covalent binding between the amino group of the APS and the amino group of the mAb. The last procedure was done in two stages. Fluorescently labelled nanoparticles were pegilated in the first stage and then bound with mAbs using 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) in the second stage. The cytotoxicity/viability of the fluorescently labelled nanoparticles was performed using flow cytometry. The size of the functionalized nanoparticles was determined with dynamic light scattering (DLS). Recognition of the epitope on the cancer cells by the mAb-marked fluorescent nanoparticles (first procedure) was investigated using MCF-7 cells. In a co-culture of breast tumor MCF-7 cells and pro-monocytic U937 cells, mAb-marked nanoparticles relatively well recognized and internalized the tumour cells.

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Reduced non-specific tissue heating with dielectric shielding for magnetic nanoparticle-mediated thermal therapy for metastatic prostate cancer

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Hyperthermia cancer treatment exposes tumors to high temperatures for a period of time to damage and kill cancer cells, or to sensitize cancer cells to the effects of radiation and anti-cancer drugs. Alternating magnetic fields (AMF) in the radiofrequency spectrum can be used to localize heat by heating antigen-targeted magnetic nanoparticles in the cancer tissue. However, direct tissue heating also results from interaction of AMF with tissue. The challenge is to minimize this non-specific power deposition over large regions of tissue to avoid overheating and damaging or killing normal surrounding tissue. Our aim is to maintain high AMF amplitudes over a large region of interest (ROI) at a select frequency of AMF, to induce heating of the magnetic nanoparticles at the tumor site, without depositing excessive power in normal surrounding tissue regions. One solution to this challenge is achieved with a dielectric material that shields the electric fields to minimize induction of eddy currents that cause non-specific heating. Water was selected because it has a high dielectric constant (80.10), is diamagnetic, electrically polarized and nearly transparent to magnetic fields. We present results obtained from computer simulations, gel phantoms, and mouse models that demonstrate a two-fold reduction of the non-specific heating. Further reductions of non-specific heating were obtained by flowing the water through the shield for active cooling. These results demonstrate the potential value of water dielectric shielding to permit high AMF amplitudes at tumor sites to activate the magnetic nanoparticle heating while controlling overall heating in normal tissues.

Influence of synthesis parameters on magnetization and size of iron oxide nanocrystals

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Superparamagnetic iron oxide nanocrystals (SPIONs) have attracted great attention in the last decade due to their promising applications in MRI as contrast agent, in drug delivery, magnetic storage, etc. For such applications, nanoparticles with controllable size, size distribution and functionality are required. During the last two decades, many researchers have been involved in studies to find out what the key points are for synthesis of uniform-sized SPIONs with narrow size distribution [1]. The nonaqueous or non-hydrolytic synthesis has thereby been presented as particularly promising technique for the controlled synthesis of such nanoparticles [2]. In spite of these efforts, many questions are still open regarding the effect of synthesis parameters such as initial precursor concentration, temperature, time, etc. on size, size distribution and magnetization.

In this work, water dispersible SPIONs were synthesized via a high temperature decomposition of iron acetylacetonate ($Fe(acac)_3$) in triethyleneglycol (TEG). The effect of synthesis parameters (iron acetylacetonate concentration: 1 mmol and 4 mmol in 40 mL TEG, reaction time: 1 and 3 h, temperature: 216 and 266 ºC, and temperature ramp rate: 3 and 15 ºC/min, on the decomposition process were studied by applying design of experiment methodology (DOE). The SPIONs magnetization in water and TEG was characterized by fluxgate magnetorelaxometry [3]. DOE analysis shows that the magnetization only depends on precursor concentration and reaction temperature (Fig. 1). The hydrodynamic size of the particles was characterized by dynamic light scattering (DLS). Analysis of DLS results and correlation between hydrodynamic size and the magnetization will be discussed.

This work was financially supported by the DFG via SFB 578.

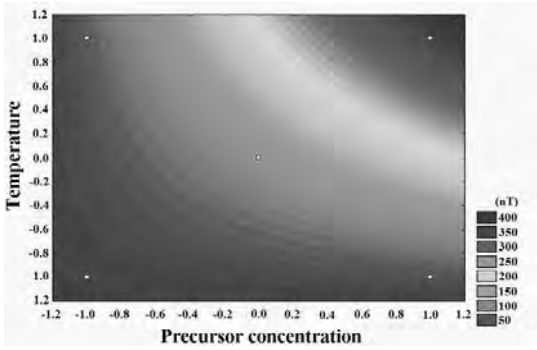

Fig. 1: Surface contour plot presenting dependency of SPIONs magnetization on precursor concentration and reaction temperature

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Magnetic core-shell fluorescent pH nanosensors

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Fluorescent nanosensors that come with a magnetic core can be readily targeted to sites of interest *in situ,* and the application of an external magnetic field may also improve cellular uptake in samples *in vitro.* Here we describe iron oxide-based nanoparticles that have been coated by two successive silica shells, embedding two fluorophores, which allow for ratiometric pH-measurements in the biologically relevant range. Iron oxide magnetic nanoparticles (10-20 nm in diameter) were formed by a co-precipitation reaction of ferrous and ferric salts. These were then added to a water-in-oil microemulsion, where the hydrophilic silica shells were attached through hydrolysis and condensation of tetraethoxyorthosilicate (TEOS), together with silylated dye derivatives. Sulforhodamine B was embedded in the inner silica shell, to be used as a reference dye; while a pH-sensitive fluorescein was incorporated into the outer shell.

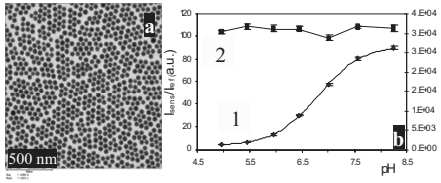

Figure 1 a) TEM; b) Changes in fluorescence intensity of the reference dye (2) in the pH-nanosensors (λ_{exc}: 557 nm, λ_{em}: 584 nm) and the fluorescence intensity ratio (1) between fluorescein and reference dye: $I_{sens}/I_{ref} = I_{517}/I_{584}$.

Once synthesized, the particles were characterized by morphology, size, composition and magnetization, using dynamic light scattering (DLS), transmission electron microscopy (TEM), X-ray diffraction (XRD) and vibrating sample magnetometry (VSM). TEM analysis showed the nanoparticles are very uniform in size (60-70 nm in diameter) and shape (Fig 1.a). Wide-angle diffractograms showed, for uncoated as well as coated nanoparticles, typical peaks for the spinel structure of magnetite at the same diffraction angle. When using VSM, we obtained the magnetization curves for our ferrimagnetic nanoparticles and the typical magnetization parameters as saturation magnetization (Ms), coercivity (Hc), and relative remanence (Mr/Ms). The resulting magnetic nanosensors show a satisfactory magnetization that is suitable for nanoparticle separation and localized targeting. The relationship between the ratio in fluorescence between sensor and reference fluorophores [1], and pH, was adjusted to a sigmoidal fit using a Boltzmann type equation giving a pK_a value of 6.8 (Fig1. b (1)). The fluorescence intensity of the reference dye does not change significantly (~3.0%) by modifying the pH of the nanoparticle dispersion in the range from pH 5 to pH 8 (Fig 1.b (2)).

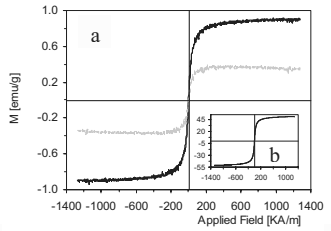

Figure2. Field-dependent magnetization curves of a) core-shell magnetic silica nanoparticles with different amount of magnetic cores. Gray line: 1 mg. Black line: 10 mg. The inset b) is a view of the magnetization curves for only the magnetic cores.

Due to their intrinsic biocompatibility, functionalization, as well as their nanometer size, these nanosensors will be suitable for pH monitoring in many relevant biological materials. Their magnetic properties also allow for the targeting specific tissues and/or microorganisms in situ.

We gratefully acknowledge the financial support from the European Union for the projects "Sensor Nanoparticles for Ions and Biomolecules" (MTKD-CT-2005-029554); and "Development of robust and quantitative biosensors based on near-infrared two-dyed silicate nanoparticles" (PIEF-GA-2008-220775). We also gratefully acknowledge the support of the Deutsche Forschungsgemeinschaft.(project MO 1062/6-1).

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Size-dependent Accumulation of PEGylated Coated Magnetic Iron Oxide Nanoparticles in Tumours

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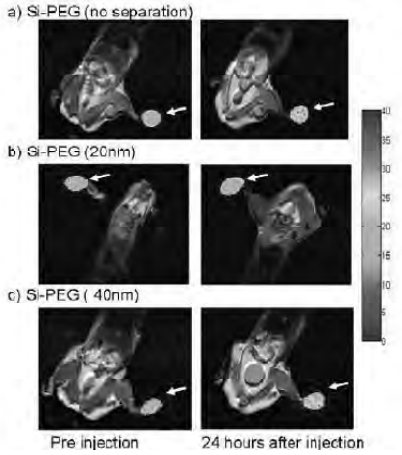
Abstract Summary: We here present results regarding synthesis of magnetic nanoparticles coated with polyethylene glycol (PEG) in two different sizes and detection of size dependent tumour accumulation in vivo using MRI.

Introduction: Nanoparticles made with an iron oxide core can function as contrast-enhancing agents in magnetic resonance imaging (MRI) for imaging of different diseases including cardiovascular and neurological diseases and cancers [1-3]. The contrast-enhancing agent allows diagnosis of both benign and malignant cancers.

Experimental Methods: Iron oxide nanoparticles coated with commercial PEG silane were made by ligand exchange of oleic acid coated nanoparticles. Size and surface charge were measured with dynamic light scattering. Different sizes of particles were prepared by running the sample through a magnetic msMACS column on a neodymium magnet. Foot subcutaneous tumour-bearing mice (SCCVII tumor n=6) were MRI scanned before and after intravenous injection with the two different sized nanoparticles or a non-separated mix. As a measure of accumulation, the R_2 relaxation rate was used.

Results and Discussion: The biocompatible PEGylated nanoparticles had a 12 nm magnetite iron oxide core with a narrow hydrodynamic size distribution around 26 nm and a slightly negative surface charge that could be magnetically separated into two distinct size subpopulations of 20nm and 40nm. Before injection the R_2 value in the tumour were around 20 s^{-1}, this increased after 24 h to ~ 25 s^{-1} for mice injected with the non-separated particles and the larger 40 nm particles. The small 20 nm particles, however, did not lead to an increase in the contrast within the tumor (Figure 1a-c).



Tumour R_2 map overlay (arrow) on a on a T_2-weighted MRI image. a) MRI of the PEGylated particles without magnetic separation, show enhanced contrast in the tumour after injection of the nanoparticles(arrow). b) The contrast level in the tumour did not change after injection of the 20 nm MNPs. c) Injection of the 40 nm MNP give rise to the highest contrast change in the tumour.

A possible explanation on the specific accumulation of the 40nm particles could be that the smaller 20 nm particles are able to extravasate from all the blood vessels into tissue whilst the extravasation of larger 40nm particles is restricted to the leaky vasculature of the tumour.

The increased entry of the 40 nm particles into tumour tissue may also partially be explained by the increased cellular uptake by macrophages. It has previously been reported that tumour-associated macrophages can capture nanoparticles at the tumour site[4]. Therefore, the accumulation of the 40 nm particles in the tumour is probably a combined effect of the particles specific extravastion and capture by macrophages in the tumour.

Conclusion: A size dependent uptake into murine tumours was observed after intravenous injection with differently sized particles, which provides important considerations for improving bioimaging approaches.

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Development and assessment of sucralfate magnetic nanosuspension for targeted ulcer therapy

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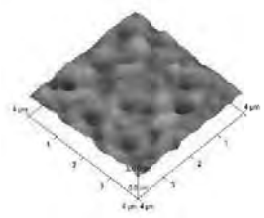
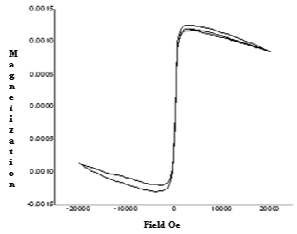
Abstract

Nanosuspension formulations tackled the tribulations associated with the delivery of poor water/lipid-soluble drugs. Nanosuspensions are unique in its simplicity and offers wide range of merits over the lacunas of conventional drug delivery[1]. Such merits would be amplified when the system could be coupled with magnetic properties[2]. Hence, the goal is to develop sucralfate magnetic nanosuspension for oral targeted, controlled drug delivery with enhanced bioavailability and stability. The drug nanoparticles were prepared by high frequency sonication; coupled with magnetite and further coated with biodegradable polymers to form sucralfate magnetic nanosuspension. The formulation was optimized through preformulation studies. Various physicochemical parameters viz. particle size, surface morphology, magnetic susceptibility, density, viscosity, sedimentation rate, assay, drug release, release kinetics, zeta potential, and stability were evaluated. The particle size falls between 300 to 400 nm; SEM and AFM images shows the spherical nature of particles; magnetic susceptibility ($50X10^{-6}$) and VSM magnetogram confirms the superparamagnetism and controlled drug release for an extended duration with zero order solubility kinetics at pH 1.2 and 7.4. Together with the zeta potential (-33.3 mV), the formulation shows excellent stability for the tested duration. The *in-vivo* targeting potential for ulcer therapy in Wistar Albino rats was effectively evaluated. The remarkable results revealed that all the evaluated parameters are well within the desirable specifications of an ideal magnetic nanosuspension and would be a potential candidate for targeted therapy of duodenal ulcer and could be explored further to develop a process for scale up.



AFM photomicrographs



Field-dependent magnetization curve

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FORMULATION DESIGN, DEVELOPMENT, OPTIMISATION AND EVALUATION OF AZITHROMYCIN MAGNETIC NANOEMULSION

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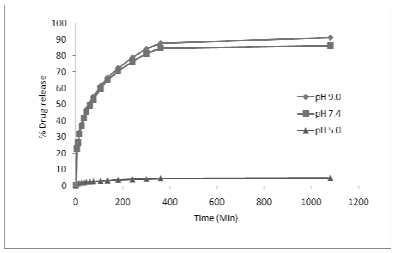
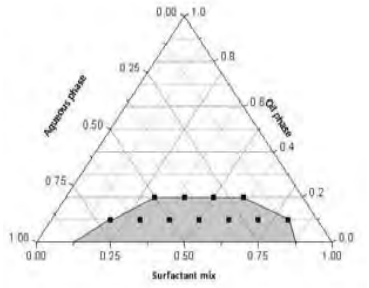
Abstract

Azithromycin is one of the leading anti-biotic that has been widely used for treating respiratory infections. Targeting to the infected area will reduce the associated systemic side effects of antibiotics. To effectively target the infected site i.e., lungs and to diminish the setbacks of the conventional drug delivery system; development of azithromycin magnetic nanoemulsion (AMNE) as a magnetic targeted drug delivery system is set as the objective of the present work. AMNE was formulated by using ethyl oleate based ferro fluid containing azithromycin as oil phase; Poloxamer 188 as surfactant; lauroglycol 90 as co-surfactant. AMNE formulation was optimized through ternary phase diagram constructed by varying the proportion of oil phase; aqueous phase and surfactant/co-surfactant mixture. The particle size of AMNE formulation ranges from 37-300nm. AMNE formulation at various compositions were evaluated for various physicochemical properties viz., magnetic susceptibility (30.46 - 40.00 emu/g); zeta-potential (-48.8 - -50.1 mV); density (0.08871-0.1575g/cm^2); viscosity (0.6520 - 0.8872cP) and conductivity (0.0999 - 0.1200 mS/cm). 60 - 90 % of azithromycin was released from AMNE in 72 hr. The drug release kinetics and release mechanism from AMNE formulations were also evaluated. With promising results such as optimal physicochemical parameters, prolonged drug release along with proven targeting efficiency in *in-vitro* system would certainly offer value addition to the prospective of the drug (azithromycin) as magnetic nanoemulsion formulation for targeting and treating respiratory infections.



Drug release profile at various pH



Ternary phase diagram of AMNE formulations

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Effects of Nanoparticle Surface Charge On Cellular Uptake and Mechanism of Internalization *In Vitro*

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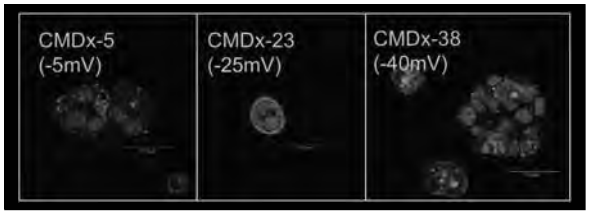
Magnetic nanoparticles are attractive in various novel applications including: a) targeted drug delivery, b) MRI contrast enhancement agents, c) magnetic cell sorting schemes, d) nano-/bio-sensors, e) agents for cancer treatment and f) magnetic assisted transfection. In order for many of the biomedical applications mentioned above to be successful, the nanoparticle's physicochemical properties (e.g., shape, size, and surface chemistry) must be tailored to promote specific interactions between the cell and the nanoparticle.

We have recently developed methods for the synthesis and modification of superparamagnetic iron-oxide nanoparticles with narrow size distributions. These magnetic nanoparticles have been functionalized with covalently-grafted fluorescent carboxymethyl-dextran (CMDx) chains with different degrees of COOH substitution. The varying degrees of COOH substitution in the CMDx chains have the effect of giving these nanoparticles different net surface charges, allowing us to systematically assess the effect of nanoparticle surface charge on nanoparticle/cell interactions. Through Confocal Microscopy and Inductively Coupled Plasma Mass Spectrometry, we have observed that more negatively charged nanoparticles have higher rates of cellular internalization than less negatively charged nanoparticles. We have also found, by inhibiting specific internalization pathways with drugs that block particular molecular mechanisms of cellular internalization, that CMDx- coated nanoparticle internalization is governed by (i) fluid-phase endocytosis, and (ii) receptor-mediated endocytosis due to non-specific protein/nanoparticle interactions. These findings and our on-going work can serve to guide design of nanoparticle surface coatings to control the extent and pathway of internalization in cells, enhancing the potential of magnetic nanoparticles in biomedical applications.



Surface charge- dependent internalization of iron-oxide nanoparticles (IONPs) coated with fluorescent carboxymethylated dextran (CMDx) into CaCo-2 cells: Dextran with 5, 23, and 38 COOH -groups per dextran chain was synthesized and covalently grafted onto IONPs. The higher the degree of substitution in the dextran chain, the more negative the nanoparticle surface charge. IONPs with a high negative surface charge have higher levels of cellular internalization than more neutral IONPs.

Poster 113

STRUCTURE AND PROPERTIES OF MAGNETIC BEADS FOR BIOLOGICAL SENSOR APPLICATIONS

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[(2)]Institute of Chemical Physics RAS, *Chernogolovka, Russia*

Nanoparticles exhibit unique physical properties due to the surface or quantum-size effects. Particular attention has been focused on magnetic nanoparticles and substantial progress has been done in this field. This is mainly due to the advances in the processing methods and development of characterization techniques. Substantial achievements in that field enabled fabrication of composite systems consisting of metallic particles embedded in various organic or inorganic matrices with prospective applications as sensors in medical and biological applications.

In this work ferromagnetic composites, consisting of elementary metals or carbides nanocrystallites, stabilised in carbon matrix, were prepared by the procedure comprising formation of appropriate metal acrylamide complexes, followed by frontal polymerization and pyrolysis of the polymer at various temperatures. The pyrolysis products were in a form of beads, which contained randomly distributed nanocrystallites having various composition and size ranging from few to tens of nanometres, depending on the pyrolysis temperature. Application of this procedure stabilizes the nanostructure and enables processing of spherical nanoparticles within a narrow window of sizes (Fig. 1).

The magnetic parameters depend on the crystallite size, determined by chemical composition of the monomer and pyrolysis temperature. The nanocrystallites pyrolysed at 773 K exhibit ferromagnetic properties for Co and Fe, and superparamagnetic behaviour for the Ni nanoparticles.

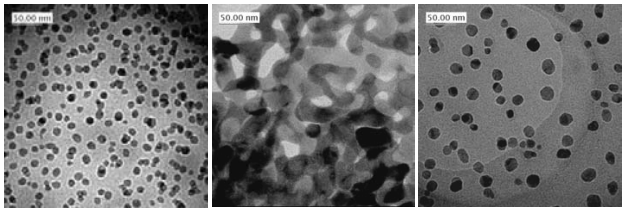

Fig. 1. TEM microstructures of the nanocrystallites pyrolysed at 773 K; left – Co, middle – Fe, right – Ni.

The nanocrystallites can potentially be applied as sensors for tagging of biological substances or for targeted drug delivery. The toxicity test showed that the beads have very low toxicity which depends on both, their concentration in the solution and type of the ferromagnetic element. Generally the iron containing beads have negligible toxicity, which slightly increase for cobalt and nickel.

Poster 114

Characterization of dispersion properties of magnetic fluids derived from nano particles by multi-sample analytical centrifugation

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The focus of our contribution is directed to the qualitative and quantitiative determination of the colloidal properties of water based magnetite magnetic fluids (MF) suitable for diagnostic and therapy applications using multisample analytical centrifugation with high resolution photometric detection [1].
Magnetite particles were prepared by the precipitation method. The samples were stabilized by different approaches (citric acid, poly aspartic acid, carboxylated dextran or by a double layer of dodecanoic acid or a nonionic surfactant).
As an example, the transmission profiles are shown for three different samples (Fig. 1).

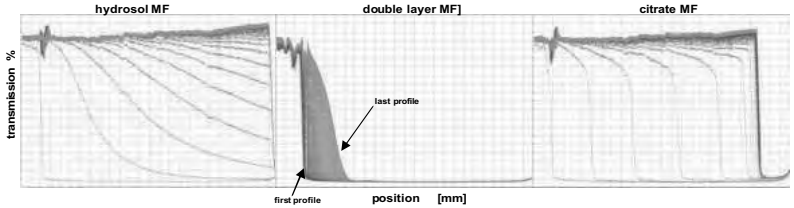
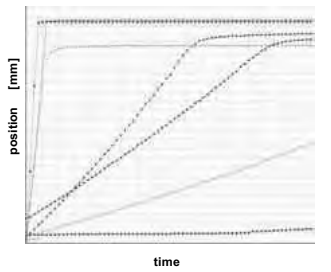


Fig. 1 Transmission profiles obtained during centrifugation at 2300 *g* for samples differently stabilized

As expected the unstabilised hydrosol separates relatively fast. The transmission profiles exhibit a gradual variation of the transmission along the sample length, i.e. the particles move individually with different velocities according to differences in the hydrodynamic size of the particle aggregates. The double layer stabilised MF shows the same principle separation behaviour but sediments much more slowly, i.e. is much more stable. The citrate stabilised MF is also relatively unstable. A sharp sedimentation front is observed characteristic for a flocculated particle network.
The samples of different stability can be easily compared quantitatively by comparing the sedimentation kinetics (movement of the boundary supernatant/dispersion Fig. 2). The highest stability was observed for the double layer MF (smallest slope) and the lowest stability for the neat hydrosol.



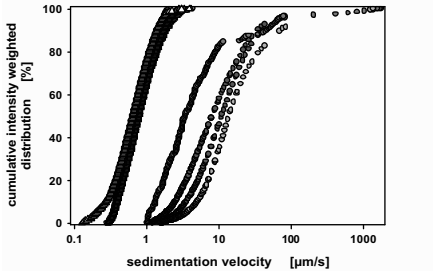


Fig. 2 (left) Sedimentation kinetics of differently stabilised MF during centrifugation at 2300 *g*
Fig. 3 (right) Velocity distribution of differently stabilised MF showing high variability in size distribution

In addition the instrument also allows to determine the sedimentation velocity distribution or particle size distribution (Fig. 3). While the velocity distribution as an integral measure of stability does not require any material data, for calculation of the size distribution the effective densities of particles are required. These might be approximately determined from the density of the core particles, of the shells and the estimated shell thickness.

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A novel method for rapid detecting *Salmonella enteritidis* and *Escherichia coli* O157 by immuno-magnetic capture / double-tagging PCR / enzyme immunoassay

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Magnetosome (bacterial magnetic nanoparticle, BMP) were bio-synthesized by magnetotactic bacteria. They were mainly composed of Fe_3O_4 or Fe_3S_4, and enclosed by biomembrane. The biomembrane offered BMP plenty of $–NH_2$ to connect with antibody through covalent bond. The resulting BMP-antibody complexes could be used to capture corresponding pathogens in many methods or processes.

A new method was developed to detect *Salmonella enteritidis* and *Escherichia coli* O157. Techniques including BMP-based immunomagnetic separation (IMS), double-tagging PCR, and enzyme immunoassay were employed in our method. Magnetosome were used in two steps of this method.

The procedure of this approach was as follows,

1) The pathogen (salmonella or *E. coli* O157) was captured by BMP-antibody complex, and collected by a magnet. DNA was extracted by boilinglysis;
2) Two sets of primers were designed to amplify the gene *invA* of salmonella and the gene *rfb* of *E. coli* O157. Each set of primers was labeled by biotin and digoxin at 5'-end respectively. Double-tagging PCR was performed and resulted in double tagged amplicon (one 5'-end labeled with biotin and the other 5'-end with digoxin).
3) The double-tagged amplicons were captured by streptavidin modified BMPs via biotin-avidin interaction.
4) Alkalinephosphatase (AP) modified anti-digoxin antibody was combined with digoxin on the amplicon. The substrate of AP was added and OD_{405} was detected after incubation.

S. enteritidis and *E. coli* O157 with different concentrations were detected by this method. The detection limit was 50-100 cfu/ml without pre-enrichment. It would be as low as 0.5 cfu/ml after 2-3 hour incubation. The results suggested this method took much less time comparing with conventional detection methods.

BMP was used as the carrier of immunomagnetic capture in double-tagging PCR and enzyme immunoassay. Different pathogens could also be detected by BMPs modified with corresponding antibodies and special primers of double tagging PCR.

Key words: Magnetosome; BMP; food-borne pathogen; *Salmonella enteritidis*; *Escherichia coli* O157; double-tagging PCR

Acknowledgements

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Kirkendall effect for fabrication of hollow MnF_2O_4 nanostructures as contrast efficacy T_2 imaging agents

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Of the methods employed in the preparation of magnesium ferrite ($MnFe_2O_4$) nanomaterials, the thermal decomposition and co-precipitation have been mainly used as a synthetic route. In this study, a self-templation approach, involving the Kirkendall effect, was employed to successfully synthesize hollow magnesium ferrite ($MnFe_2O_4$) nanoparticles. Depending on the molar ratio of magnesium stearate ($Mn(SA)_2$) and iron stearate ($Fe(SA)_3$), the hollow nanostructures of $MnFe_2O_4$ nanoparticles were easy tunable from nanospheres (Mn:Fe = 1:2) to nanorods (Mn:Fe = 1:1). Time-dependent observation indicated that the hollowing mechanism proposed is followed with a consumption of original manganese oxide templates (sphere or rod) and these voids condensed in the interior. TEM, XRD, FT-IR, and XPS, measurements were carried out to characterize the resulting hollow precipitates (hollow nanoparticles and nanotubes). The magnetization measurements including ZFC-FC curves and magnetization vs *H*/*T* as well as their usefulness for in vitro MR imaging were investigated for both $MnFe_2O_4$ hollow nanoparticles and nanorods. On the basis of in vitro MR assays, both $MnFe_2O_4$ hollow nanoparticles and nanorods were found great evolution in negative-contrast ability of MR images, whereas the original manganese oxide templates acted as paramagnetic T_1 MRI contrast agents.

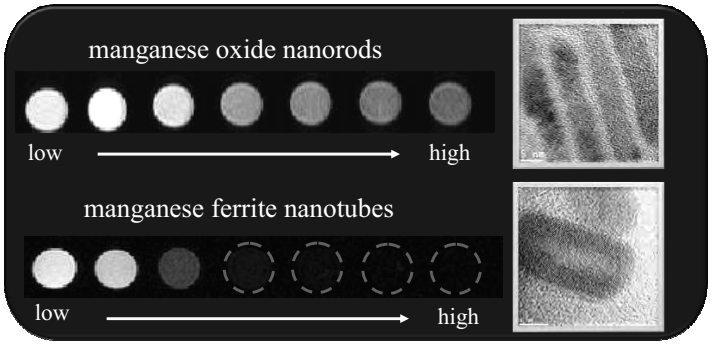

Figure 1. TEM images (right) and T_2-weighted MR images (left) of the manganese oxide nanorods (template) and manganese ferrite nanotubes (Kirkendall effect prodcut).

Poster 117

Characterization of oscillation dynamics of magnetic nanoparticles at ultra-low frequencies

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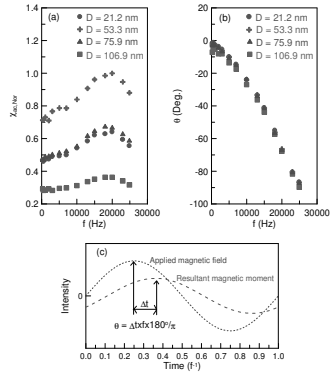
For magnetic nanoparticles dispersed in water, the time constant of Brownian relaxation is the orders of μs'. As a low-frequency magnetic field is applied, magnetic nanoparticles oscillate with the applied field. In this work, the frequency dependent oscillating properties of magnetic nanoparticles are characterized. The analyzer (XacQuan, MagQu) is used to detect the frequency dependent complex ac magnetic susceptibility χ_{ac} of magnetic fluid with magnetic nanoparticles. The frequency f of the applied magnetic field is varied from 300 Hz to 25000 Hz. The magnetic nanoparticles are consisted of Fe_3O_4 core coated with lauric acid to make nanoparticles dispersed in water. Different sizes of magnetic nanoparticles are synthesized for χ_{ac} analysis. According to the experimental results, the amplitude $\chi_{ac,o}$ of χ_{ac} increases with increasing the frequency of the applied field, and reaches to the maximum value at frequency around 20000 Hz, and then decreases as frequencies higher than 20000 Hz. This implies that there exists a resonant oscillation for magnetic nanoparticles at around 20000 Hz. The phase difference θ between the resultant magnetic moment of magnetic fluid and the applied field continuously was found to decrease as the frequency of the applied field increases. It is worthy noting that the phase difference θ is always negative for every frequency of the applied field. This evidences that the phase of resultant magnetic moment of magnetic fluid is retarded with respect to that of the applied ac magnetic field.



Driven frequency f dependency of (a) the normalized amplitude $\chi_{ac,\,Nor}$ and (b) the retarded phase θ of the complex alternative-current magnetic susceptibility of magnetic fluids under alternative-current magnetic fields. The scheme to illustrate the physical meaning for the retarded phase θ is shown in (c).

The particle-size dependent $\chi_{ac,o}$-f and θ-f curves are also investigated. The mean diameters of magnetic nanoparticles are ranging from 20 to 100 nm. As the mean diameter of particles increases from 20 nm to 50 nm, the χ_{ac}-f curve is shifted to the region with higher $\chi_{ac,o}$. However, the χ_{ac}-f curve is shifted to the region with lower $\chi_{ac,o}$ once the mean diameter is larger than 50 nm. Hence, there exists a suitable particle size to optimize the oscillation of magnetic nanoparticles under ac magnetic fields. All of these results can be explained by taking the following causes into account: magnetic interaction between applied field and magnetic nanoparticles, thermal energy, and shearing friction of magnetic nanoparticles during oscillation in water.

Poster 118

Magnetoresponsive Lanthanide Doped Phospholipid Vesicles

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We have investigated the influence of magnetic fields on different types of phospholipid based self-assembly structures in water. Phospholipids are amphiphilic molecules and are important components of biological membranes. In general, molecular assemblies are more promising to react to magnetic fields than individual molecules, because the magnetic energy acting on a single molecule is much smaller than the randomizing thermal fluctuation. We use magnetic fields as a non-invasive structure inducing or orienting force. Paramagnetic lanthanide ions were anchored to the membrane and used as magnetic handles to increase the magneto responsiveness. The different lipid mixtures were studied with SANS in magnetic fields of up to 8 T and temperatures from 2.5 to 30°C. Cryo-TEM micrographs were taken from the same samples. Additional information was gained by ^{31}P-NMR spectroscopy, DLS, and permeability measurements with and without magnetic fields of calcein, a water soluble fluorescence marker. Vesicles are self-closed phospholipid bilayers with a spherical shape, and are used as a model system to study physico-chemical properties of lipid membranes. In particular lipid rafts and domain formation on vesicles have received a lot of attention in recent research. [1,2] Domain formation was observed on small unilamellar vesicles with a size of 100 nm consisting of 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC) and a chelator lipid, 1, 2-dimyristoyl-sn-glycero-3-phosphoethanolamine-diethylenetriaminepentaacetate (DMPE-DTPA) with complexed lanthanides. Lipid segregation was temperature dependent and the solid domains were orientable in magnetic fields. Figure 1 shows a sketch illustrating the magnetic field alignable domain model. Parallel orientation of the domain normal to the magnetic field was observed if the lanthanide thulium was used and perpendicular orientation with the lanthanide dysprosium. [3]

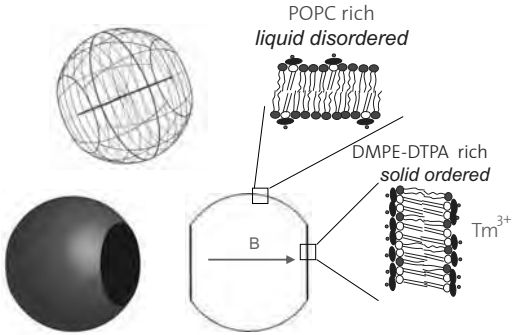

Figure 1. Sketch illustrating the magnetic field alignable domain model. DMPE-DTPA·Tm is assumed to form solid domains surrounded by POPC in the liquid-disordered state.

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Preparation of Silica Coated Superparamagnetic Nanoparticles and Its Application in Immobilization of Endo-Xylanase

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Superparamagnetic iron oxide (Fe_3O_4) nanoparticles were prepared by the co-precipitation method and then coated with amino-functionalized silica by reacting with tetraethyl orthosilicate (TEOS) and 3-aminopropyl trimethoxylsilane (APTMS) in an aqueous solution containing 2-propanol, Triton X-100, and ammonia. The resultant core/shell nanocomposites were characterized by SEM and XPS. Based SEM observations, the average particle size of amino group-containing core/shell nanoparticles was ca. 24 nm, which was larger than that (ca. 14 nm) of non-coated Fe_3O_4 nanoparticles. Peaks for elements of N, C and Si were all found in the spectra of XPS, suggesting that the coating of amino-functionalized silica was on the surface of iron oxide nanoparticles. By the activation of glutaraldehyde, the amino groups on core/shell nanoparticles were converted to reactive aldehyde groups. The glutaraldehyde-activated nanoparticles were then coupled with endo-xylanase, which were produced from *Bacillus halodurans* using corn cobs as the substrate. Both the amount of protein and enzymatic activity of xylanase immobilized on the core/shell nanoparticles increased with the amount of enzyme applied for immobilization (Figure 1). The immobilized endo-xylanase was able to catalyze the production of xylo-oligosaccharides from xylan.

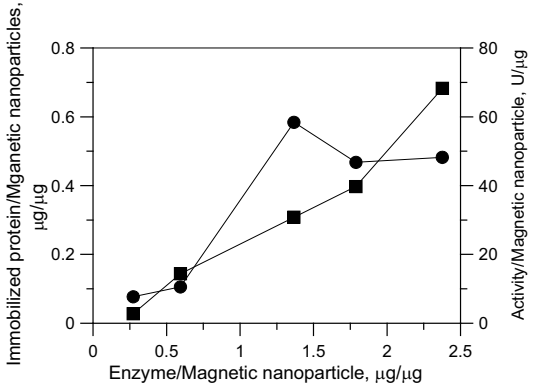

Figure 1. Amount of endo-xylanase (in terms of protein ● and activity ■) immobilized onto amino group-containing silica-coated superparamagnetic nanoparticles changes with the amount of enzyme applied to magnetic nanoparticles.

INVESTIGATION OF THE MAGNETIC BEHAVIOUR IN Fe_3O_4 FERROFLUID

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[2]Departamento de Física, Universidade Federal de Minas Gerais, C.P. 702, 30123-970 Belo Horizonte, Brazil.
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A ferrofluid based on Fe_3O_4 has been synthesized using the condensation method by coprecipitating aqueous solutions of $FeSO_4$ and $FeCl_3$ mixtures in NH_4OH and treated further in order to obtain colloidal sols by creating a charge density on their surface and functionalized by carapa guianensis (andiroba oil). Aqueous sample with an average particle diameter of about 7 nm were studied by Mössbauer spectroscopy and dc magnetization measurements in the range of 4.2-250 K. The saturation magnetization (M_s) at 4.2 K was determined from M vs 1/H plots by extrapolating the value of magnetizations to infinite fields, to 5.6 emu/g and coercivity to 344Oe. The low saturation magnetization value was attributed to spin noncollinearity predominantly at the surface. From the magnetization measurements a magnetic anisotropy energy constant (K) of 1×10^4 J/m^3 was calculated. Fe_3O_4 spectra at room temperature showed a singlet due to superparamagnetic relaxation and a sextet at low temperature. Mössbauer (Figure 1)

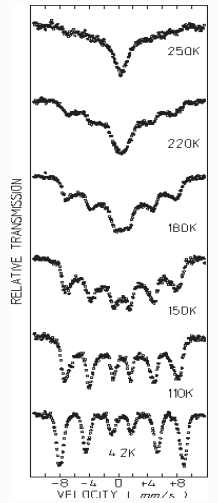

Figure 1 Mössbauer spectra of Fe_3O_4 ferroluid functionalized using andiroba oil.

Characterization of magnetic core-shell nanoparticle suspensions using ac susceptibility for frequencies up to 1 MHz

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Magnetic nanoparticles (MNPs) find wide application in medicine and bioanalytics. As has been demonstrated in [1-3], the measurement of the magnetorelaxometry (MRX) and the analysis of the experimental curves with the moment superposition model (MSM) is a quick and powerful tool for the estimation of structure parameters like core and hydrodynamic size distributions. The accessible range of relaxation time constants of the MNPs is limited by the switch-off time of the magnetizing field, by the bandwidth of the magnetic field sensors, and by the deadtime between switching off the magnetizing field and data acquisition for SQUID MRX systems. Consequently, relaxation processes with time constants below about 100 µs can not be detected.

An alternative magnetic technique is the measurement of the complex magnetic susceptibility. Measurements of the ac susceptibility have successfully been used for the study of MNP properties as well as for the realization of homogeneous bioassays. Here we report on measurements of the real and imaginary part of the ac susceptibility on different magnetic core-shell nanoparticle samples in the frequency range from 100 Hz up to 1 MHz. In the analysis of the experimental susceptibility spectra both the distribution of core and of hydrodynamic sizes are taken into account. To investigate the ac susceptibility of suspensions of $CoFe_2O_4$ nanoparticles with hydrate shell when a static magnetic field is superimposed, an additional cylindrical coil with its axis parallel to the ac excitation field coils was used. It is shown that – taking into account the magnetic field dependence of the Brownian relaxation time [4] – both hydrodynamic and core size distribution can be determined from measurements on MNP suspensions. The estimated core and hydrodynamic size distributions of the aqueous $CoFe_2O_4$ nanoparticle suspension agree well with those obtained from TEM and dynamic light scattering, respectively. For a correct determination of the core size distribution from the shift of the peak of the imaginary part χ'' caused by a static magnetic field, it is important that the MNP suspensions are sufficiently strong diluted, thus minimizing magnetic dipole-dipole interactions and the formation of dimers and larger agglomerates.

This work was financially supported by the DFG via SFB 578 and by the BMBF under contract number 13N9174.

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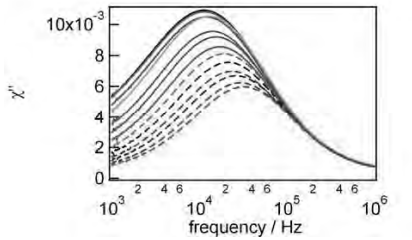

Imaginary part of ac susceptibility of $CoFe_2O_4$ nanoparticle suspension for different static magnetic field values. Peak position in χ'' shifts to higher frequencies with increasing static field.

Thermal Destruction on the Nanoscale:

Cell Membrane Hyperthermia with Functionalized Magnetic Nanoparticles

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Due to enhanced sensitivity of tumor cells to increased temperature, cancer therapy with hyperthermia might be a promising approach for the treatment of resistant tumors[1]. Recently magnetic nanoparticles (MNPs) exposed to alternating high frequency magnetic field have been used for local hyperthermia. Heat generated by MNPs depends strongly on the specific heating power of MNPs and particle concentration[2]. Hence, heat generation could be increased by increasing the concentrations of MNPs in tumor tissues, by enhancing the power of magnetic fields, and/or the time the patient is exposed to the field[3]. However, MNPs are a rather weak source of heat, and the MNPs internalized by a cell might not provide the amount of heat required to destroy a tumor cell[4]. In this work the clusters of superparamagnetic iron oxide MNPs captured by receptors on the cell membrane have been studied as potential systems to deliver heat to the membrane of tumor cell. We propose a new mode of hyperthermia – a temperature-induced cell membrane destruction with functionalized MNPs. The clusters of MNPs targeted to the membrane of a cancer cell by functionalization with receptor ligands could provide the heat sufficient for the cell membrane damage. A theoretical heat transfer analysis shows that the proposed mechanism of the membrane destruction via hyperthermia with MNPs is feasible. The optimal size of the MNPs was calculated. Studies on the receptor internalization revealed that treatment of cells with inhibitors of endocytosis prevents the uptake of MNPs by the cells, and cluster them on the cell membrane surface. Further, the surface density of MNPs on the cell membrane was analyzed as a function of time. The obtained preliminary results confirmed the possibility of cell membrane hyperthermia with functionalized magnetic nanoparticles.

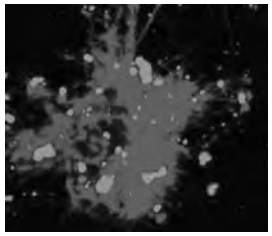

Figure 1: Human macrophage (membrane in red) pre-treated with an inhibitor of clathrin-mediated endocytosis and SPIO (green) on the cell surface

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Interaction of Partially Iron Saturated Human Transferrin with Superparamagnetic Iron Oxide Nanoparticles

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Protein binding to nanoparticles is recognized as one of the key elements that affect both the toxicity and biodistribution of nanoparticles throughout the body.[1] The biological response of the body is influenced by this nanoparticle-protein complex. Since SPIONs are injected almost directly to the blood for targeted imaging and drug delivery applications, we studied the interaction of human transferrin (which is a protein found in the blood of all vertebrate species) with superparamagnetic iron oxide nanoparticles (SPIONs). Bare SPIONs with an average size of 5 nm with a very narrow size distribution have been produced according to optimal synthesis parameters.[2] Poly vinyl alcohol (PVA) was employed as a coating of SPIONs (total core shell size of 8 nm). Both bare and PVA-coated SPIONs were incubated with the partially iron saturated human transferrin protein for a period of 2 h in physiological conditions (i.e. 37°C and 5% CO_2). In order to achieve reliable results, the amount of protein was fixed at 2.8 ml per m^2 of SPION surface.[3] In order to immobilize SPIONs in the magnetic column after the incubation period, the protein-treated SPIONs were run through a strong magnetic field using MACS® (see Figure 1(a)). Consequently, the flow-through fraction was collected and nanoparticles were washed with 1 M KCl solution. SDS-PAGE was used to analyse the interaction of human transferrin with SPIONs. Human transferrin showed a tight cluster of bands corresponding to transferrin molecular weight of 77 KDa (see Figure 1(b)). According to Figure 1(b), there is no detectable protein breaking down due to interactions with magnetic nanoparticles. Probing the bands in 1M KCl solutions reveals the attachment tendency of human transferrin to both the bare and coated SPIONs. After washing with 1KCl solution, the fixed SPIONs were collected by removing the column from the high magnetic field and analysed using gel electrophoresis. According to the results, the trace of protein attachment to the bare SPIONs was detected even after washing with 1 KCl solution, whereas there was no detectable band in the coated one, confirming the strong interaction between human transferrin and bare SPIONs. Hence, the attachment quality of human transferrin to the SPIONs is highly dependent to the surface characteristics of magnetic nanoparticles together with their sizes.

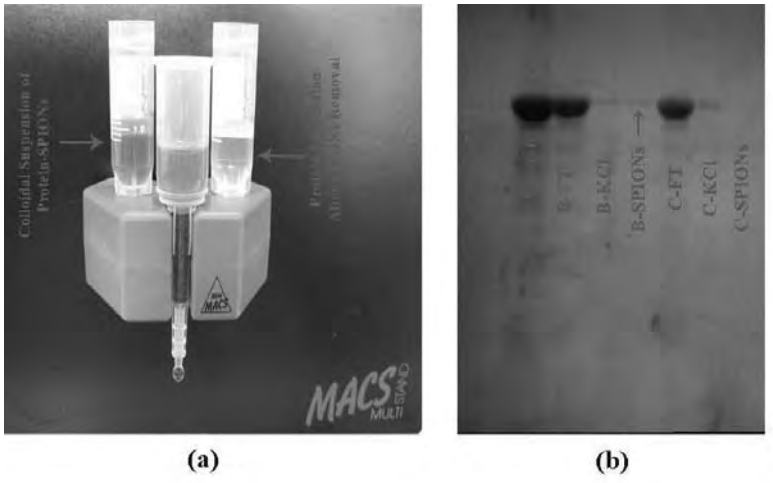

Figure 1: (a) Separation process with MACS®; (b) SDS-PAGE (FT: flow through, B: bare, C: coated)

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Superparamagnetic clusters as carriers for an anatase photocatalyst

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Oxidative decomposition using titanium dioxide (TiO_2) as the photocatalyst can be effectively used for the purification of water polluted with organic pollutants. In the process of photocatalysis a large surface area of the photocatalyst should be provided. This can be easily achieved when the photocatalyst is prepared in the form of nanoparticles and dispersed in the polluted water. However, there is a problem related to the difficulty in completely eliminating the nanoparticles from the water after the purification. One of the possible solutions to this problem involves the immobilization of the photocatalysts on magnetic carriers, which allows them to be eliminated from the water suspension after cleaning using an external magnetic field.

The idea of immobilizing the photocatalyst with a magnetic carrier is not new. Generally, two types of magnetic carriers have been used: superparamagnetic nanoparticles, or larger, ferrimagnetic particles of different ferrites. For magnetic separation, both types of carriers are rather ineffective. Because of the attractive magnetic forces, the ferrimagnetic particles are very difficult to be dispersed in the polluted water, whereas the magnetic forces acting on the superparamagnetic nanoparticles are generally too weak for their efficient magnetic separation.

In this study, multi-core magnetic carriers were applied. The carriers had the form of clusters (50–200 nm in size) of superparamagnetic maghemite (γ-Fe_2O_3) primary nanoparticles (15 nm). The clusters were prepared using controlled agglomeration of the maghemite nanoparticles with adapted surface properties in their suspensions. The nanoparticles' surfaces were amino modified by coating them with 3-(2-aminoethylamino)propylmethyldimethoxysilane (APMS), or carboxyl modified using adsorption of citric acid or bonding succinic acid to the amino-modified nanoparticles. The nanoparticles in their aqueous suspensions were agglomerated into the clusters by applying electrostatic attractive forces between the nanoparticles with an opposite surface charge, or by direct chemical reactions between amino and carboxyl functional groups at the nanoparticles surfaces.

The photocatalyst was deposited onto the magnetic clusters using coating of the nanocrystalline anatase layers onto the magnetic clusters during the hydrolysis of aqueous $TiOSO_4$.

Antibody and Fc-fusion Protein Purification using Protein A/G coated Magnetic Beads

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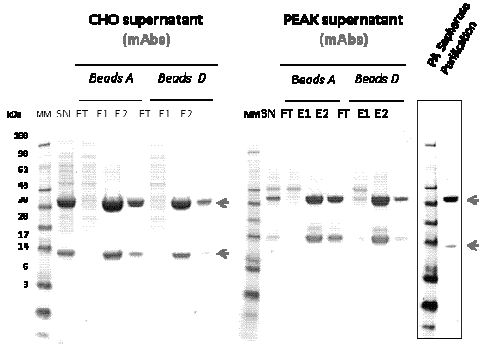
*Merck Chimie SAS, Estapor Microspheres - 201 rue Carnot - F-94126 Fontenay sous Bois Cedex. France. § Email: pmalinge@novimmune.com

Magnetic beads are a well established alternative to conventional chromatography resins in automated high-throughput protocols replacing centrifugation and filtration by simple magnetic separation.. Estapor® beads combine this advantage with unique features like high magnetite content and unporous surface which allows them to migrate very fast in magnetic fields while binding target molecules with a low unspecific adsorption. In order to select the best solid support, Magnetic Beads are modified with Protein A or Protein G by means of different coupling methods (covalent or passive) and on different sizes (small and medium).

Estapor Ref. (Beads)	Size (µm)	Surface (µEq/g)	Ferrite (%)	Coupling Strategy
M1-030/40 (A & D)	0,36	COOH (85)	37	Covalent coupling
EM1-100/40 (B & E)	0,99	COOH (82)	47	Covalent coupling
MS-070/40 (C & F)	0,95	Plain (none)	52	Passive coupling

Purity by SDS PAGE – mAbs purified from Mammalian cells supernatant



These results clearly show that our coated magnetic beads (A and D) are well suited for IgGs purification for both supernatants CHO and PEAK. Heavy and Light chains are similar to Protein A Sepharose purified products. IgGs purified from PEAK supernatant using beads A and D are biologically active. Same dose response was obtained for magnetic beads and Sepharose purification.

ImmunoMagnetic Separations are very common in Cell Biology, ImmunoAssays and Nucleic Acid Technology. These technologies are expanding into new fields including Proteomics and as new and efficient approach to separate Immunoglobulins. It is clear that people who have never used ImmunoMagnetic Separation should seriously consider evaluating this technology.

Biomedical examination of Fe-Co oxide nanoparticles for local ferromagnetic hyperthermia

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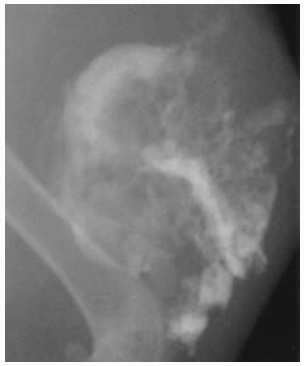
Much attention has been paid to the behavior of endovenously introduced colloidal superparamagnetic particles, in particular, cobalt ferrite particles [1] which are capable of traveling in the circulatory system. By using small amounts of coated particles and modern protocols of biological evaluation, cobalt ferrite particles are reported as biocompatible. We consider the case of uncoated particles introduced into the tumor in relatively large quantities. The particles under investigation are used for thermal destruction of tumors by low-frequency ferromagnetic hyperthermia as described in another report [2]. They are 50-80 nm sized maghemite particles with small addition of Co (2.7 mass % of the total amount of metals). Experiments were carried out on 2–3-months old Af female mice of 20 g body weight, which were kept under steady conditions of a vivarium at 22±1.0 ◦C with free access to water and food.



Distribution of nanoparticles injected in a tumor

After per oral and subcutaneous introduction no abnormalities were observed in their appearance and behavior. In 2 months, there were no magnetic particles in the stomachs of the animals and no signs of necrosis or inflammation at the site of injection. When injected in body of hyperploid strain of Erlich carcinoma the particles are well distributed in tumor without penetration into the healthy tissues (figure). We also found that subcutaneously introduced particles do not exert cytostatic, mutagenic and carcinogenic effects. Also, no effects on the spontaneous tumor formation level in mice were detected of the AC magnetic field with frequency 3.7 kHz and amplitude of 700 Oe.

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Poster 127

Functionalization of magnetic nanoparticles using curcumin conjugates: A multidrug carrying vehicles for targeted drug delivery applications.

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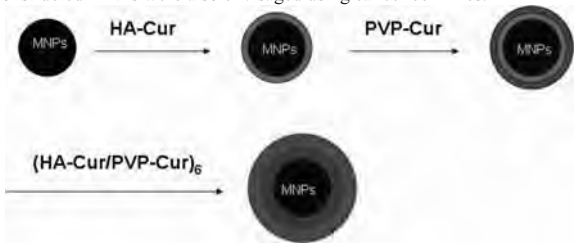
The success of chemotherapy depends on the drug as well as how it is delivered to its target. Because of the relatively non-specific action of chemotherapeutic agent, there is always some toxicity to normal tissue even under optimal conditions. Therefore it is of great importance to be able to selectively target the anti proliferating agent to tumor target as precisely as possible. Over these years, different approaches have been explored for drug targeting. Among them magnetic nanoparticle based drug targeting, the targeting of drug immobilized on the magnetic materials under the action of an external magnetic field, has gained considerable attention. Nanoparticle that possess magnetic properties offer exciting new opportunities including site specific drug delivery, magnetic resonance imaging and hypothermic treatment of malignant cells.

The present invention relates to the functionalization of MNPs by layer by layer construction of water soluble curcumin conjugates on the surface of aminated magnetic nanoparticles and the encapsulation of other commonly used anticancer agents in between these layers to impart high potency in cancer treatment. Briefly, Magnetic nanoparticles (MNPs) having size in the range of 20-50 nm were synthesized by the co-precipitation of ferrous chloride and ferric chloride in ammonium hydroxide solution. Hydrophillicity and charge were imparted on the surface of magnetic nanoparticles using trimethoxy silyl derivatives such as N-[3-trimethoxy silyl) propyl)-ethylene diamine]. Surface charge created on MNPS will act as a driving force for the construction of different layers of curcumin conjugates on its surface. Simultaneously curcumin conjugates of hyaluronic acid (HA-Cur) and Polyvinylpyrrolidone (PVP-Cur) were synthesized by esterification reaction. Finally different layers of HA-Cur and PVP-Cur conjugates were constructed on the surface of MNPs using 10 wt % solutions of HA-Cur and PVP-Cur in deionized water. Each layer formation on the surface of MNPs was confirmed by zeta potential measurements. Antiproliferative ativity and cellular uptake efficacy of these layer by layer constructed MNPs were also envisaged using cancer cell lines.



Poster 128

Mathematical Modelling of the Magnetic Carrier Collection Efficiency with Magnetisable Stent Implant in a Mechanically Stretched Vessel. Future Application of Implant Assisted Magnetic Drug Targeting.

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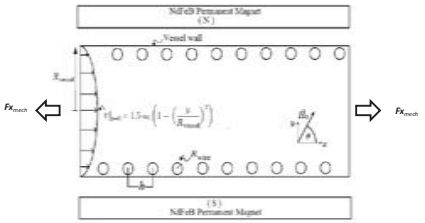
In the past ten years there has been a growing interest in the scientific and clinical application of magnetic carriers as drug targeting vehicles [1-6]. In general, Magnetic Drug Targeting (MDT) uses an external magnetic field source to capture and retain Magnetic Drug Carrier Particles (MDCPs) at a specific site after being injected into the body. Studies have shown that MDT is a relatively safe and effective methodology for targeting drugs to a specific site in the body [2]. Due to the gradient problem in MDT, ferromagnetic materials such as wires, seeds and stents are implanted into the body and this process is called Implant Assisted Magnetic Drug Targeting (IA-MDT). Recently Cregg and co-workers have extended the model to deal with many particles [5]. This has enabled the possibility of large scale models such as the delivery of drug therapy into tissues and into more complex biological systems. In that work, the extended mathematical model of the IA-MDT is presented and is shown to compare favourably with in vitro experiments. Results show that under suitable conditions for flow rate and magnetic field strength, the MDCPs' collection efficiency reaches almost 100%. This suggests promising opportunities in clinical applications. However, in the work to date, the vessels were considered to be rigid. In this work that assumption is removed and the main objective of this study is to present the mathematical modelling results of such a complex biological system which encompasses of magnetic forces, fluid shear stresses, and mechanical stresses applied at both particles and stent level. This will demonstrate the possibility of using IA-MDT as a successful approach for targeting the drug delivery to a specific location in the human body and to understand the parameters that govern the performance of the systems. In this work, the basic model geometry comprises a magnetisable coiled wire stent (flexible implant) in close contact with the inner wall of a vessel to mimic the mechanical constraint and strain of both stent and vessel as shown in Figure 1. MDCPs containing single domain magnetic nanoparticles are precisely injected under controlled fluid flow conditions and a simple sinusoidal waveform is applied to the system for stretching the vessel (frequency = 10 hertz and strain rate 10%). We model the behaviour of N (N < 25) MDCPs under the influence of i) Stokes drag, ii) hydrodynamic interaction forces, iii) magnetic forces that account for the mutual magnetic dipole-dipole interactions. We also investigate the effect of other forces acting on the system such as inertia and gravity and consider them as negligible forces. Furthermore, we account for the agglomeration of the particles which are known to occur in the IA-MDT system. Simulations based on this mathematical model were performed using the open source C++ finite volume library OpenFOAM. The static results are in agreement with previously published work [5]. Whereas when under dynamic cyclic mechanical straining the collection efficiency of the IA-MDT system is dependent on the initial MDCPs concentration and the fluid shear stress as in the static case. Finally, in this study, we expand the previously published work and we present the next experimental challenge to demonstrate the clinical relevance of IA-MDT, to measure the IA-MDT collection efficiency within an in vitro model that mimics arterial vessel stretching.



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Magnetoresponsive Biocatalysts

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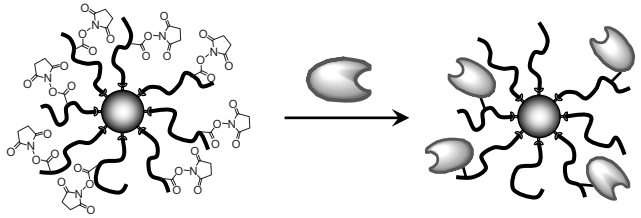
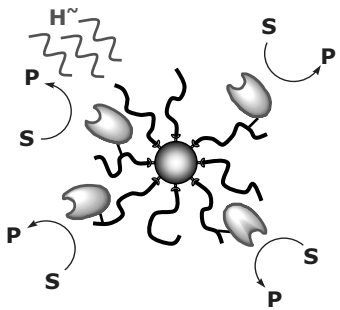
Controllable catalytic systems are a growing part of scientific interest. They offer the possibility to control chemical reactions by an outer stimulus, opening interesting opportunities for a row of technological and biological applications. In this context, the use of electromagnetic radiation provides great potential for fast and local reaction control. An encouraging approach is the use of magnetic nanoparticles, which can be heated locally in an alternating field at frequencies in the kHz range. The combination of magnetic control with the high selectivity of enzymatically catalyzed reactions affords great potential in biological and biotechnological reaction systems with exact regulation.



Scheme 1: Immobilization of enzyme to magnetic nanoparticles

The concept of our work is based on the combination of enzyme immobilization, magnetic heating and a thermoreversible dispersion behavior resulting in a magnetically switchable biocatalytic system. Therefore we combine magnetic FeO_x nanoparticles (d_{core} = 12 nm) via surface initiated ATRP with carboxy-functional copolymer shells showing a LCST in water[1]. The obtained particles are characterized with respect to their functionalization, composition, thermal and magnetic properties and their dispersibility in water or physiological buffer.

The carboxy-functional groups are introduced into the polymers by the use of succinimidyl methacrylate (SIMA)[2] as a functional comonomer. In an immobilization step, enzymatic catalysts can be covalently bond to the polymeric shell. By extrinsic heating in an oil bath and intrinsic heating in an alternating magnetic field, the enzymatic activity can be influenced by a change of the solubility of the polymer shell.



Scheme 2: Magnetoresponsive activity of the immobilized enzymes

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Physical properties and giant magnetoresistance in perovskites

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Abstract

The results of magnetic transport measurements with neutron-diffraction data [1] are combined, to calculate the magnetic properties of the entire family of $SrMn_{1-x}Ru_xO_3$ with $0 \leq x \leq 1$ perovskites the mean field theory and the high temperature series expansions. We have found antiferromagnetic (AF) ordering of the C type for lightly Ru-substituted materials $0.06 \leq x \leq 0.5$, due to the generation of Mn3+ in both families of manganite perovskites by either B-site substitution of Ru^{5+} for Mn^{4+} or A-site substitution of R^{3+} for Sr^{2+}. Heavily substi-tuted $SrMn_{1-x}Ru_xO_3$ materials are ferromagnetic due to dominating exchange interactions between the Ru^{4+} ions. Intermediate substitution $0.6 \leq x \leq 1$ leads to a spin-glass behavior instead of a quantum critical point reported previously in single crystals due to enhanced disorder.

Keywords: high temperature series expansions; $SrMn_{1-x}Ru_xO_3$; magnetoresistance. Magnetic phase diagram.

PACS: 73.43.Qt ; 71.45.Gm ; 75.50.Ee ; 75.50.Cc.

Au_{55} clusters and magnetic particles for biomedical applications

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Keywords: magnetic nanoparticles, surface modification, biomedical application.

Recently, in STREM Nanolab located in Uni-Strasbourg we establish all things for Synthesis of Au_{55}-cluster ($Au_{55}[P(C_6H_5)_3]_{12}Cl_6$) according G. Schmid's method [1G. Schmid, "Inorganic Synthesis" 1990, 27, p.214-218.], using diborane, which is obtained during the synthesis. We can produce Au_{55}-clusters soluble in dichlormetane (DCM) and in other non-polar media. Via the ligand-exchange the water soluble Au_{55}-clusters were prepared too.
Au55 – clusters have very specific properties and have a great interest for bio-medicine.
We will present bio-friendly surface-engineering pathways for Fe_3O_4- Co-, and Fe/Co-nanoparticles dispersed in a plethora of carrier liquids using suitable surfactants. L-cysteine ethyl ester is very suitable to cover the particle surface. While - according to IR- and XANES evidence - the -SH group binds to the particle surface the $-NH_2$ functionality remains free and is available as an anchoring group for further coupling reactions with bioactive molecules. Similar bio-friendly surface coating was achieved using dendrimer formation or particle surface coverage with dextrane. [2].
Via co-polymerisation of methacrylate and divinylbenzene in the presence of concentrated magnetic fluid microspheres were grown at the surface to protect the magnetic nanoparticles.
Around the metallic Fe_2Co core which is highly magnetic a dense non-magnetic protective shell is formed. Since a rich methodology is known for binding biomolecules, drugs, antibodies or oligonucleotides to nontoxic ironoxide surfaces, this finding conjures hopes of applying highly magnetic metallic-core nanoparticles in the field of medical diagnosis and therapy

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New method of synthesis of SPIONs: *in vitro* and *in vivo* biological applications as a MRI contrast agent

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Nanosized magnetite Fe_3O_4 and other ferrites ($Fe_{3-x}M_xO_4$) as magnetic cores are classically synthesized by coprecipitation and used as target agents for detection or drug delivery. In this work, new SuperParamagnetic Iron Oxides Nanoparticles (SPIONs) are studied for an eventual use in MRI detection in cardiovascular pathology.

The synthesis of coated SPIONs at an industrial scale requires alternative methods. Among them, continuous hydrothermal syntheses, in subcritical and supercritical water conditions, are currently developed in our group [1]. The size of the particles and their size distribution are smaller and narrower than those of powders obtained with other common methods. The production is around 50 g of nanoparticles per hour. With this process we studied the influence of Fe (II) and Fe (III) molar ratio in sub and supercritical conditions on the morphology, cristallinity and magnetic properties of the nano-objects. Furthermore the surface modification of SPIONs via citric acid and polyethylene glycol addition was possible using a one step procedure. The influence of these agents was also studied on the same parameters. Our results show a drastic decrease of crystallite size, a change of particle shape and shifts in the SPIONs isoelectric points. Moreover, the surface-modified nanoparticles have a Fe_3O_4 magnetic core and are well-dispersed in physiological pH thereby offering potential for biological applications.

In a second approach, batch-synthesized nanoparticles were coated with several molecules (polyethylene glycol (PEG); meso-2,3-dimercaptosuccinic acid (DMSA)) or with silica to prevent aggregation and sedimentation. A new stabilization and functionalization method was developed in order to obtain stable nano-objects that carry free thiol sites for biological post-functionalization. We have shown that the stability of thiol groups can be significantly increased when DMSA is protected by PEG chains on the surface of SPIONs [2]. The thiol groups of DMSA were stable even after 10 weeks of storage under ambient conditions. These thiols can be used to attach proteins or thiol reactive dyes without using regeneration agent or stringent storage conditions. Furthermore, these new SPIONs are stable under physiological conditions. This synthesis allows us to handle well-dispersed nano-objects with functionalizable sites durable for a long period of time compared with the classical strategies.

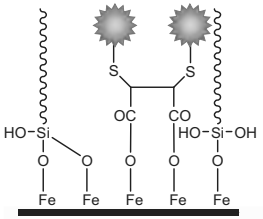

Figure 1: SPIONs bifunctionalized by DMSA and silanated $mPEG_{2000}$-$mPEG_{2000}$-Si stabilized nanoparticles and protected the thiol functions of DMSA.

Finally, the magnetic properties of these SPIONs were tested *in vitro* on a clinical 3 Tesla MRI. The magnetic efficiencies (relaxivities) of our suspensions are better than those of a commercial product (Cliavist®) and MTT assays proved that our iron oxide suspensions have no apparent cytotoxicity on different cells (cardiomyocytes, macrophages and hepatic cells). The *in vivo* biodistribution shows reduced capture of our SPIONs by mice liver compared with the commercial product and suggesting that our contrast agents freely circulate longer in mice than Cliavist®. These results open the way for specific pathology detection such as myocardial infarction or cardiotoxicity induced by several carcinoma drugs [3].

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Poster 133

Magnetic Beads in Mixture Screens for Modulators of Protein-Protein Interactions

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The high frequency of functional protein complexes make them desirable targets for compounds that can selectively enhance or suppress protein-protein interactions. The development of a small molecule screening method with applicability to a wide variety of protein complexes would be invaluable for both the discovery of new chemical probes of cellular pathways and the identification of leads that could be developed into therapeutics.

Magnetic beads (MB) are a multifunctional assay platform, providing an easy method for the simultaneous isolation and concentration of bound molecules. Proteins are easily immobilized on the particle surface, leading MB to be frequently applied to mixture screens for both ligand and target identification[1-4]. Immunoaffinity assays[5-7] and the purification of intact complexes[8] using MB demonstrate that protein-based interactions are possible on the bead surface; however ligand identification and the analysis of protein complexes have not yet been combined into a mixture screen for modulators of such interactions.

Here we present an efficient magnetic bead-based screening platform coupled to ESI-MS-MS to assess and identify potential modulators of protein-protein interactions. Initial work has been done with a Calmodulin-Melittin (CaM-Mel) model system and a selection of known modulators. Wide applicability of this method to a variety of targets is achieved by using affinity-based immobilization of histidine-fusion CaM onto Ni(II)-coated MB. Our data suggest that the ability to screen for modulators using CaM-bound MB is less effective than modulation of the CaM-Mel interaction in solution before isolation of the pre-formed complex using MB. A preliminary screen of mass-encoded mixtures of bioactive compounds determined both the activity and the identity of a known inhibitor from a single sample using ESI-MS analyses. Furthermore, the utility of this assay in quantitative inhibition studies was shown through the development of dose-dependant response curves and the determination of IC_{50} values for ligands of varying affinities. Future studies will focus on increasing the throughput by automation and multiplexing the assay.

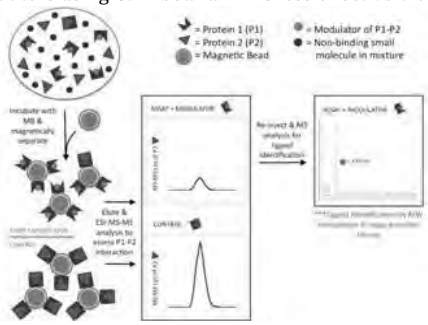

Figure 1: Schematic representation of the magnetic bead-based screening platform for modulators of protein-protein interactions.

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Poster 134

Size effects of polymer-nanoparticles systems in magnetic hyperthermia

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In 1979, Gordon et al. first proposed the heating of cancerous cells by magnetic nanoparticles.[1] Targeted cells are introduced with ferromagnetic or superparamagnetic particles. The tissue is then irradiated by an alternating electromagnetic field. The particles convert the electromagnetic energy applied to thermal energy, raising the temperature of the cells to above 40ºC, resulting in the death of target cells. Particles typically studied for magnetic hyperthermia are produced via co-precipitation of iron salts. This method results in a mean particle diameter around 10 nm, but with a wide polydispersity.[2] Polydispersity results in inefficient heating rates[3], and makes a study of the fundamental mechanics of hyperthermia difficult. Therefore, chemistries to produce monodisperse systems are necessary. In addition, a stabilizing layer is also necessary to insure that these particles are stabile in the body, and can also be used for tissue targeting and drug delivery.

While many authors have investigated the size effects of the magnetic particles[4-6], the effect of the surrounding stabilizing layer has not been sufficiently studied. To investigate the effects of both the size of the core magnetic particles and the surrounding polymer brush thickness, we have produced a matrix of samples of different core particle sizes and varying molecular weights of poly(ethylene oxide) stabilizing polymers. The resulting samples have been characterized by number of methods. These include transmission electron microscopy (TEM), thermogravimetric analysis (TGA), dynamic light scattering (DLS).

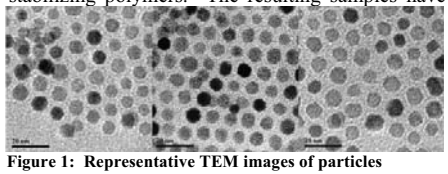

Figure 1: Representative TEM images of particles produced in varying core magnetite sizes.

In addition the magnetic properties, specifically those in the presence of an alternating magnetic field were characterized by two different methods. First the real and the imaginary susceptibilities were measured via an AC susceptometer from 10 kHz to 250 kHz. Second the specific absorption rate was measured via AC calorimetry. Finally, to investigate biological effects, MTS assays were performed on fibroblasts in culture to probe for potential toxicological issues associated with the polymer-nanoparticle complexes. To determine the effectiveness as potential hyperthermia agents, live-dead cell assays were performed for cells containing particles exposed and not exposed to alternating magnetic fields.

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The impact of surface modifications on the biological activity of superparamagnetic magnetite nanoparticles.

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Magnetic nanoparticles (MNPs) are the subject of fast-moving developmental efforts aimed at the improvement of diagnosis and treatment of cancer. MNPs are physiologically well tolerated and biocompatible, hence they have been widely used for various biological applications. In nanomedicine, a great effort has been focused on development of magnetic resonance imaging contrast enhancement, hyperthermia cancer therapy and nanovectors for targeted drug delivery to increase cytostatic drug uptake and accumulation in malignant tissue. Surface coating reduces agglomeration and adsorbtion of the nanoparticles on plasma protein and should make MNPs more stable, biodegradable and non-toxic.
The objective of this study was to investigate the uptake and distribution as well as the biological activity of coated nanospheric superparamagnetic magnetite particles (Fe_3O_4, 10 nm in diameter) in the human alveolar epithelial carcinoma cell line A549 after short-term (4h) and long-term (24h) exposure. MNPs were prepared by the coprecipitation of ferric and ferrous salts in an alkali aqueous medium. The magnetic properties of prepared MNPs were characterized by SQUID magnetometer at room temperature. Two surfactans - sodium oleate ($C_{17}H_{33}COONa$) and polyethylene glycol (PEG Mw=1000) were applied for MNPs coating. Distribution of SO-MNPs (sodium oleate-coated MNPs) and SO-PEG-MNPs (sodium oleate- plus PEG-coated MNPs) has been investigated by transmission electron microscopy (TEM). Cytotoxicity of individual MNPs and surfactants was evaluated using MTT, trypan blue exclusion test and LDH, the genotoxic activity of MNPs was investigated by the alkaline single cell gel electrophoresis (comet assay) and the micronucleus assay. The oxidative status of exposed vs. control cells was analyzed, too.
Differences in the biological activity of nanosphered magnetite particles were found in dependence on surface modifications.
This study was supported by grants: VEGA 2/0051/09, HEALTH-F5-2008-201335 (NanoTEST), APVV 99-026505 and CEX SAS NANOFLUID, INTARESE GOCE 018385.

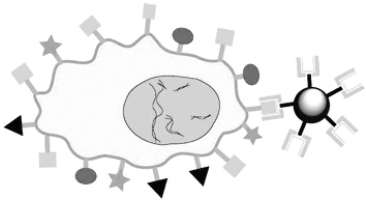

Figure 1: Upon controlled surface functionalization and coupling with fragments of DNA strands, proteins, peptides or antibodies, MNPs can be used for drug delivery, magnetic separation, magnetic resonance imaging contrast enhancement and magnetic fluid hyperthermia (Xu and Sun, 2009).

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Bio-activated magnetic beads for a cost effective isolation of biological molecules: Fast separation and reusability using Low Field Gradient Magnetophoresis

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Magnetic micro- and nano-particles coated with bio-affinity ligands have been successfully used in multiple applications for the separation of cells and other biological molecules, such as antigens or antibodies, from crude extracts [1]. The increasing demand for magnetic beads for isolation and purification in biotechnology and biomedicine applications [2],[3] has prompted particle producers to develop bio-activated beads with optimized binding capacity that can cope with high concentrations of the molecule of interest.

Beyond the improved binding capacity, in order for the application to be cost effective magnetic beads need to be reused several times in successive separation and elution processes. Two key parameters define the reusability of the magnetic beads: (i) the aggregation of the beads after repeated magnetic separation processes and (ii) the stability of the coating after a number of consecutive capture and elution processes. The focus of the present study is on the analysis of the performance of a new range of bio-activated magnetic beads of sizes 0.86µm and 2.37µm and coated with three different ligands: Streptavidin, anti-Mouse IgG and anti-Rabbit IgG.

Nanoparticles can be easily separated from a dispersion using Low Gradient Magnetic Separation due to a field-induced phenomenon of reversible aggregation [4]. Reversible aggregation times under Horizontal Low Gradient Magnetic Field were estimated for the three types of beads presented in this work and correlated with their size and magnetic content. The stability of the suspension was studied by performing several consecutive separation processes for each one of the samples under constant magnetophoretical conditions. Monitoring of the magnetophoresis process showed a good reproducibility in the successive separation tests, suggesting that no change in the average particle size had taken place and thus aggregation was completely reversible. The absence of irreversible aggregation in all three samples after 10 magnetic separation processes was confirmed through optical microscopy visualization.

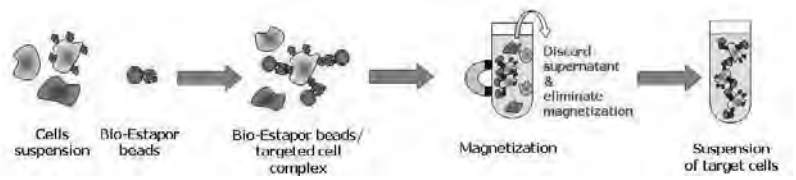

Figure 1 Capture, separation and elution process of a target molecule using Bio-Estapor beads activated with an affinity ligand.

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Coupling of a planar Halbach array to a step-SPLITT channel for continuous sorting of magnetic microspheres

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Continuous immunomagnetic sorting is widely used for stem cell enrichment and cancer cell isolation and identification. The previously reported Quadrupole Magnetic Sorter (QMS), developed for high throughput separations of magnetized cells, combined a quadrupole magnet that generates an axi-symmetric magnetic field, to an annular flow channel. Here we report the first pairing of a parallel plate channel, of a type known as step-Split-flow thin-cell (s-SPLITT), to a planar Halbach magnet array. The Halbach array generates uniform magnetic fields and gradients on planes orthogonal to the magnet surface. It comprises elementary permanent magnets (in this case, neodymium cylinders) whose polarization angles between successive units produce magnetic field lines that are reinforced on one side, while cancelled on the other side of the array. This is illustrated by the color contours of the magnitude of B in Figure 1, for an array of 16 diametrically polarized cylinders. A magnetically inducible particle will experience a force toward the surface of the array independent of axial or depth location. The s-SPLITT is a ribbon-like channel with two inlets and two outlets, each separated by a "step". The dimensions are 19 cm long, 1 cm wide and 400 or 650 µm thick. A suspension of a binary mixture of magnetic microspheres (6.2 µm latex, custom made by Micromod Partikeltechnologie GmbH) and non magnetic particles (15 µm latex, Duke Scientific) is injected at the inlet labeled **a'** and a carrier fluid (free of particles) is injected at the inlet **b'**. The sample is axially transported by the flow as a selective transversal magnetic force drives the magnetic species (shown in red) toward the wall opposite that of the injection. Studies were conducted with magnetic microspheres alone in which we varied the distance between the channel and Halbach array, and varied the total flow rate at fixed distance; in other studies we varied the distance with the above-described binary mixture. At optimal distance, a feed of 41.8% purity in magnetic microspheres was enriched to 93% in outlet **b**, and depleted to 9.0% in outlet **a**. These results are corroborated by theoretical predictions with the software Maple 10, with inputs of field measurements and modeling and particle susceptibility measurements.

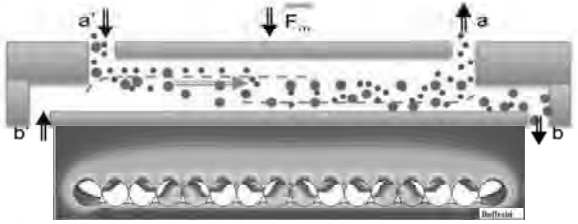

Figure 1. Planar Halbach magnet array and s-SPLITT channel.

Recent advances in the synthesis and characterization of magnetic iron oxide nanoparticles prepared by laser pyrolysis

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Nanoscale iron oxide nanoparticles, often showing a superparamagnetic behavior have been intensively studied these past years for biomedical applications. Nano iron oxides with spinel-like structure (maghemite/magnetite), small particle sizes (2-6 nm) and narrow size distributions have been prepared by the laser pyrolysis of gas mixtures containing the precursors iron pentacarbonyl and air (as oxidizer). The method is based on the resonance between the emission of a CW CO2 laser line and the infrared absorption band of a gas precursor and on subsequent heating of precursors by collisional energy transfer. A sensitizer serves as an energy transfer agent. We have reported recently[1] on the controlled use of air as oxidizer for the production of iron oxide nanoparticles in the gamma phase. In this paper we further demonstrate the possibility to vary the chemical composition and the iron oxide nanoparticle dimensions by varying the nature of the sensitizer (ethylene or sulfur hexafluoride). The comparative study was performed by characterization tests on the compositional, morphological and magnetic properties of the as-prepared nanoparticles. High resolution transmission electron micrograph images (Figure 1) suggest lower mean particle sizes in case of SF_6 than that obtained with C_2H_2 ethylene sensitizer. For samples synthesized with C_2H_4 sensitizer sharper particle size distributions were found. For both types of nano-iron oxide samples, polycrystalline morphology and mostly faceted particles were observed. Coalescent features and a cubic structure (spinel) of the iron oxide (namely maghemite/magnetite) were identified (by XRD and SAED analysis). It seems that there is an inversion between the particle dimensions and the magnetic characteristics of the nanomaterial, due to the fact that the highest magnetization should belong to the samples with the higher particle sizes. The samples synthesized from ethylene –containing mixtures demonstrated a much higher magnetization and a saturation-like behavior in a magnetic field**.**

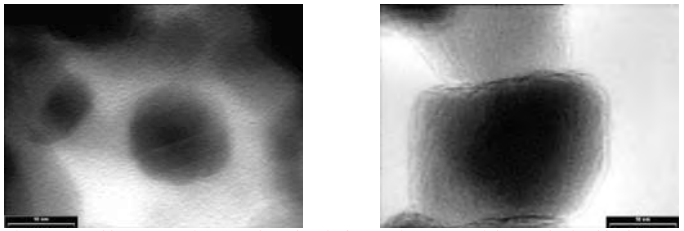

Figure 1 a and b. HRTEM images of maghemite/magnetite nanoparticles synthesized by laser pyrolysis from gas mixtures containing SF_6 (a) or C_2H_4 (b) as sensitizers

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Poster 139

Investigations on controlling the crystal modification of Fe_2O_3 nanoparticles produced by CO_2 laser vaporization

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By vaporization of coarse crystalline hematite (α-Fe_2O_3) in the focus of a CO_2 laser beam and recondensation in a flowing carrier gas whose composition can be varied, iron oxide nanopowders with average particle sizes of 20 nm…30 nm were obtained (fig. 1). Our aim was the production of nanoscale γ-Fe_2O_3 (maghemite), which is of interest for medical applications due to its ferrimagnetic properties. If pure air was chosen as the process and carrier gas, the X-ray diffractograms (fig. 2) revealed beside a dominant ratio of the desired maghemite, which is typical for the Fe_2O_3 formation from the gas phase, however, also about 10 vol% hematite. The formation of hematite could be suppressed if the process gas has been enriched with an inert gas (N_2, Ar, or He). In contrast, if the process gas was additionally enriched with O_2, the hematite portion could be increased significantly.

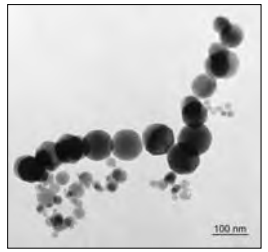

Fig. 1. TEM micrograph of laser generated Fe_xO_y nanoparticles

Although in each case an overstoichiometric portion of O_2 was present in the condensation zone, it was initially thought that the depletion of atmospheric oxygen within the process zone was the cause for the reduction of Fe_2O_3 in favour of a higher ratio of magnetite (Fe_3O_4). However, this was not confirmed by XRD measurements. Especially Mössbauer spectra revealed an apparently paradoxical result: With increasing excess of O_2 the weight of the sub-spectrum, which has to be assigned to the Fe^{2+} ions, also increased. The increase of the hematite portion thus correlates with the increasing portion of the Fe^{2+} ions. A lattice expansion of hematite by about 0.6% simultaneously observed in the XRD spectra, however, is independent of the amount of supplemental oxygen, i.e. the relative amount of the Fe^{2+} ions built in the hematite lattice is approximately constant. An explanation for the phenomena observed here is seen in the fact that under laser irradiation with increasing O_2 concentration, the probability of the ozone formation in the process zone also increases. Also present in the laser generated plasma are Fe^{2+} ions with a slightly greater radius compared to Fe^{2+} ions. Bent ozone molecules, however, should be able to sterically stabilise Fe^{2+} ions preferred by forming the preform of an oxygen octahedron around a Fe^{2+} ion. During the crystallization process of the nanoscale melt droplets such octahedrally coordinated iron ions act as nuclei for the formation of hematite, whose crystal structure is characterized by containing only octahedrally coordinated Fe ions, whereas in maghemite also tetrahedrally coordinated Fe ions are existent.

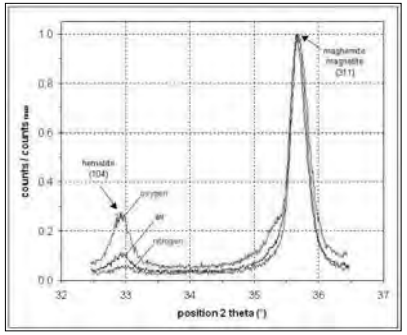

Fig. 2. XRD measurements of Fe_xO_y nanopowders generated with the additional gases O_2, air, and N_2

Poster 140

Magnetite submicron particles coated with rifampicin and fluorescent chlortetracycline for drug delivery applications

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In vivo magnetic delivery, based on drug targeting by means of magnetic nanoparticle carriers represents more and more an intensely studied medical technique, since chemotherapy effectiveness could be considerably improved compared to classical methods. Also, identification of a tumor boundaries or biopsies performing using fluorescence imaging can be accomplished with higher efficiency if the molecules attached to the magnetic carrier posses fluorescent properties. In this experimental study, submicron magnetite particles (Fe_3O_4) were prepared by chemical co-precipitation from ferrous and ferric salts in the presence of NaOH solution, in basic (BM), as well as in acid medium (AM). Following further functionalization with antibiotic molecules – rifampicin (RIF) and chlortetracycline (CTC) four types of biocompatible aqueous magnetic fluids (BMF) were obtained.

BMF	Reaction conditions	Drug shell
1	Basic (BM)	RIF
2		CTC
3	Acid (AM)	RIF
4		CTC

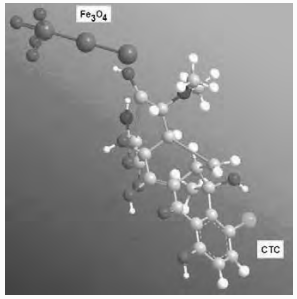

Fig.1 Structural model of magnetite - chlortetracycline interaction without intermediate organic

The choice of these pharmaceutical items was motivated not only by their ability to fight against bacterial infections, but also by their molecular structure – since, due to the high electro-negativity of some oxygen atoms, they exhibit several potential binding sites able to form complexes with iron ions [1-3]. Thus, the obtained magnetite-core/drug-shell systems - that avoid intermediate organic coating of magnetic nanoparticles, represent potential candidates for *in vivo* biomedical applications. Comparative analysis of the structural properties was accomplished by magnetization measurements (VSM), X-ray diffraction (XRD) and scanning electron microscopy (SEM). Typical spinel structure was evidenced for all samples by XRD investigation as well as uniform spheric shape of colloidal nanoparticles. Physical size distribution and saturation magnetization were related to longer stability in time of the BMF3 and BMF4 samples that recommend them for further drug delivery applications.

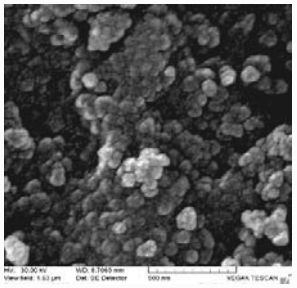

Fig. 2 SEM picture of BMF3 sample

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Double-layered magnetic nanoparticles for biological applications

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One of the most widely used and explored methods for nanoparticle synthesis is the aqueous co-precipitation method. Highly crystalline nanoparticles with a large size distribution can be obtained using this technique, though aggregation of particles often occurs with aqueous methods. Magnetic nanoparticles intended for biological applications are usually biocompatible ferrite powders coated in organic molecular shells and dispersed in water, due to their chemical stability, biodegradability and lack of toxicity.

The purpose of the study was to obtain a new stable biocompatible magnetic fluid, based on magnetite nanoparticles (Fe_3O_4 NP), stabilized with oleic acid and further coated with a biologically active molecule dispersed in chloroform - in this case rifampicin (RIF); this bacteriostatic antibiotic drug was chosen since its molecular structure was found to posses several sites of interaction, with high electronegativity, thus being able to bond metal ions [1,2].

Magnetite was prepared by a modified Massart method [3,4] from the chemical reaction among aqueous solutions of ferric and ferrous chloride salts using aqueous sodium hydroxide as precipitation agent. After magnetic separation, the ferrophase was resuspended in 2 ml of ethanol and further coated with 6 ml of oleic acid (OA), heating the mixture up to 80°C with continuous magnetic stirring for 30 minutes; 4 ml of water dropwise addition was used for removing the excess oleic acid and the lipid-coated magnetite was washed twice with 30 ml ethanol. The obtained OA-coated iron oxide nanocrystals were further dispersed in 6 ml of chloroform, containing 30 mg rifampicin, with vigorous magnetic stirring for one hour.

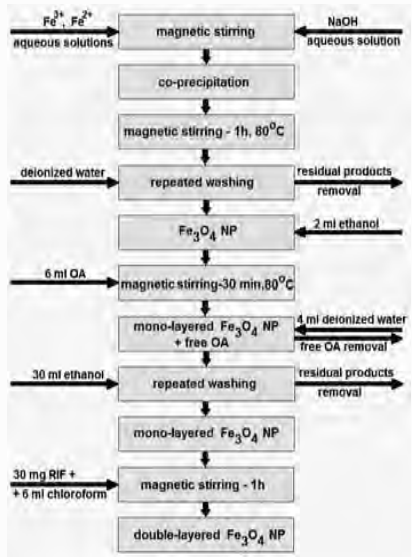

Fig. 1 Preparation steps of the double-layered magnetite nanoparticles

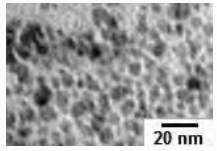

Fig. 2 TEM image of the magnetite grains

The final product resulted in a stable ferrofluid with good crystalinity and fine granularity of the submicron magnetic grains (7 to 15 nm average diameter); rare aggregates or short chains were also present. Microstructural investigations of the double-layer coated magnetite nanoparticles were carried out by X-ray diffraction (XRD) and transmission electron microscopy (TEM) and their magnetic properties were measured by vibrating sample magnetometry (VSM); rheological features were also performed, by measurements of superficial tension coefficient, dynamic viscosity, density and magnetite volume fraction.

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Application of click chemistry for functionalization of polypyrrole coating the magnetic nanoparticles

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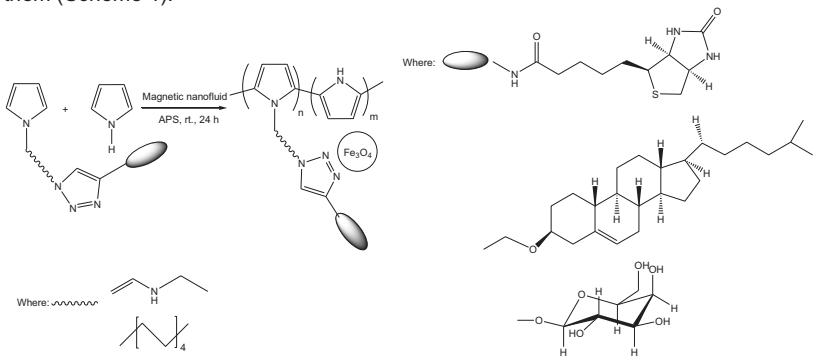
Synthesis of conducting polypyrroles is especially promising for many commercial applications because of its unique optical, electric, thermal and mechanical properties. The combination of conducting polymers and inorganic magnetic nanoparticles represents a new strategy to obtain nanocomposites with the specific properties required by a wide range of applications like: biotechnology, electromagnetic interference shielding, microwave absorbing and magnetic separation.

We report the synthesis and characterization of novel pyrrole functionalized monomers and also core-shell hybrid nanostructures, consisting in the deposition of a conjugated polymer layer (functionalized pyrrole copolymers) on the magnetic nanoparticles Fe_3O_4 surface. Water based magnetic nanofluids, pyrrole and functionalized pyrrole monomers were used as starting materials. We focussed on the covalent attachment of biomolecules at the nitrogen atom of pyrrole ring and to investigate the explicit properties of magnetic core-shell nanostructures derived from them (Scheme 1).



Scheme 1

The properties of the magnetic nanoparticles based on functionalized polypyrrole were investigated by TEM, FTIR spectroscopy and magnetization measurements.

The resulting functionalized polypyrrole layers which cover the magnetic nanoparticles can interact with other biomolecules in solution to detect structure-response relationships.

Extraction and purification of Nanomagnetic particles from bacteria

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Abstract:

Nowadays medical sciences tools and related subject (in the fields of molecular biology, prognosis, diagnostic, isolation, purification, diseases treatment and particular in for cancer therapy etc.) has been greatly developed. In fact goals of these new methods are simultaneously detection of high sample throughput or drug targeting with at least side effect.

For establishment of mentioned techniques and methods, the most important section of work is to find small volume materials with high efficacy and versatile. The super paramagnetic nanoparticles (50-100nm) magnetite (Fe_3O_4), greigite (Fe_3S_4), maghemite (γ-Fe_2O_3), and various types of ferrites ($MeO.Fe_2O_3$, where Me = Ni, Co, Mg, Zn, Mn, etc.) are the most applied materials among them.

Today's these nanoparticles are used to isolation and detection of biological molecules such as specific proteins, genetic material and drug targeting.

These nanomagnetic particles can be prepared with chemical reactions and natural source too. Natural source of this Magnetic nanoparticles are Magnetotactic bacteria (mtb) that was detected by Blakemore at time. Drug delivery potential of these Bacterial magnetic particles is 1.7 times higher than the artificial magnetic particles.

In this study we experienced a modified and successful procedure for bacteria Magnetosomes extraction and purification by reviewing of the most purification procedures.

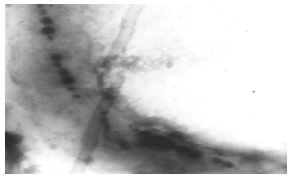

Fig.1: Transmission electron micrograph of Magnetospirillum magneticum AMB-1 reveals a long chain of nanometer-sized magnetic particles

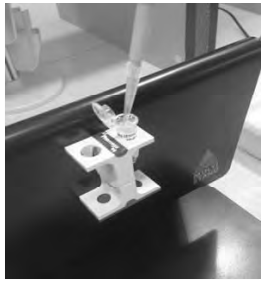

Fig.2: Extraction and purification of BMPs by magnetic field

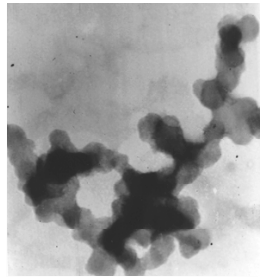

Fig.3: Transmission electron micrograph of BMPs extracted of the Magnetospirillum magneticum AMB-1

Key words: extraction, purification, Magnetotactic bacteria, nanomagnetic particles

Endothelialization of Magnetic Graft Materials using SPM-labeled Endothelial Cells

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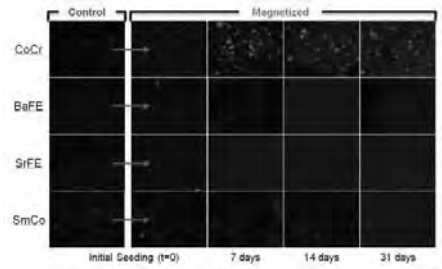
Susceptibility of small diameter (<5 mm ID) synthetic vascular grafts to rapid occlusion precludes clinical use, particularly in coronary artery bypass grafting[1]. Post-operative occlusion of synthetic conduits often results from acute thrombosis or neointimal hyperplasia, processes which are regulated by the vascular endothelium in healthy blood vessels[2]. Tissue mimicking grafts, seeded on the lumen with autologous endothelial cells, show improved in vivo patency[3]. However, lengthy cell isolation and seeding procedures have prevented these methods from reaching fruition. Pislaru, et. al. recently demonstrated that endothelial progenitor cells endocytose superparamagnetic iron oxide microspheres (SPM) in culture. Subsequently, they are rapidly attracted to magnets[4].

We have employed this method using a functional modality; flexible, polymer-based magnetic composites that capture and sustain porcine endothelial outgrowth cells (EOC) *in vitro*. Specialized casting fixtures were used to fabricate and magnetize patches of material comprised of poly(ether urethane) embedded with metal particles (strontium ferrite, barium ferrite, samarium cobalt, and cobalt chromium). Using a multi-factorial design of experiments (DoE) approach, the amount and spatial distribution of metal particles were systematically varied to produce unique material configurations. Then, porcine EOCs were labeled with SPMs, fluorescently tagged, and seeded onto the samples. Cell coverage was quantified at various post-seeding time intervals using micrographic image analysis. The effects of changing design parameters on cell capture and sustained cell viability on magnetic substrates were quantitatively examined.



Following exposure to a 3 Tesla magnetizing field, treatments containing strontium ferrite, barium ferrite, and samarium cobalt showed appreciable surface residual magnetism. These formulations had increased cell coverage immediately following seeding, relative to their non-magnetized counterparts. Hard ferrites and samarium cobalt failed to achieve appreciable cell coverage, however. Cobalt chromium substrates showed significant cell growth for up to one month. The current work indicates strong potential for the use of magnetic particles, particularly cobalt chromium, to quickly and effectively endothelialize synthetic vascular grafts.

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Study of magnetic particle *in vivo* degradation by Mössbauer spectroscopy

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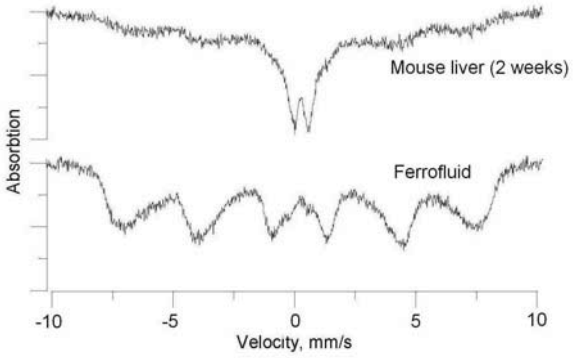
Magnetic nanoparticles (MP) belong to the most promising and interesting nanosized objects for biomedical applications not only because of the possibility to remotely control their position for drug delivery applications and temperature for hyperthermia purposes, but also because of the possibility of simultaneous visualization by MRI and highly-sensitive quantitative detection [1,2]. However, behavior of magnetic particles *in vivo* is an important issue to be raised. Despite the fact that MP are already approved by FDA for intravenous injection as MRI contrast agents, little is known about MP clearance from the organism or their biodegradation/biotransformation. In this work we propose using Mössbauer spectroscopy, one of the most informative methods for MP characterization *in vitro* [3], for investigation of MP biotransformation *in vivo*.



Magnetic particles were injected in mice and at different time intervals after injection they were sacrificed and organs were extracted. Then the organs were lyophilized and their Mössbauer spectra were recorded (see figure). The study showed that the magnetic particles were well-detectable in liver and spleen spectra along with contribution from ferritin–like pattern. The spectra of other organs did not reveal any significant presence of magnetic particles; therefore, we focused mainly on the study of liver and spleen samples. Investigation of time evolution of the shapes of liver spectra depending on the time of mice sacrifice clearly demonstrated that after magnetic particle injection and their absorption by liver, MP underwent a chemical transformation from superparamagnetic state to paramagnetic forms of iron. Although the method yields unique and interesting data, its sensitivity needs to be improved – it is hard to understand what would be the difference in biodegradation of the magnetic particles if smaller quantities are injected.

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Preparation of magnetic iron oxide nanoparticles in water-in-oil micro-emulsion stabilized by nonionic surfactant Tween 81 for MRI diagnostics

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The ternary system comprising hydrocarbon $C_{15}H_{32}$, oil-soluble non-ionic surfactant Tween 81 and water was studied as reaction medium for superparamagnetic nanoparticles (NPs) preparation. The phases were detected by observing cloudy points during titration procedure. The structure of formulation was studied by dynamic light scattering, SEM, ^{1}H-NMR spectroscopy and microscopy. The results obtained are in good agreement with phase behaviour presented in Fig.1, where microemulsion phase was found to fit structure of inverse micellar solution in area A of phase diagram. Microemulsion is suitable for solubilization of iron salts Fe (2,3) and their co-precipitation into NPs composed of Fe_3O_4 under basic pH conditions. The hydrodynamic diameter of microemulsion droplets and magnetic NPs were measured by means of dynamic light scattering (laser wavelength λ=645 nm, scattering angle θ=45^o). The mean particle diameter of NPs was found to be equal to 15 nm, which is close to the diameter of inverse non-loaded micelles in microemulsion. Magnetophoretic properties of particles were studied in capillary microfluidic system with defined geometry in order to evaluate their mobility in various media, imitating biological tissues and vessels. Mobility observations were carried out by means of Doppler laser light scattering. The suspension of NPs in microemulsion media was stable for 60 days at 20^{0}C. Magnetic properties of NPs were analyzed by measuring water proton relaxation with CXP-300 spectrometer (Bruker) at resonance frequency of 300 MHz. The data confirm the superparamagnetic nature of prepared NPs. The rates of magnetic relaxation depend linearly on concentration of iron in suspension. The relaxivity of NPs derived from the slopes of the plots were in interval (21,73 + 1,08) *l/mMs* which corresponds to negative contrast agents for MRI. The safety of parenteral application of synthesized NPs was studied on murine model. The NPs suspension in microemulsion media was diluted with the physiological solution to concentration level of MRI contrast preparations. Intravenous injection of NPs to mice did not decrease life span of animals compared to control group. It can be concluded that iron NPs prepared by microemulsion method with Tween 81 used as stabilizer could be used for parenteral contrast enhancement in MRI measurements *in vivo.*

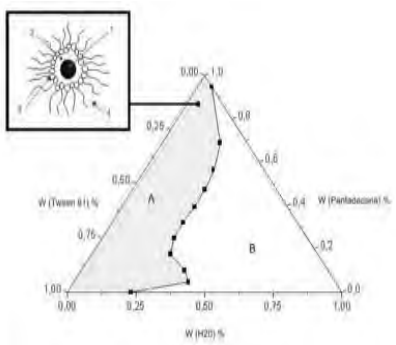

Fig. 1 Phase diagram of ternary system: Tween 81–pentadecane–water. (A) – reverse micellar zone, (B) – biphase zone; 1 – Fe_3O_4 NP, 2 – water, 3 –surfactant, 4 – hydrocarbon

Colloid preparation of maghemite nanoparticles functionalized with dirhodium(II) citrate

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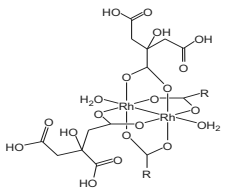
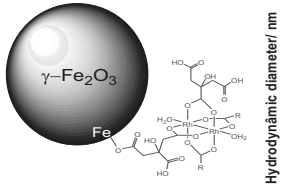
Superparamagnetic iron oxide nanoparticles (SPIO) are potential tools for biomedical applications aiming both diagnosis and therapy [1]. SPIO can be surface-functionalized with bioactive molecules to act as drug carrier agents. Dirhodium(II) citrate, $[Rh_2(H_2cit)_4]$, is a binuclear complex with significant antitumor activity and low toxicity when compared to similar dirhodium(II) carboxylates, thus being very promising for cancer chemotherapy [2]. This compound possess functional free groups (-COOH and -OH) on its structure (Scheme 1) that are appropriate to interact with biomolecules and SPIO's surface. Thus, $[Rh_2(H_2cit)_4]$ can be attached to SPIO to produce a nanoparticle-based drug formulation, with stable colloidal dispersion, biocompatibility, and stability in physiological media for drug delivery applications (Scheme 2). This study investigates surface changes of nanosized maghemite due to the adsorption of $[Rh_2(H_2cit)_4]$ and evaluate the effect of the dispersion media on the colloidal stability of the funcionalized nanoparticles. Dirhodium(II) citrate was synthetized by an exchange reaction of trifluoroacetate ligands from the precursor dirhodium(II) trifluoroacetate by citrate ligands. Maghemite nanoparticles (7.8 nm average diameter) were prepared as described in the literature [3]. Nanoparticle's funcionalization were performed by adding the aqueous dirhodium(II) compound solution into the maghemite-based colloid. After centrifugation, washing and pH adjustment, the nanoparticles were dispersed in saline water, producing a stable colloid. Dried powder samples were characterized using XRD, VSM, FTIR, and rhodium elemental analysis. Zeta potential and hydrodynamic diameter (DLS) were used for suspension's characterization. The bare and functionalized maghemite nanoparticles showed characteristic diffraction patterns of spinel structure and magnetic saturation of 48 emu/g. The FTIR spectra showed intense absorptions at 1724, 1630, and 1564 cm^{-1} assigned to carbonyl stretching mode, asymmetrical and symmetrical carboxylate stretching modes, respectively. Functionalized $[Rh_2(H_2cit)_4]$ nanoparticles present deep changes on surface properties. The isoeletric point (IEP) shifts to lower values of pH, reaching pH3 when the surface-adsorbed rhodium complex reaches saturation. We found that the modified surface presents negative zeta potential in a wide range of pH with magnitude of -38 mV. The magnetic sols were diluted with saline solution, PBS buffer solution, fetal bovine serum (FBS), and Dulbeco's modified Eagle's culture medium (DMEM). Stabilization in DMEM media was realized by adding PEG-8000 diluted solution to the precursor sol. No significant changes in hydrodynamic diameter were observed within 20 days time (Figure 1). However, the FBS and DMEM dispersed sols undergo aggregation in a more prolonged time. Therefore, sols produced by $[Rh_2(H_2cit)_4]$-functionalized maghemite are suitable for biological *in vitro* and *in vivo* studies.



Scheme 1



Scheme 2

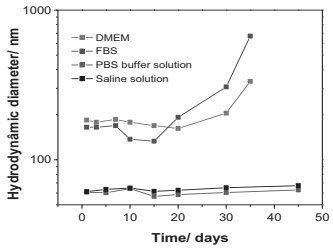


Figure 1: Effect of dispersion media on the colloidal stability of dirhodium(II) citrate functionalized nanoparticles.

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Magnetic label detection in paper chromatography using planar hall resistance sensor

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In the last few decades, magnetic beads (micro/nano) based bioassay technology has been widely investigated due to its fast and accurate detection capabilities. However, until now, a simple, cost-effective and high sensitivity magnetic sensor, where the magnetic beads can be detected by means of electrical signal, is not well developed. Among the various magnetic beads detecting method, magnetoresistive sensors have aroused much interest among the researchers due to its rapid response and high sensitivity to detect magnetically labelled biomolecules. Especially, the planar Hall resistance (PHR) sensor has unique advantages of measuring the magnetoresistance in a transverse geometry that leads to reducing the temperature effect by four orders of magnitude resulting in increase the thermal stability and resolution of the sensor.

In this study, we have investigated PHR sensor based lateral flow technologies to detect the low concentration of cTnI-target biomolecules causing Acute Myocardial Infarction (AMI) below the optical visible range. Firstly, the amine ligand magnetic beads (Dyna bead® M-280) was immobilized with different concentration of target molecules and disperse it on chromatographic membrane which contains desired probe molecules at specific point (test line). Scanning electron microscopy (SEM) study reveals that the magnetic beads are captured onto chromatographic paper with specific biomolecules as shown in figure 1 and the concentration of magnetic beads at the test line is depends on the concentration of the target molecules. Finally, magnetic stray field from the captured magnetic beads were detected by scanning the PHR sensor with a sensitivity of 7μV/Oe through the chromatographic paper. It was found that the sensor signal is proportional to the concentration of the target molecules and enough to detect the low concentration below the optical visible range. In figure 2, we demonstrate the PHR signals for 4 ng/ml and 16 ng/ml concentration of target molecules.

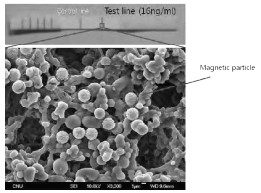

Fig. 1. SEM image showing the presence of hybridized magnetic beads in the chromatography paper.

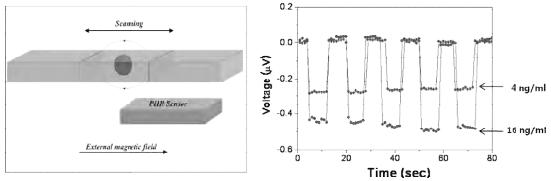

Fig. 2. (a) Schematic representation of the scanning method on the chromatography paper using PHR sensor (b) The sensor output voltage signal data with two different concentrations of the magnetic beads

Poster 149

INTERACTION OF J774A1 MACROPHAGES WITH IRON OXIDE NANOPARTICLES COATED WITH DEXTRAN.

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Recent advances in nanotechnology, such as the development of Super Paramagnetic Iron Oxide Nanoparticles (SPION), are providing new options for medicine and science. A biocompatible SPION, coated with dextran, (Endorem ® - Guebert), associated with magnetic resonance imaging (MRI), has been used for the study of cell distribution and fate, but conditions for uptake, action and elimination in cells other than hepatocytes have not been established yet. In this work we analyze Endorem uptake both qualitatively and quantitatively, as well as the survival and proliferation of macrophages.

J774A1 macrophages, 10^5cells/well, medium RPMI supplemented with 10% bovine fetal serum, were exposed to Endorem at 100, 200, 350, 500, 750 and 1000 μg/ml, for 45 min, washed once in Hank's solution and counted at incubation times of 1, 3, 5, 7 and 10 days. The presence of SPIONs in cells adhered on the coverslips was detected by Prussian blue staining at the light microscope and by transmission electron microscopy. Controls were done in cultures without Endorem. The presence of apoptotic and necrotic cells was evaluated through flow cytometry using Annexin V-FITC apoptosis detection Kit I and the data acquisition was carried out in a FACSARIA flow cytometry equipment (BD Biosciences), and data analyses were performed using the FlowJo software (TreeStar). SPIONs were detected by Ferromagnetic Resonance (FMR) in supernatants from cultures incubated, after 8, 7, 5, 3 and 1 day respectively, using a Bruker EMX homodyne spectrometer operating in the X band at a frequency of 9.428 GHz.

The presence of SPIONs in cells was detected by Prussian blue staining in all days of culture and even with exposures to the highest concentrations tested. Mitotic figures were seen in cultures incubated with SPIONs at 100 to 500 μg/ml indicating that the cells keep proliferating as in the control groups. SPIONs were observed in the macrophages cytoplasm, loading ramified tubular structures, irregular vacuoles, or fused with lysosomes containing or not cell debris; even apoptotic and cells in mitosis presented endocytosed iron particles. The number and area of structures containing endocytosed SPIONs increased in cells incubated with 350 μg/ml nanoparticles. The control cells differed from the cells incubated with iron particles by the presence of only cell debris in the phagolysosomes. In the 7th day of culture, both in control and in cultures incubated with the nanoparticles, active macrophages were found phagocytosing degenerating cells, some of them in apoptosis. Flow cytometry showed, that the phagocytic cells J774A1 are naturally driven to apoptosis after a few days in culture. However, we observed that, besides being time dependent, early apoptosis was also dependent on the nanoparticles dose added to the culture. On the other hand, a straight correlation between early apoptosis and necrosis or late apoptosis was not observed, probably because the viable macrophages phagocytosed part of the degenerating cells. The FMR results are in accordance to those of flow cytometry indicating elimination of the SPIONs, due to cell death, in the supernatants from cultures at the concentration of 200 μg /ml thereafter. This loss of nanoparticles represents about 10^5 SPIONs/μl or only 10^{-6} of the initial administered dose. The bulk of the nanoparticles remains in the macrophages that had phagocytosed the apoptotic or necrotic cells carrying uptake SPIONs. We conclude that professional phagocytes not only can phagocyte huge amounts of SPIONs, but they also keep proliferating for at least 10 days thereafter. Elimination of SPIONs appears to be dose dependent.

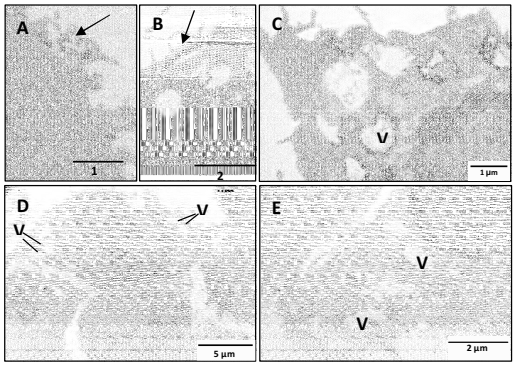

Figure 1 – Transmission electron micrographs of J774 macrophage cells culture incubated with SPIONs during 45 minutes: A-C - 200 μg/ml; D, E - 500 μg/ml. Nanoparticles are internalized through macropinocytosis (arrow in A) originating large cytoplasmic vacuoles (V) with iron nanoparticles close to their inner border. In B, arrow points to a bar-shaped agregate of nanoparticles internalized in a cytoplasmic vacuole.

Poster 150

FUNCTIONALISED MAGNETIC NANOPARTICLES FOR NUCLEIC ACIDS SEPARATION IN PLANT DISEASE DIAGNOSTICS

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Magnetic nanoparticles (MNPs) can be employed for magnetic separation of complex mixtures in a wide range of biotechnology applications [1, 2]. Commercially available magnetic 'beads' for magnetic separation are generally expensive and not tailored to any specific application. The demand for beads with higher binding capacity and lower sedimentation rates encourages the development of smaller magnetic particles with higher surface area and tailored surface functional properties.

Aim of this work is the production and characterization of functionalised magnetic nanoparticles for nucleic acid separation of plant pathogens and their comparison with commercially available products. For this purpose, superparamagnetic iron oxide nanocrystals were synthesized in an aqueous solution [3]. The magnetic and structural properties of the produced MNPs were characterized by means of magnetometry, X-ray diffraction and scanning electron microscopy. A thin silica layer was deposited on the MNPs surface and subsequently the functionalisation with probe oligonucleotides was attained [4]. The surface functionalisation was aimed at detection of phytoplasmas, unculturable, wall-less prokaryotes that cause disease in hundreds of plant species World-wide, often causing serious economic losses. Common detection techniques for phytoplasmas involve total DNA extraction from symptomatic tissues, amplification of phytoplasma DNA by Polymerase Chain Reaction (PCR) followed by a nested step of amplification, to increase sensitivity [5]. These procedures are very time expensive, labor intensive and have a high risk of false positives results due to sample contamination.

Binding ability testing on the functionalised MNPs was performed by hybridization with specific phytoplasma PCR amplicons and a comparison with commercial magnetic separation products was made. The tests performed involved capture of single and double strand DNA fragments of different lengths. The preliminary results obtained show that custom-made MNPs efficiently capture single strand fragments up to 20 bases. At the moment both commercial and home-made functionalised magnetic particles are not suitable to capture specific double DNA strands of 100–2000 base pairs. This may be ascribed to the steric hindrance of the MNPs that may interfere with the hybridization, since, in general, short oligonucleotides bind to complementary target faster and more efficiently than longer ones.

Further activities, directed to test the binding ability of MNPs on single-strand templates, such as RNA of different lengths, will be presented.

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Optimization of Immunoassays based on Magnetic Nanolabels by Optical Biosensor Picoscope™

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The goal of our research was the development of combined opto-magnetic immunoassays based on magnetic nanoparticles and direct optical biosensors with affordable biochips [1].

As an optical label-free biosensor, a Picoscope™ [2,3] was used for detection of different antigens with assistance of magnetic nanoparticles. With the Picoscope™, we registered in real time consequent biochemical binding of different immunoreagents on the biochip surface by measuring the biolayer thickness change at a picometer scale (averaged over the sensor's surface) as shown in Fig.1. The biochip represented a conventional microscopic glass slip without deposition of any metal or dielectric films. Sensogram in Fig. 1 demonstrates that using magnetic labels it is possible to improve limit of detection of Picoscopes™ by several orders of magnitude due to thickness amplification.

The experiments demonstrated that combination of the direct optical biosensor with the functionalized magnetic nanoparticles benefits the assay due to possibilities for:

- pre-enrichment of antigen and sample "cleaning" using magnetic separation,
- increased sensitivity for amplification of optical response of the Picoscope™,
- active acceleration of binding of immunoreagents by external magnetic field manipulation,
- decreased non-specific binding,
- realization of "magnetic washing" by magnetic field gradients, etc.

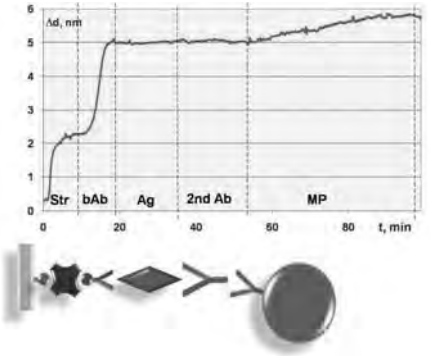

Fig. 1. Sensogram of monitoring of binding of streptavidin (Str) to biotinylated glass surface, then biotinylated antibody (bAB), antigen (Ag) at small concentrations, 2nd mouse antibody and MP (10 nm of iron oxide coved by 50 nm shell) with anti-mouse antibody.

The protocol of the Picoscope™ immunoassay on microscope glass slip was transferred to the 3D glass capillary structures, where the magnetic nanolabels were detected electronically by non-linear magnetization at two frequencies of magnetic field for development of Magnetic ImmunoAssays (MIAtek®) [4].

It has been shown that the optical label-free biosensor is an efficient tool for investigation of chemical properties of magnetic nanoparticles and for rapid selection of protocols, reagents and buffers for highly sensitive magnetic immunoassays for detection of soluble proteins and bacteria.

The research was supported by grants of Russian Foundation of Fundamental Research.

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Nanoscale Imaging of Magnetically Loaded Stem Cells as Diagnostic and Therapeutic Vectors for Lung Cancer

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The relatively small change in mortality of the lung cancer over the years despite the recent achievements in new chemotherapies is encouraging the search for new methods for its treatment. The introduction of bio-compatible iron-oxide magnetic nanoparticles into modified mesenchymal stem cells (MSCs) to enable localised cellular-level sensing and heating, is one of the most promising techniques. This is partially due to the fact that the MSCs are inmuno-privileged and home preferentially to tumour tissues [1].

To assess the uptake of iron oxide nanoparticles as well as the homing and kinetics of MSCs integration into lung tumours, both conventional (CTEM) and cryo-transmission electron microscopy (cryo-TEM) are used. These methods cover a vast range of imaging conditions that allows the recognition of intracellular structures relevant to the study of tumours that are not detected by light-microscopy techniques. Additionally, the different contrast mechanisms involved in nanoparticle and tissue imaging, require special sample preparation and imaging conditions.

We have found that a preparation protocol similar to that used in gametes and embryos [2] can be successfully used in these samples. The new protocol comprises modified chemical fixation (introducing the Karnovsky's fixative for a better penetration), postfixation (studying the effects of buffered osmium tetroxide concentration for contrast enhancing) and embedding steps. Fig. 1 illustrates these effects.

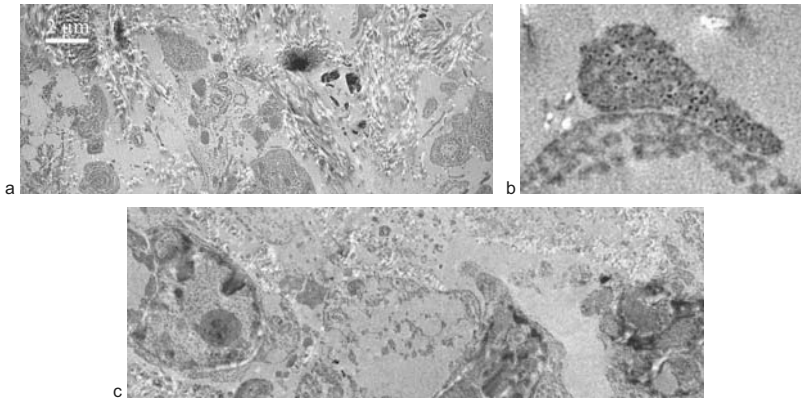

Figure 1 CTEM images of osmicated (a, b) and non-osmiocated (c) lung tumour tissue previously incubated with iron oxide-MSC complexes.

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GROWTH OF BLOCK COPOLYMERS FROM THE SURFACE OF MAGNETIC NANOPARTICLES

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Abstract: We study controled polymerizations using ATRP technique from the surface of superparamagnetic iron oxide nanoparticles (SPIOs). SPIOs with different coating tickness and chemistry as well as responsive SPIOs will be discussed.

Tremendous improvements were achieved in the synthesis of nanoparticles over the last 20 years. This created a wider application area for the nanoparticles. Polymers, once as hype as nanotechnology, frequently get together with nanoparticles either as templates for synthesis, or as a coating or as a carrier matrix. Nanoparticle coatings has many functions: suspends particles in a solvent or makes particles miscible with other materials such as polymers, passify and protect surface on the crystal, stabilize particles, prevent aggregation and provide a functional surface (if desired). A polymeric coating on nanoparticles enable steric stabilization. Depending on the chemistry of the polymers, they can provide electrostatic stabilization and functional surfaces where molecules or other particles of interest can be conjugated. Polymers can be used as coatings during the synthesis of nanoparticles or can be added as a coating material after the nanoparticles are formed. Later usually requires a ligand exchange mechanism. Particle size (actually the aggregate size) depends on many factors including the chemistry and the molecular weight of the coating polymer.

Polymer growth from the surface of nanoparticles have been reported in the literature.[1] Some studies report block copolymer synthesis from the surface of nanoparticles, as well.[1] Yet, in dept studies on the synthesis of block copolymers from the surface of nanoparticles are few. We are interested in growing polymer chains using ATRP method from the surface of superparamagnetic iron oxide nanoparticles (SPIOs) and study the details of polymerization. This would allow controlling the coating thickness around the SPIO crystal. As a continuation we are interested in forming block copolymers on the nanoparticle surface (Figure 1).

SPIOs have been widely studied in a variety of areas from medical diagnostics to cancer therapy, cell sorting to sensors. [3]

Small nanoparticles with polymeric surface coating can be achieved with surface initiated polymerizations. The nature of the polymer and the molecular weight would not only influence the aggregate size and the coating thickness but also the stability. We also aim to prepare smart particles which can respond to stimuli such as pH and temperature. As an example the stability fo the particles may change as a response to temperature of pH change and this can be utilized in magnetic separation, sensing, diagnostics or therapy.

For the described study, halogenated molecules are anchored to the surface of SPIOs and polymers were grown from these initiating sites in appropriate solvents with a ligand such as bipyridine and CuCl. Polymerizations were stopped at different time intervals to follow the molecular weight development with conversion, and to obtain polymers of different molecular weights. Polymerization of styrene and HEMA will be described. For the responsive systems polymers of dimethylaminoethyl methacrylate will be discussed. Block copolymers of these monomers will also be described.

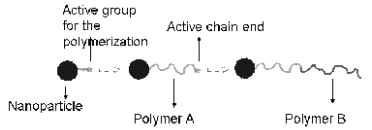

Figure1: Schematic representation of block copolymer growth from SPIO surface

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Functionalized titanate nanotubes as potential carriers in nanomedicine.

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Titanate nanotubes provide more and more attention since their pioneer discovery [1]. Several applications of these nanomaterials are developed in fields of photocatalysts, sensitized solar cells, lithium-ions batteries, gaz sensors. Recently, they are implied in biomedical fields like dopamine detection and bone regeneration but not yet as a therapeutic molecular carrier.

The cultures of neonatal rat cardiomyocytes (CM) represent a very useful tool for the observation and the understanding of the cellular aspects of cardiac diseases. However, their uses are limited by low transfection efficiency with conventional techniques unless viral vectors which provide stable long-term transgene expression.

This study presents the development of a novel non-viral carrier for cardiac myocytes based on the use of functionalized titanate nanotubes (TiONts). TiONts are synthesized by an hydrothermal treatment (150°C, 5 bar) in strongly basic conditions (NaOH, 10 mol/L) [2]. These nanotubes have a tubular morphology being rolled up in spiral with an inner cavity: they develop large specific surfaces and they are charged in surface with hydroxide groups at physiological pH. This particularity inferes an increase of possible interactions with their outside environment and increases possibilities of grafting.

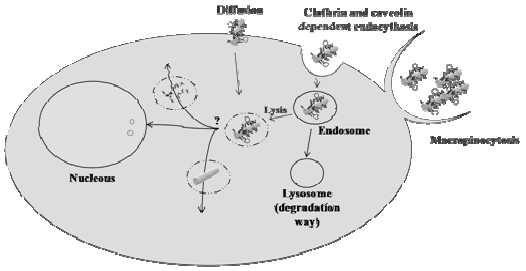

Figure 1: Scheme of different internalization pathways of functionalized titanate nanotubes as a potentiel carrier in nanomedicine.

Grafting of dye molecules (e. g. rhodamin) on nanotubes allows the detection of the TiONts internalized in CM cells by fluorescence microscopic technique and flow cytometry. Transmission Electron Microscopy datas show, as for them, that up to 80% of CM internalized TiONts without sign of toxicity. TiONts are located in vesicles near the nucleous. TEM observations suggest possible coupling of both endocytosis and diffusion internalization process.

Nanomaterial dispersion step is an important parameter, especially for biomedical fields. PEG (polyethylene glycol) and PEI (polyethylene imine) functionalized nanotubes are very well dispersed. The main advantage in PEI use for biological application is its proton sponge effect. This property permits a direct access to cytosol after cellular internalization. That is the reason why this polymer was selected to study TiONts as delivery system [3]. For example, functionalizing these TiONts with appropriate amount of appropriate PEI allow DNA binding and do not change internalization rate and toxicity. It is also of interest to decorate TiONts with Fe_3O_4 nanoparticles in order to induce magnetic behaviors. TiONts-Fe_3O_4 carriers would thus be detectable by MRI imaging.

Our results suggest that further development of these TiONts may provide a new useful tool for research and clinical therapy in the field of cardiovascular disease.

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Synthesis and Clinical Application of Magnetic Nanoparticles

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Magnetic nanoparticles in different size ranges are very important for technical and medical applications. We focused on the synthesis, characterization, and application of magnetite-based nanoparticles prepared by wet chemical precipitation. Two common methods were used:

(1) Partial oxidation of Fe (II) salt solution under alkaline conditions[1]
(2) Co-precipitation of Fe (III) and Fe (II) solution with stoichiometric ratio[2].

Using method (1) in-situ pH measurements were carried out and it was discovered that the pH-time graph is a suitable tool for controlling particle size. The other parameters, such as injection time of oxidant/base and heating rate, were adjusted to the requirements. With this method we are able to obtain magnetite particles in the following size ranges: 30-70 nm (40 nm), 40-90 nm (70 nm), and 70-150 nm (120 nm).
With method (2) we utilized constant pH for precipitation. The position of the precipitation pH-value compared to point zero surface tension (PZC) and ionic strength have a leading influence on particle size and distribution according to thermodynamic aspects (Vayssieres et al.[2]). Working near the PZC with low ionic strength we are able to prepare magnetite/ maghemite-nanoparticles in the range of 15-20 nm with relative homogeneous particle size distribution.
Depending on size and magnetic properties REM/TEM, X-ray, SQUID- and/or VSM-measurements were carried out.
For medical applications the particles produced by method (1) and (2) were coated with Carboxymethyl Dextrane (CMD). The use of ultrasound before and after the coating process is necessary for the successful stabilization of the particles.
Magnetite nanoparticles with a size range of 30-60 nm are used for non-specific separation (without antibodies) of tumor cells (Mamma-Ca) from peripheral blood. This method is based on a method developed by Clement et al. [3] which utilizes superparamagnetic nanoparticles and magnetic separation in a high magnetic field gradient (MACS). Our new aspect consists of using larger magnetite particles along with a lower field gradient separator and external separation column (blood bag). This method allows a mild separation of cells to increase the vitality of healthy cells. The development of this method consists of both improvements to the incubation conditions (plasma addition, temperature, concentration of nanoparticles, incubation time) and to the conditions of separation (pump, separation velocity, blood bag). In the beginning of this new development we optimized separately MCF-7 cells and leukocytes (analyses with cell counter) and continued with cell mixtures in the ratios 15/85, 10/90, 5/95 (analyses with FACS). Currently, we are testing the peripheral blood of different patients with the result of selective depletion of tumor cells (analyses with LSC).

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Fast and Efficient Proteolysis Using Trypsin Immobilized on Magnetic Nanoparticles Isolated from Magnetotactic Bacteria

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Superparamagnetic nanoparticles of iron oxides have shown great potential applications in many biological fields such as biomolecules immobilization, bioseparation, magnetic resonance imaging, tumor hyperthermia, magnetically guided site-specific drug delivery, DNA and RNA purification and immunoassays[1]. The suitable magnetic nanoparticles (mainly γ-Fe_2O_3 or Fe_3O_4) need to be stabilized to avoid agglomeration. They are usually coated with a polymeric shell such as chitosan, dextran, PEG etc. that make them biocompatible, biodegradable, stable and non-toxic. These shells possess active groups which can be bound to biocomponents such as proteins or enzymes, hormones, drugs and cells. This is advantageous namely for trypsin, which is commonly applied for the digestion of protein samples in proteomics. There are two major drawbacks, which hamper the use of native trypsin. The enzyme shows only marginal thermostability at typical working conditions and undergoes rapid autolysis. Chemical modifications of trypsin like reductive methylation or glycation with oligosaccharides have been employed to enhance its stability and prevent from autolysis[2]. A considerable effort has been also dedicated to the development of trypsin conjugates with water-soluble polymers like polysaccharides, polyethylene glycol or polyacrylamide. The use of trypsin immobilized onto solid supports including glass beads, membranes, silica and polymeric monoliths and predominantly magnetic carriers can minimize disadvantages of a conventional in-solution digestion by increasing enzyme-to-substrate ratios, significant speeding up digestion times and reducing autolysis.

In this study, biogenic magnetite nanoparticles isolated from *Magnetospirillum gryphiswaldense* were used as magnetic carriers for covalent immobilization of proteolytic enzymes (trypsin, chymotrypsin). First, the nanoparticles were coated by chitosan, which contains appropriate functional groups for protein immobilization. Egg white avidin was then covalently immobilized on the chitosan magnetic carriers using carbodiimide as a coupling agent. Trypsin, alpha-chymotrypsin and raffinose-modified trypsin were simultaneously modified by biotinylation reagents. Finally, the biotinylated proteolytic enzymes were coupled to the avidin magnetite nanoparticles. The resulting conjugates were studied by biochemical methods and employed for a rapid protein digestion. Both free and immobilized enzymes were characterized by their kinetic parameters, thermostability, optimal pH and reusability. The size and morphological properties of the magnetic carriers before and after attaching proteolytic enzymes were determined by TEM. Magnetic properties were measured by SQUID.

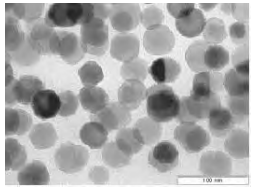

Figure 1: Magnetite nanoparticles isolated from magnetotactic bacteria

[1] Kluchova K., Zboril R., Tucek J., Pecova M., Zajoncova L., Safarik I., Mashlan M., Markova I., Jancik D., Sebela M., Bartonkova H., Bellesi V., Novak P., Petridis D., Biomaterials **30** (15), 2855 (2009).
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Real-time PCR to study the sequence specific magnetic purification of DNA

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The performance of various molecular techniques using complex biological samples greatly depends on the efficient separation and purification of DNA targets. In recent years, magnetic separation technology making use of small magnetic beads, has gained immense popularity Most of these methods rely on the non-specific adsorption of DNA/RNA. However, when functionalizing the beads with complementary DNA probes, the target of interest can selectively be isolated. This sequence specific purification was thoroughly studied. The hybridization of short DNA targets was evaluated by means of simple fluorescent measurements, resulting in purification efficiencies around 80%. Besides standard fluorescent techniques, a real-time quantitative PCR (qPCR) method was applied for monitoring longer DNA targets (Figure 1). This qPCR method also enabled to quantify the purification efficiency of lower target concentrations. Additionally, parameters possibly affecting the magnetic isolation, such as for example the length of the used capture probe or the hybridization location, were investigated. Using the sensitive quantification method together with the optimized conditions, purification efficiencies between 60% and 80% were observed; indicating the magnetic isolation of long DNA fragments is almost as efficient as for short DNA probes. These data together with the optimized qPCR method clearly show the great potential of the sequence specific magnetic purification of DNA, even for trace amounts of target DNA.

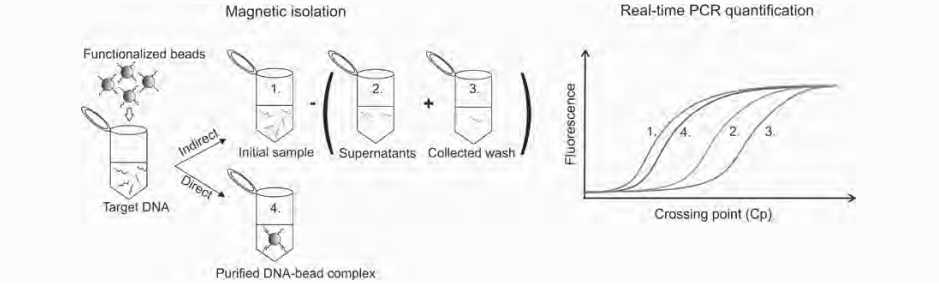

Figure 1. Magnetic purification of target DNA measured by real-time PCR. Using the direct approach the purified target is amplified while attached to the beads. On the other hand, for the indirect approach the target is measured before (i.e. initial sample) and after (i.e. supernatants + wash) magnetic isolation. The difference between these two measurements was found to correspond well with the on-bead amplification.

Immobilization of enzyme bovine enterokinase on biopolymeric magnetic shells

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Recombinant proteins (e.g. antibodies, growth hormones, etc) constitute an important class of biopharmaceuticals. The processes to produce and purify these proteins are often facilitated by the use of affinity tags [1]. For most applications, there is the need to remove the affinity tag upon fusion protein purification. Tag cleavage can be a critical step considering yield, protein purity and manufacturing costs [1-2] Tag removal can be carried out by harsh chemical treatments (e.g. cyanogen bromide) or by enzymatic cleavage, which is by far more specific and less detrimental for the target protein due to milder reaction conditions [1]. However, enzymes are usually costly and unstable. Several methods for enzyme immobilization on solid supports have been developed in order to improve stability and easy recovery and reutilization of the biocatalyst [3]. Recently, magnetic nanoparticles (MNPs) attracted attention as putative solid supports for enzyme immobilization, as they provide minimum diffusion limitation, maximum surface area per unit mass, easy recovery and high enzyme loading [3]. This work reports a novel support based on gum arabic coated iron oxide magnetic nanoparticles (GA_MNP) [4] for enterokinase (EK) covalent immobilization. EK is an endopeptidase that recognizes the DDDDK sequence without requiring a specific sequence at C-terminal, and is commonly utilised for the cleavage of tags in recombinant fusion proteins [1]. GA_MNPs were synthesized by using the Massart method in the presence of gum arabic solution, aminated with 3-(aminopropyl) triethoxy silane, after which the covalent immobilization of EK was carried out [2, 4]. Once immobilized, an EK fluorimetry activity assay was performed with the EK synthetic substrate Gly-Asp-Asp-Asp-Asp-Lys-2-naphtylamide. The EK enzyme cleaved the substrate, releasing 2-naphtylamide, a fluorophore that was monitored at different reaction times. The activity and stability of the immobilized EK was compared with those for the free EK. In general, the immobilization procedure lowers the initial activity but increases the stability of the enzyme.

Fig 1: EK Immobilization on GA_MNP (PDB id: 1ekb)

1 Arnau, J., et al. Protein Expression and Purification, 2006. **48**(1): p. 1-13.
2 Kubitzki, T., et al. Bioprocess and Biosystems Engineering, 2008. **31**: p. 173-182.
3 Kim, J., et al. Chemical Engineering Science, 2006. **61**(3): p. 1017-1026.
4 Roque, A.C.A., et al., Journal of Biotechnology, 2009. **144**: p. 313–320

Trapping of magnetically-labelled liposomes on flat micro-patterned hard magnetic films

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Despite remarkable advances in the past ten years, cell trapping on surfaces remains an important challenge in biotechnology. Indeed, current diagnostic techniques or pharmacological screening require parallel analysis of millions of cells. Improving the technology for gathering cells into clumps makes it possible to treat and control them differently while keeping them under observation. Cluster trapping can also be useful for studying cell-cell interactions. Precise positioning of individual cells is also required in many situations. Contact-free manipulation techniques using electric, magnetic, optical or acoustic forces present the advantage of being intrinsically sterile and enable fine control of the exerted force. In this work, we used giant liposomes as cellular models to illustrate the possibility to trap magnetically-marked cells on a flat micro-patterned hard magnetic film by magnetophoresis. Liposomes can encapsulate nano- or micro-functional materials during the electroformation process (fig. 1.A) and the amount of nanoparticles incorporated can be controlled by regulating the concentration of magnetic nanoparticles in the solution used for liposome electroformation.
Flat hard magnetic films were magnetically micro-patterned (chessboard like features of area 100 x 100µm) using the thermo-magnetic patterning technique [1]. The magnetic patterns were revealed by magneto-optic imaging of a uniaxial Magneto-Optic-Indicator-Film (MOIF) placed on the NdFeB film (Fig. 2B.). The Liposomes incorporating superparamagnetic nanoparticles (100 nm) were selectively attracted towards the boundaries separating adjacent oppositely magnetised magnets, where the field gradient is maximum (Fig. 2.A), as revealed by the fact that the liposome distribution followed the magnetic chessboard pattern (Fig. 2C.).. Separation possibilities were demonstrated using a mixture of magnetically-marked and unmarked liposomes obtained from fluorescent phospholipids respectively labelled with NBD and pyrene (principle illustrated in Fig. 1B.).

[1] F. Dumas-Bouchiat et al., Appl. Phys. Lett. **96**, 102511 (2010)

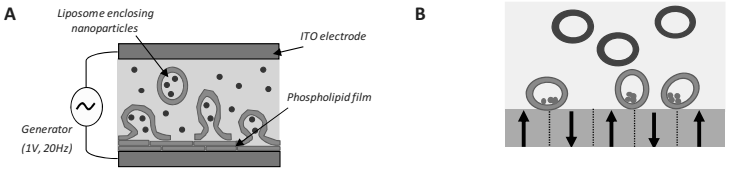

***Fig. 1 A.** Electroformation of magnetically-labelled liposomes – **B.** Labelled liposome attraction towards magnetic field maxima.*

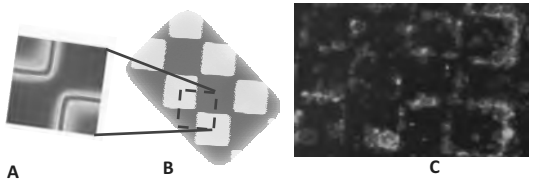

***Fig. 2 A.** Norme of Magnetic induction in the plan situated right at the surface of the magnetic film – **B.** Magneto-optic image of the multipole pattern obtained in the flat NdFeB film – **C.** Fluorescent and magnetic liposomes attracted towards fieldgradient maxima.*

Trapping of spherical and elongated magnetic particles in microfluidic systems

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Magnetic manipulation of particles in microfluidic systems has been applied only recently. Magnetic forces can be used for transport, positioning, separation and sorting of magnetic particles and magnetically tagged cells and eg proteins. Most commonly magnetic nanoparticles in microfluidics serve as a surface for bio- or immunoassays, which can therefore be done in a small volume and very localised [1]. In this study magnetic trapping of commonly used spherical super paramagnetic particles (Endorem®) and elongated nanoparticles, Fe filled multi walled carbon nanotubes (Fe-MWNTs) is studied. Suspensions of both types of particles are in a microfluidic chip and trapping is achieved by the use of a magnetic needle with a high gradient magnetic field (7500 T/m). Although the mass magnetization of the SPIO particles is a factor five higher compared to the Fe-MWNTs (figure 1) due to the low filling grade of the nanotubes trapping is still possible (figure 2). Furthermore Fe-MWNTs have potential in lab-on-a-chip-devices thanks to there large surface area for binding of proteins etc.

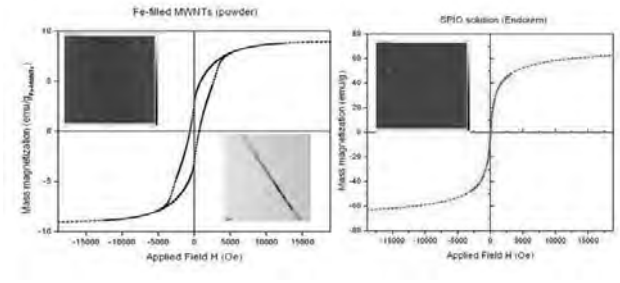

Figure 1: *Hysteresis loops of Fe-filled MWNTs,inset AFM and TEM image of Fe-MWNTs and Endorem® suspension, inset AFM image*

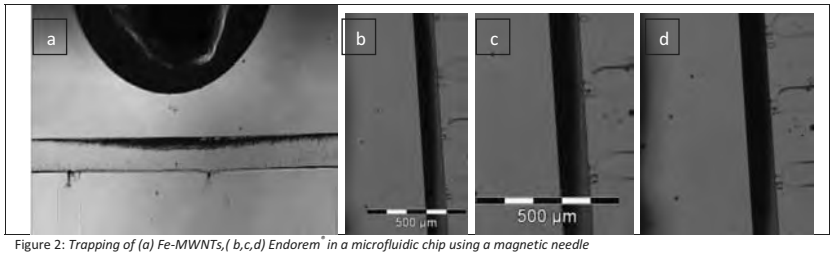

Figure 2: *Trapping of (a) Fe-MWNTs,(b,c,d) Endorem® in a microfluidic chip using a magnetic needle*

1. Pamme, N., *Magnetism and microfluidics.* Lab on a Chip, 2006. **6**(1): p. 24-38.

FeCo nanoparticles encapsulated into biodegradable microparticles for targeted liver chemoembolization

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To improve the therapeutic efficiency of the liver tumor chemoembolization, we are developing therapeutic magnetic micro carriers (TMMC) which can be steered in the blood vessels from the hepatic artery to the tumor area by an upgraded clinical magnetic resonance imaging (MRI) system[1,2]. The magnetic field of the MRI system saturates the magnetization (M_s) of the carrier in deep tissues and it could be used to track in real time the carrier in the blood vessels. A magnetic gradient generated by an insert of coils placed in the MRI tunnel is used to steer the carrier in the tumor blood vessels. TMMC, are biodegradable microparticles with proper size required for the embolization and they are loaded with a high amount of magnetic nanoparticles and an antitumor drug. As a proof of concept, we previously encapsulated FeCo nanoparticles (M_s= 209 emu/g) into poly(D,L-lactic-*co*-glycolic acid) (PLGA) microparticles (FeCo-PLGA microparticles-V1, M_s= 61 ± 2.5 emu/g, diameter = 58 ± 17 µm, FeCo nanoparticle loading = 33% *w*/*w*) by a simple emulsification process[2]. These microparticles were successfully steered in a phantom mimicking the hepatic artery[2]. According to our steering model, the FeCo-PLGA microparticles could be steered with a 400-mT/m magnetic gradient in a rabbit hepatic artery of 2 mm diameter with a blood flow of 11 mL/min. However, the hepatic artery blood flow can be higher depending on the physiopathological conditions. Consequently, we improved the microparticle properties for the steering. We encapsulated FeCo nanoparticles coated with a graphite shell (M_s= 212 ± 3 emu/g) into PLGA microparticles having a mean diameter of 69 ± 20 µm, an M_s of 87 ± 3 emu/g and a loading of FeCo nanoparticles of 43% (*w*/*w*) (Figure 1). With these properties, FeCo-PLGA microparticles-V2 could be steered with a blood flow of 22 mL/min. These findings indicate that the loading of FeCo nanoparticles and the size of the microparticles can be increased in order to improve the steering. This optimization of the microparticle properties was an important step for the preparation of the *in vivo* steering assays.

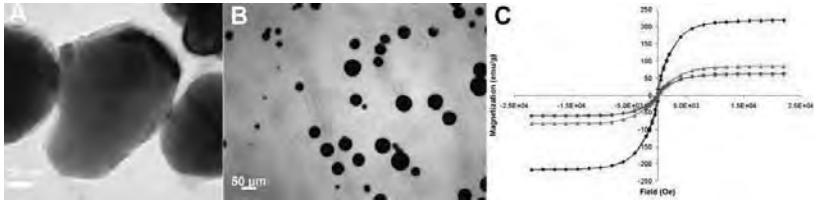

Figure 1. FeCo-PLGA microparticle properties. A) TEM image of FeCo nanoparticles coated with a graphite shell. B) Optical microscopy image of FeCo-PLGA microparticles-V2. C) Hysteresis loop recorded at 20°C of ◆ FeCo nanoparticles (M_s= 212 emu/g), ● FeCo-PLGA-microparticles-V1 (M_s= 61 emu/g) and ▲ = FeCo-PLGA microparticles-V2 (M_s= 87 emu/g)

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Synthesis and Antibacterial Activity of Multifunctional Fe_3O_4–Ag Heterodimer Nanoparticles

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ABSTRACT

Multifunctional Fe_3O_4-Ag heterodimer nanoparticles with both superparamagnetic and antibacterial properties were prepared by reducing silver acetate on the surface of Fe_3O_4 nanoparticles in toluene medium. The formation of bifunctional Fe_3O_4-Ag heterodimer nanocomposites (12 nm Fe_3O_4 - 6 nm Ag) was confirmed by transmission electron microscopy. X-ray diffraction patterns indicated the co-existence of both Fe_3O_4 and Ag phase as well as their nanocrystalline features. The appearance of surface plasmon resonance band of silver at 418 nm in nanocomposites and their superparamagnetic nature confirmed the presence of Fe_3O_4 and Ag. In order to make aqueous stable suspension, amine and carboxylic moiety was introduced onto the surface of as-synthesized nanocomposites by well-known surface conjugation chemistry. These biocompatible nanostructures are highly toxic to microorganisms. Their antibacterial activity was evaluated by means of minimum inhibitory concentration value showed that Fe_3O_4-Ag heterodimer nanoparticles presented good antibacterial performance against *Escherichia coli* (gram-negative bacteria) and *Staphylococcus epidermidis* (gram-positive bacteria). Furthermore, Fe_3O_4-Ag nanoparticles can be easily removed from water by using a magnetic field to avoid deposition of silver nanoparticles.

Visualization of magnetic microparticles in blood vessels using synchronous ultrasonic Doppler imaging.

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[b]*NanoSynthesis, Columbus, OH, USA*

We considered applicability of acoustic imaging technology for the detection of magnetic microparticles and nanoparticles inside soft biological tissues. Such particles are widely used for magnetically targeted drug delivery and magnetic hyperthermia. We developed a new method of ultrasonic synchronous tissue Doppler imaging with magnetic modulation for *in vitro* and *in vivo* detection and visualization of magnetic ultradisperse objects in soft tissues [1]. Prototype hardware with appropriate software was produced and the method was successfully tested on magnetic microparticles injected into an excised pig liver.

The method is based on inducing periodic movement of the particles and surrounding tissues using low frequency (dozens of Hz) periodic (e.g., rotating) magnetic field, and reading acoustic Doppler signal at the frequency of the modulating magnetic field [1]. The main experimental challenge of the method is the spread of the particle oscillations through soft biological tissues, reducing the spatial resolution. Increasing the frequency of the modulating magnetic field improves spatial resolution and reduces artifacts, but also reduces the sensitivity of the method [1]. To achieve better sensitivity at the modulating frequency of 25-50 Hz, a new industrial prototype of ultrasound medical scanner УДС-10-ЦМА (Medical Acoustic Imaging Center, Ltd.) was developed. It has 3-5 times higher Doppler sensitivity than the earlier version, and its working frequency is 6.5 MHz.

An excised pig liver was used as a phantom. A blood vessel with 2-3 mm diameter was filled with a suspension of nanoparticles. Alternating magnetic field was generated by a rectangular (40x40x20 mm) NdFeB alloy magnet, which was rotated by a step motor at the rate of 12-25 revolutions per second. Peak magnetic field in the area with the particles was about 1500 Gauss. Magnetic field vector was perpendicular to the direction of rotation, and was almost parallel to acoustic scanning plane. Adjusting the modulating field frequency we were able to achieve Doppler images of the blood vessel filled with the suspension of the particles with limited amount of artifacts.

1. M.Ph. Pyshnyi, O.A. Kuznetsov, S.V. Pyshnaya, G.S. Nechitailo, A.A. Kuznetsov, Synchronous ultrasonic Doppler imaging of magnetic microparticles in biological tissues. Journal of Magnetism and Magnetic Materials 321 (2009) 1552–1556

Theory of the magneto-inductive hyperthermia in a rotating field

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The nanoparticle-mediated magneto-inductive hyperthermia is quite a popular subject. Somehow, in common mind it is associated with a linearly polarized AC field, as if it were the only type of induction possible. Meanwhile, even at the first sight, a rotating field should be at least twice as efficient. Indeed, as well known[1], if a container is entirely filled up with a magnetic suspension (no free surface), then a rotating field does not induce a hydrodynamic flow and spends all its energy on the motion of individual particles. Given that, the particles experience the field of amplitude H instead of effective $H/\sqrt{2}$ that is the case of an AC field.

In the present work we use the kinetic approach, once applied to the case of linear polarized field oscillations[2], to account for the energy dissipation in a suspension of magnetically hard particles subjected to a rotating field of an arbitrary amplitude and to rotary Brownian motion. Physically, the context of the problem is quite close to that of magnetic viscosity, but the points of interest and goals are different. The specific loss power is analyzed in terms of the applied field amplitude and frequency as well as temperature. Comparative diagrams obtained are presented in Fig.1. We note that the results on the rotating field effect are much poorer described with simple single relaxation-time approximations, like [3], and indeed need to be considered in a full statement, as is done here.

As seen, the absorption level for a rotating field is normally substantially higher than for oscillating one. This means that the same absorption rate could be achieved with lower field amplitudes without enhancing the field frequency. The latter is quite important given the physiological restrictions imposed on the hyperthermia techniques.

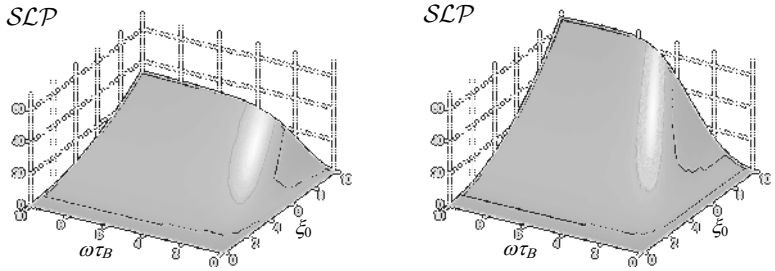

Fig.1. Specific loss power per unit mass of a single-domain particle of magnetic moment μ in cases of a linearly polarized (left) and rotating (right) fields of the same amplitude H; here τ_B is the Brownian rotational time and $\xi_0 = \mu H/kT$ the Langevin argument

Acknowledgements. The work was done under auspices of projects: 08-02-00802 (RFBR), 09-02-91070 (RFBR-CNRS, PICS 4825), AATP 2.1.1/4463 (Russian Ministry of Education and Science) and 21394NH (ECONET).

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Multichannel magnetorelaxometry *in vivo* monitoring of magnetic nanoparticle quantity for thermal ablation studies

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To inactivate cancer cells with minimal side-effects to the normal tissue, cancer therapy as magnetic thermal ablation utilizes superparamagnetic iron oxide nanoparticles (MNP) injected into the tumor, which are generating heat when exposed to an externally applied alternating magnetic field. Hence, for thermal ablation the intratumoral quantity of MNP needs to be thoroughly controlled to assure adequate heat production in the carcinoma region[2].

In our recent work[1], we presented multichannel magnetorelaxometry (MRX) measurements as a powerful tool for the quantification and localization of MNP accumulation in carcinoma bearing mice *post mortem*. Here, we expanded our method to demonstrate that multichannel MRX enables *in vivo* quantitative monitoring of MNP accumulation in the tumor region via contactless, non-invasive measurement on conscious laboratory animals. We present results of a carcinoma bearing mouse, which previously got an intratumoral injection of MNP (fluidMAG-D by chemicell GmbH, Berlin, with solid content β = 200 mg/ml, hydrodynamic diameter d = 200 nm, total core size d_c = 70 nm, composed of several particles of about 10–12 nm) followed by a thermal ablation treatment 24h after MNP injection.Utilizing the biomagnetic 304-multichannel SQUID system in the magnetically shielded room BMSR-2 of the PTB, we performed MRX measurements in the pre and post phase of thermal ablation. During the measurements, the mouse was located in a non-magnetic container (with vent holes) wherein it could freely move. First, the container was magnetized with a magnetic field of 1.0 mT using a Helmholtz coil (d ≈85 cm). After switching off the magnetic field the container was rapidly transferred underneath the measurement device, which caused a delay of about 7 s. The decaying magnetic induction $B(t)$ in the z-direction was subsequently measured for 70 s with a 250 Hz sampling rate. All experiments were approved by the regional animal care committee and were in accordance with international guidelines on the ethical use of animals.

The measured spatial magnetic field distribution of the multichannel MRX data was subsequently fitted applying a Levenberg–Marquardt algorithm, modeling the magnetic nanoparticle accumulation as a point-like magnetic dipole. As a result, we get the magnetic moment and position of the dipole. Via normalization to a reference sample with known MNP amount (measured and fitted under the same conditions), we therewith quantified the nanoparticle amount in the tumor region of the mouse in repeated measurements in pre and post phase of thermal ablation. In conclusion, we assess MRX as well-suited to quantitatively monitor the MNP accumulation *in vivo* in non-anesthetized laboratory animals, being an important tool to support long-term thermal ablation studies.

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Nonlinear Energy Dissipation of Magnetic Nanoparticles in Oscillating Magnetic Fields

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Magnetic nanoparticles are of increasing interest in biomedical applications such as cancer treatment and triggered drug release due to their capacity to dissipate energy in the form of heat when subjected to an oscillating magnetic field. The rate of energy dissipation P has been described according to a model derived by Rosensweig [1] for collections of non-interacting particles with a characteristic relaxation time τ

$$P_R = \frac{\chi_0 H^2 \Omega^2 \tau}{2\left(1+\Omega^2\tau^2\right)}, \quad (1)$$

where χ_0 is the initial susceptibility, H is the applied field amplitude, and Ω is the applied field frequency. However, as has been pointed out by others [2] this analysis is limited to small applied magnetic field amplitude and frequency due to the use of the phenomenological magnetization relaxation equation, derived by Shliomis [3], in the linear magnetization limit. This implies that the expression derived by Rosensweig should only be applicable to values of the Langevin parameter less than unity and frequencies below the inverse relaxation time. These limitations led Raikher and Stepanov [2] to investigate the absorption of ac field energy in suspensions of magnetic dipoles using a formulation based on solution of the Fokker-Planck equation, predicting that the expression due to Rosensweig ceases to be valid for large values of the applied field frequency. In this contribution we approach this problem from an alternative phenomenological point of view by solving the phenomenological magnetization relaxation equation exactly for the case of arbitrary magnetic field amplitude and frequency and by solving the magnetization relaxation equation of Martsenyuk, Raikher, and Shliomis [4] (MRSh) numerically. The results are summarized in terms of a non-dimensional energy dissipation rate

$$\tilde{P} = \frac{P}{2\pi\mu_0 M_s H \Omega\left(\frac{\Omega\tau}{1+\Omega^2\tau^2}\right)} \quad (2)$$

which is a function of the applied field amplitude, parameterized by the Langevin parameter, and the product of field frequency and relaxation time (**Figure 1**). In (2) μ_0 is the permeability of free space and M_s is the saturation magnetization of the suspension of particles.

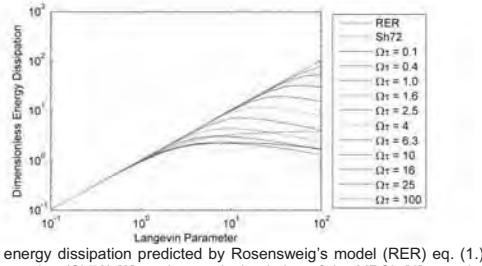

Figure 1: Dimensionless energy dissipation predicted by Rosensweig's model (RER) eq. (1.), exact solution of the magnetization relaxation equation (Sh72) [3], and numerical solution of the MRSh [4] equation for various values of the applied field frequency.

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RF susceptibility of magnetic nanoparticles system with application in Biomedicine

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Magnetic nanoparticles (MNPs) find wide application in medicine and bioanalytics. Magnetic nanoparticles being subjected to a magnetic AC field may show remarkable heating effects related to losses during the magnetization reversal process of the particles. In this work is presented the design and set up of a very sensitive RF susceptometer based on a Robinson NMR oscillator. This home-made susceptometer operates in a broad RF frequency range (~10-40 MHz), depending mainly on number of turns the inductive coil. The validity of this RF susceptometer is tested in some magnetic nanoparticles systems in order to study the thermal relaxation and particle interaction effects on dynamic properties. It is demonstrated that this unconventional system can be used to study the spin dynamics and charge/spin ordering related properties. Field-dependence of the peak susceptibility. Solid line represents the best curve-fitting. The inset presents a typical imaginary component of the RF susceptibility at H_{DC}=600 Oe (Figure 1). Real part of the RF-susceptibility (χ') plotted as a function of the temperature for $Co_xFe_{3-x}O_4$ nanoparticles. The inset shows the imaginary component (χ'') as a function of the temperature for the same set of samples (Figure 2). Magnetic nanoparticles offer some attractive possibilities in biomedicine.

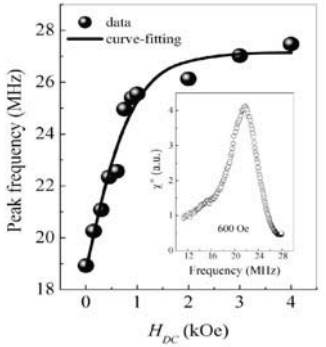

Figure 1

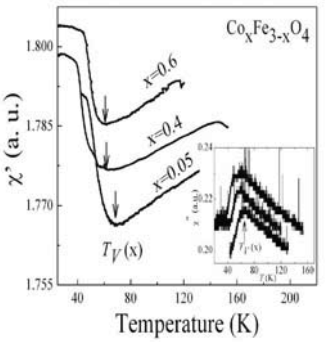

Figure 2

Fast detection of *Legionella pneumophila* in cooling towers by immunomagnetic microspheres.

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Summary

Legionnaires´disease is a severe form of pneumonia caused by bacteria of the genus *Legionella*. It is a notifiable disease worldwide, with relevant clinical aspects, such as a fatality rate of 12-15 %, easily extended up to 30-50% in immuno-compromised patients (1, 2). Predominant species responsible for illness is *L. pneumophila*, a virulent pathogen which is responsible of 90-98 % of the cases (3,4). Rapid, cost-effective, reliable and simple test is a key issue for monitoring water quality and prevention of the outbreaks of *Legionella* infections. Cooling towers are known to be one of potential sources of harboring, amplifying and disseminating *L. pneumophila*. Composition of water in cooling towers can be very complex, causing a loss of culturability in the cells, so they can not recovered by culture technique. Moreover, this water can contain toxic substances, such as heavy metals, inhibiting polymerase reaction in the PCR. Immuno-sensing techniques addressed to detection of intact cell can be good approaches to restrict detection to the viable cells. In this study, kit Bioalarm Legionella based on Estapor® magnetic microspheres is applied to environmental monitoring of cooling towers, overcoming drawbacks of the above mentioned techniques. Methodology used is enzyme-linked immunomagnetic colorimetry.

Figure 1 Micro-device (MD) (A) for easy to handle Estapor® magnetic microspheres(B)

A

B

Figure 2 Comparison of culture, PCR and Kit Bioalarm results.

Water samples from cooling towers		Kit Bioalarm (*)		
Culture (cfu/L)	PCR	No. of Positive	No. of negatives	Positives (%)
260	[illegible]	9	1	90
340	+	10	0	100
1600	+	10	0	100
2800	+	10	0	100
5400	+	10	0	100
27600	+	10	0	100

(*) Ten replicates for each sample

Iron oxide nanoparticles in microporous silica shell presenting enhanced r_2 relaxivity values

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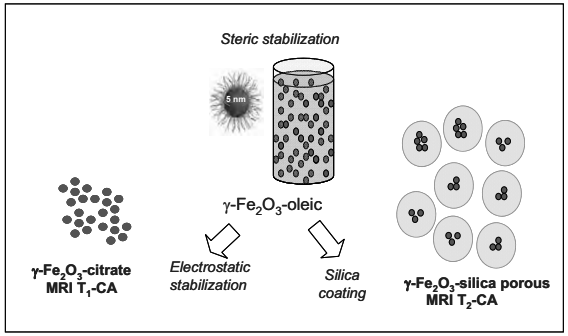
Actual trends in MRI are towards visualization of events at cellular level which require very high detection sensitivity. Special attention is directed at fabricating novel superparamagnetic iron oxide systems with higher relaxivity values.

Maghemite nanoparticles were synthesized in organic media by thermal decomposition of iron pentacarbonyl in presence of oleic acid to obtain best quality (7 nm) maghemite nanoparticles, which in turn can act as positive contrast agents[1]. Such nanoparticles were later coated with silica by a combination of sol-gel chemistry and supercritical fluids. The resulting nanocomposite particles have a mean size of 100 nm with a magnetic core formed by non-contacting iron oxide nanoparticles sourronded by a silica microporous shell. The material is superparamagnetic at RT with improved saturation magnetization values. The method is relatively fast and straight forward producing materials in a dry form and with good yield. Moreover, the process has potentiality for being scaled-up. The particles are monodisperse in size and readily dispersable in water.

It will be shown that composite particles are promising T_2-agents with relaxivity values larger than Endorem and thus enhanced sensitivity. The silica shell could be easily functionalized via surface silanols and due to their microporosity there is the possibility to use the material for theranostic purposes.



1. Taboada et al. *Langmuir* 23 (2007) 458.
2 Taboada et al. *Advanced Functional Material* 19 (2009) 2319.

Title: Selective noble metals extraction from dilute, acidic streams using functionalized carbon-coated nanomagnets

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Magnetic filtration provides an efficient way to control large liquid volumes with a small amount of a specific reagent. At present, the lack of sufficiently stable nanomagnets has limited the magnetic filtration to routine processing in biochemical separations, particularly in diagnostics. Unfortunately these high-price applications are limited to water and neutral pH as the present oxide-based nanomagnets have a poor stability and low binding capacities.

Recently we prepared metallic nanoparticles with a graphene-like carbon coating of 1-2 nm by reducing flame spray synthesis. This coating protects the metal nanoparticles from oxidation up to temperatures of 190°C and against dissolution in acids. On the other hand it allows the introduction of covalently bound functional groups via diazonium chemistry or physical adsorption, giving access to organic chemistry as a method to design the particle surface.

The functionalized magnetic particles represent a promising modular platform for removing a wide range of contaminants. Attaching a thiourea-like chelating agent allows the efficient and selective removal of precious metal ions in highly acidic solutions. The low costs and high particle stability favor this processing method and associated material for large-scale separation application where metal ions are present at ultra low concentrations.

This contribution will show how noble metal can be rapidly collected from acidic mining streams down to the microgram per liter level.

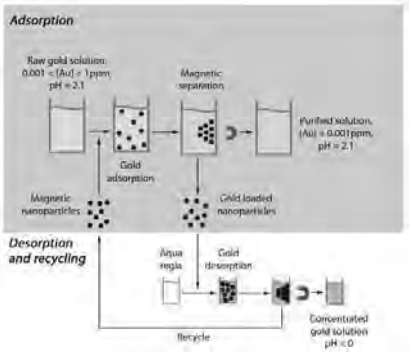

Figure 1: Scheme of the extraction process using carbon-coated nanomagnets [1]

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Poster 171

MagnaFy: the use of iron oxide nanoparticles enabling MRI visibility of medical devices

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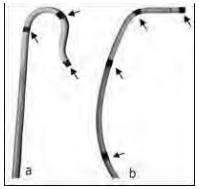
Magnetic nanoparticles have become more and more important for applications in biotechnology and biomedicine over the past few years. During the last decade many efforts have been undertaken to advance the field of magnetic resonance imaging (MRI). Excellent soft tissue contrast, the ability to perform vessel wall imaging, more biocompatible contrast agents, and the lack of radiation for patient and physician are some of the many advantages for using MRI instead of X-ray technology in the interventional suite. However the biggest hurdle for MRI based interventions is the shortage of MR-compatible devices. Magnets and metal device not match, and therefore there is a need for devices made of synthetic materials that are made visible in MRI. MagnaFy® and MagnaFy-Hybrid are specially designed technologies by MagnaMedics to facilitate the use of medical devices in magnetic resonance imaging (MRI) and multimodal imaging. By use of a chemical process and a specific coating we are able to modify most existing quidewires, catheters and (micro)drains with small markers that become visual in a normal 1.5 Tesla MRI. The first studies of MagnaFy-enabled PEEK-guidewire have been performed in pre-clinical studies in collaboration with the University of Basel and are currently published [1,2]. Below as example are images of MagnaFy marked non-braided pig-tail catheter.



In order to optimize the MRI visibility, we are currently working on testing various iron oxides nanoparicles in MRI phantoms models in gels at 1.5 T and higher magnetic fields. We are also investigating the influence of marker size and shape and particle concentration on MRI artefact size and estimate the iron oxides nanoparticles concentration that gives an acceptable MRI artefact size. In the future, application of the described MagnaFy passive markings of any medical devices like balloons, stents, filters, introducer sheaths, guidewires and catheters (if needed) will facilitate the use of MRI in interventional medicine.

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Poster 172

Effects of Magnetohyperthermia on Ehrlich Solid Tumor

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Magnetohyperthermia (MHT) represents a promising approach for cancer treatment especially due to the possibility of side effects reduction in comparison to conventional therapies. As such, MHT is one of the most attractive biomedical applications of magnetic nanoparticles (MNPs). This study was developed with the aim to investigate the biological and therapeutic outcomes of the MHT procedure applied to the treatment of tumors. To perform the experiments a new magnetic fluid (MF) sample based on magnetite nanoparticles (7.9 nm dimeter @ 1.2×10^{16} particle/mL) surface-coated with polyaspartic acid (PAMF) and stabilized in physiological medium was developed. This surface coating was selected because aspartic acid by itself seems to inhibit tumor cell growth or increase antitumor activities of several chemotherapeutic drugs [1]. PAMF was biologically tested as described elsewhere [2]. In a second stage an animal model for Ehrlich solid tumor was developed. The tumor-bearing animals received intra-tumor injections of PAMF and were subsequently exposed one or three times to AC magnetic fields generated by an appropriated equipment described elsewhere [3]. Although complete tumor remission was not obtained, AgNOR tests showed tumor volume and proliferative activity reduction. Anti-tumor activity was more evidenced 30 days after the threefold AC field treatment. Histology analysis showed wide necrotic areas near magnetic nanoparticles regions (Fig.1), thus confirming AgNOR results. Further, histology observations found no alterations in any other organ except at the tumor. The results suggest that the treatment mediated through MHT is viable and possibly advantageous in relation to other cancer treatments.

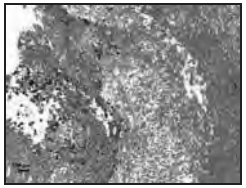

Fig.1 shows tumor section stained with hematoxylin-eosin. Magnetic nanoparticles (MNPs) are evident as dark-brown spots. Figure shows wide necrotic areas near MNPs.

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Multifunctional magnetic nanomedicine based on poly(ε-caprolactone)

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Active targeting strategies are one of the most significant approaches to assure a maximum antitumor effect with negligible toxicity. In this way, the introduction of magnetic colloids has revolutionized the formulation of stimuli-sensitive carriers [1, 2]. Even more, the use of superparamagnetic materials has facilitated the design of multifunctional nanomedicines that not only can very efficiently accumulate any given antitumor drug into the tumor site, but also can kill the cancer cells by a complementary hyperthermia effect. Interestingly, such magnetic materials have shown very surprising possibilities in clinical diagnostic of malignancies, as they are efficient contrast agents in magnetic resonance imaging (MRI) [3]. Thus, the present investigation is focussed on the formulation of multifunctional superparamagnetic magnetite/poly(ε-caprolactone) (core/shell) nanoplatforms.

Nanomagnetite (Fe_3O_4, mean diameter: 12 ± 2 nm) was prepared following the chemical co-precipitation method proposed by Massart [4]. Superparamagnetic Fe_3O_4/poly(ε-caprolactone) (Fe_3O_4/PCL) (core/shell) nanoplatforms (mean diameter: 86 ± 12 nm) were prepared by interfacial polymer disposition, a well-known procedure for the synthesis of pure PCL [5], except that the aqueous phase was a Fe_3O_4 suspension (0.3 %, w/v). Compared to other procedures [6], our methodology avoids the use hydrophobic Fe_3O_4 and, as a result, simplifies the formulation.

The physicochemical characterization of the magnetic nanocomposites confirmed the efficiency of the PCL coating onto the Fe_3O_4 core. For instance, the zeta potential (ζ)-pH and ζ-ionic strength trends of the magnetic composites was almost indistinguishable from those of the pure polymer. In addition, the analysis of the surface thermodynamics of the materials also ascertained the efficiency of the PCL coating: the hydrophilic nature of Fe_3O_4 changed to hydrophobic (just like PCL) when it is covered by the polymer. It was hypothesized that an attractive electrostatic interaction between the positively charged Fe_3O_4 particles and the negatively charged polymer (note that the reaction occurs at pH ≈ 6) will tend to concentrate the latter in the vicinity of the Fe_3O_4 surface, thus leading to the formation of the polymeric layer onto the Fe_3O_4 cores.

We determined the initial susceptibility of Fe_3O_4 ($\chi_i = 0.16 \pm 0.03$), and Fe_3O_4/PCL ($\chi_i = 3.13 \pm 0.17$) for the magnetic composite particles. The enhancement of saturation magnetization when the magnetic nuclei are merged under a PCL layer was also significant: 12 ± 3 kA/m for Fe_3O_4 and 258 ± 7 kA/m for Fe_3O_4/PCL. Interestingly, the magnetic properties of the nanocomposites were found to be much larger than those found previously for particles of similar composition but consisting of ≈ 160 nm [6].

Two drugs were successfully adsorbed onto the nanocomposite surface: 5-fluorouracil and diclofenac sodium (entrapment efficiency > 25 %, under the best drug loading conditions). Preliminary *in vitro* experiments also suggested that the magnetic composites provided MR contrast enhancement by shortening both the longitudinal (T_1-recovery) and transverse (T_2-decay) relaxation of surrounding protons. T_2-weigthed pulse sequences of Fe_3O_4/PCL aqueous suspensions provided negative [hypointense (dark)] contrast enhancement compared to controls (water, PCL aqueous suspension, and Fe_3O_4 aqueous suspension).

Conclusions: In this work we have shown that it is possible to reproducibly coat ultrasmall superparamagnetic iron oxide nanoparticles with a shell of the biodegradable polymer poly(ε-caprolactone). Although the existence of the PCL shell is observable under the electron microscope, the efficiency of the coating is demonstrated by the surface analysis of the mixed particles compared to that of their components. Extensive research is under process to incorporate the drugs into the polymeric matrix. This will enhance the drug loading capacity and, additionally, will lead to a sustained (biphasic) drug release profile.

Acknowledgements: Financial support from Junta de Andalucía, Spain, under Project PE-2008-FQM-3993 is gratefully acknowledged.

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Magnetic affinity starch adsorbent for the purification of cyclodextrin glucanotransferase

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Cyclodextrin glucanotransferase (CGTase, EC 2.4.1.19) is a unique enzyme converting starch into cyclodextrins (Fig. 1). These compounds possess the ability to form inclusion complexes with many organic and inorganic molecules, changing their physical and chemical properties, which determines their wide applications in environmental protection, medicine, food, cosmetic, pharmaceutical and chemical industries.

An efficient isolation and purification of the enzyme from culture medium has still been of high importance. This enzyme contains „raw starch binding domain“ exhibiting affinity to different starch derivatives. In this work one-step, affinity isolation/purification of CGTase produced by an alkalophilic strain of *Bacillus circulans* ATCC 21783 on magnetic starch has been developed.

Fig.1 β-cyclodextrin

Several types of magnetic derivatives of starch were prepared as affinity adsorbents and different kinds of elution systems were tested in two different volume batches of culture medium (100 and 400 ml). Larger scale (400 ml) magnetic separation process was performed using a magnetic separator SEPMAG Q for 500 ml bottles (SEPMAG Q500ml 2042, precision magnetophoresis system; Fig. 2). The best results were obtained with magnetic porous corn starch gel particles (size ca 300 - 600 μm) as an adsorbent and two alkaline buffers (pH 8 and pH 10) for the elution. Under the optimized conditions majority of accompanying proteins was removed (Fig. 3) and substantially purified enzyme was obtained. The specific activity of the isolated CGTase increased ca 29 times after the magnetic affinity separation and the CGTase activity yield was 80-95 %. No differences were shown in 100 ml and 400 ml volumes of culture medium used for CGTase purification. Batch isolation/purification of the enzyme thus could be scaled up to 20 l working volume using SEPMAG Q20L system, which is currently also available.

Fig. 2 SEPMAG Q500ml 2042, precision magnetophoresis system (SEPMAG Tecnologies, Spain)

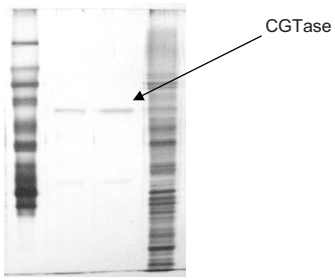

Fig. 3 SDS electrophoresis (from left: markers, eluate pH 8, eluate pH 10, culture medium)

Poster 175

Homogeneous Catalysts immobilized on Carbon Coated Cobalt Nanoparticles

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Schematic representation of a chiral ligand/transition metal- complex covalently grafted on magnetic Co/C-nanoparticles.

Catalysis is among the most important applications within the field of nanoscience. [1] The large surface area of nanoparticles qualifies them quite naturally to act either as heterogeneous promotors for catalytic reactions[2] or as a support for homogeneous catalysts.[3] Carbon coated cobalt nanoparticles[4] are predestined to act as such a scaffold for “heterogenized” transition metal complexes due to the impressive thermal and chemical stability provided by the only 1 nm thick graphene layers.[5] Moreover, the high magnetization (158 emu/g) of the pure metal cores, make them a suited choice when applications demand superior immanent magnetism to iron oxide nanoparticles (e.g magnetite). In addition to the pronounced ferromagnetism, the specific properties of the carbon surface allow either irreversible covalent grafting of functional compounds through C-C-bonds[6] or a reversible tagging via non-covalent π- π stacking interactions, e.g. for a thermally triggered catch/release-system.[7]

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Poster 176

Magnetic optical sensor particles for pH measurement

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The incorporation of magnetic nano-particles into dye-doped polymer beads yields in magnetically controllable sensors. Such magnetic optical sensor particles (MOSePs) can be directed by an external magnetic field to any region of interest inside a reaction vessel. Magnetic collection in front of fibre optics enhances the signal intensity, yielding in a lower particle concentration needed for measurement as well as lower background signals. Furthermore, they are characterised by high flexibility and ease of operation as they can be applied as dispersion.

Here, we report on MOSePs consisting of a magnetic core and a hydrogel-shell with an incorporated pH-sensitive dye. The core is generated by a nano-precipitation method which produces particles with diameters from 50 to 250 nm. The carboxyl groups of the polymer matrix, a polystyrene-maleic acid anhydride co-polymer, ensure highly stable aqueous dispersions.

A subsequent radical chain polymerization using the core particles as seeds resulted in a 30 nm shell, consisting of acrylamide crosslinked with *N,N'*-methylenebis(acrylamide) (BIS). For pH measurements we co-polymerized *N*-fluorescein-acrylamide (FLAC) and an acryloyl-piperazinyl sulforhodamine B isomer as reference dye for ratiometric measurements. Figure 1a shows a zero-loss TEM image of the particles, with magnetite as dark spots. As the particles were dried before measurement, the hydrogel-shell is invisible. The dynamic range of those particles is in between pH 6 and 8, with a pK_a value of 7. Their cross-sensitivity to ionic strength is very low (Figure 1b). The response time regarding a change of pH within the dynamic range is in average 3.2 seconds. The reversible response of MOSePs to a changing pH is shown in figure 1c.

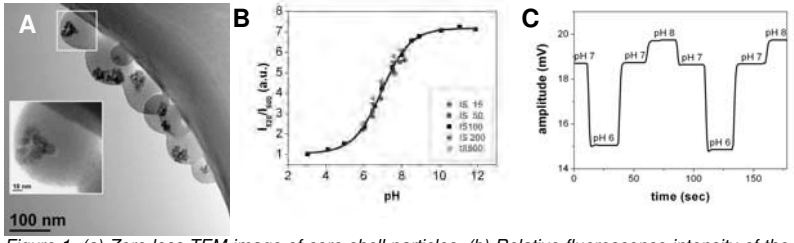

Figure 1. (a) Zero-loss TEM image of core-shell particles. (b) Relative fluorescence intensity of the particles in dependency of the pH and ionic strength (IS). (c) Signal reversibility of the pH-sensitive MOSePs.

These nanoscaled pH sensor particles may be adequate for medical applications, as their dynamic range covers physiological conditions. They are capable for applications in imaging techniques including microfluidics. Furthermore, they can be directed to a desired spot of interest by an external magnetic field. Also, due to the ratiometric approach, the obtained fluorescence signal is independent on the particle concentration.

Poster 177

Towards stem cell sorting by differential surface marker expression

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Cell separation and fractionation based on fluorescent and magnetic labeling procedures are common tools in contemporary research. These techniques rely on binding of fluorophores or magnetic particles conjugated antibodies to target cells. Cell surface marker expression levels within cell populations vary with differences in proliferation. In an earlier work we showed the reproducible separation of binary Jurkat cell populations based on their characteristic surface marker expression into unlabeled and immuno-magnetically labeled fractions, while the latter was further fractionated based on the level of surface marker expression.

Here we present a study on a stem and progenitor cells (SPC) cell model, the acute myelogenous leukemia cell line KG-1a. The cells were immuno-magnetically labeled for the CD34 surface marker in a two-step protocol and fractionated by continuous flow dipole magnetophoresis based on antibodies bound per cell as quantified by QuantiBRITE™ PE beads. By modifying the successive increments to transport lamina thickness, corresponding to successive outlet flow streams, it was possible to influence the distribution of the fractionated sample between the outlet streams. This was verified by flow cytometry and cell tracking velocimetry (CTV) measurements. Furthermore, the distributions showed good agreement with numerical simulations of the fractionation.

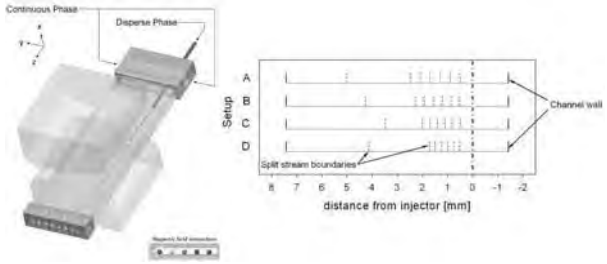

Figure. Sequential fractionation of an immunomagnetically labeled stem and progenitor cell model was achieved by modifying the successive increments to transport lamina thickness, which requires no solid physical boundaries and allows the fraction collection from different regions of the channel breadth.

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Comparison of Mitoxantrone and Nanoparticle Distribution after Magnetic Drug Targeting in an *ex vivo* Bovine Artery Model

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Introduction
A main problem during cancer treatment with chemotherapeutic agents represents the frequent disproportion between tumor response and unwanted side effects. With Magnetic Drug Targeting (MDT) chemotherapeutic agents like Mitoxantrone (MTO) bound to magnetic nanoparticles (MNP) can be directed to desired body compartments using an external magnetic field. This system makes a clear increase of anticancer drug concentration in specific organ regions compared to regular chemotherapy.

Aim
The aim of this study was the measurement and comparison of Mitoxantrone and Nanoparticle distribution after MDT.

Materials and Methods
In an experimental artery model, isolated bovine arteries were rinsed thoroughly with albumin substituted buffer in direct proximity to an external magnetic field (Magnetic field strength: 1,08 Tesla [T]; magnetic field gradient: 72 T/m). During the influence of the external magnetic field, the nanoparticles were injected into the flush medium and afterwards the arteries were dissected in 11 equal sections and examined with HPLC and magnetorelaxometry (SQUID).
MTO extraction: Each segment of 1 cm length was treated 4 times for 1 hour with ultrasound in the extraction mixture (5ml water, 500µl ascorbic acid (20%) in citrate buffer (pH 3.0) 2ml methanol, 2ml formic acid, 1ml 20% trichloroacetic acid and 4ml chloroform) followed by centrifugation for 10 minutes at 8000 x g. The combined supernatants were concentrated using a Bond Elut Plexa cartridge after conditioning with methanol and 2% formic acid in water. The analyte was eluted with 3ml 2% formic acid in methanol and dried under airstream. The water resolved residue was measured by HPLC.
HPLC: Separation was carried out using a 3.0 x 100 mm X-Bridge™ Phenyl column (Waters®, Germany) with a particle diameter of 3.5 µm; the guard column consisted of the same material and was 3.0 x 20 mm of size. The column temperature was 55°C, the mobile phase was made up of buffer (80 nM sodium formate and formic acid, pH 3.0) and methanol (80:20 v/v). The flow rate was 1 ml/min and the injection volume 50µl; the eluate was monitored at 254 nm.
SQUID: The amount of MNP was quantified by magnetorelaxometry. At this, highly sensitive superconductand quantuminterferometer (SQUID) serve as sensor to detect the signal of relaxation on magnetization.

Results
HPLC and Magnetorelaxometry showed corresponding results. The maximum amount of MTO as well as MNP is detected under the poleshoe. Fig.1

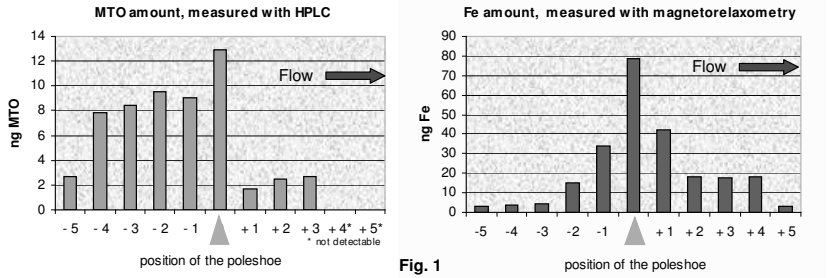

Fig. 1

Conclusion
This pilot study shows clearly that with MDT a high concentration of Mitoxantrone and Nanoparticles can be achieved under a focused external magnetic field. With this experimental set up important physical parameters of MDT can be investigated

Acknowledgements
The authors thank the Else Kröner-Fresenius-Foundation, Bad Homburg v. d. H., Germany and the DFG (AL552/3-1) for their support

Poster 179

Optical relaxation measurements of novel hybrid nanoparticles for homogeneous biosensing

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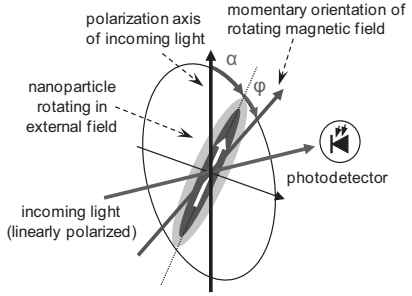
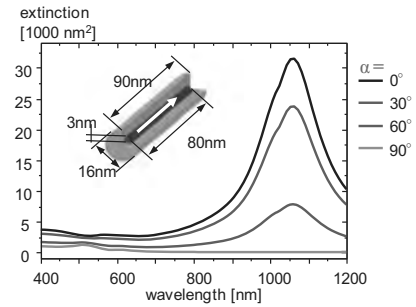
The term Point-of-Care (PoC) diagnostics generally describes biomedical testing outside of clinical laboratories. Possible applications include rapid testing in clinics, at doctors' surgeries or even directly at the patient's home. All these scenarios require automated and robust analysis systems, and specific and sensitive results have to be displayed in a comprehensive format within a short time.
We present a new approach for real-time molecular detection that can be directly carried out in serum or even whole blood, thus promising to circumvent the need of sample preparation altogether. It relies on highly sensitive plasmon-optical detection of the relaxation time of magnetic nanoparticles immersed in the sample solution, which increases when target molecules bind to their surfaces due to their larger hydrodynamic radius. Contrary to classical magnetorelaxation techniques that pick up the decaying total magnetic moment of the nanoparticle ensemble after turning off an aligning magnetic field, in our approach excitation and detection of nanoparticle orientation are independent. Thus, we can employ constant-amplitude rotating magnetic fields for alignment control, and the increased hydrodynamic diameter of analyte-carrying nanoparticles translates into an easily measurable phase shift that is picked up sensitively by lock-in techniques.
Our method requires complex multi-component nanoparticles that combine both magnetically and optically anisotropic properties. A suitable nanoparticle type consists, for example, of an elongated core-shell structure with magnetic core and noble metal shell functionalized by specific antibodies against the target molecules. Thereby, the magnetic core enables control of the nanoparticle alignment by external magnetic fields, while the anisotropic plasmon resonances within the noble metal shell allow optical detection of the momentary nanoparticle orientation in linearly polarized light (see sketch in figure below).
We present model calculations of both the magnetic and optical properties of suitable hybrid nanoparticles along with estimates concerning their relaxation behavior and sensitivity to molecular detection. These ab initio calculations are related to first experimental results demonstrating the evolution of the angle phase lag of plain Co-nanorods relative to the rotating magnetic field under varying conditions.



Nanorod follows magnetic field by a certain phase lag, which depends on its hydrodynamic radius and increases with molecular binding.



The orientation of the rotating nanorods is measured optically by monitoring the periodic change of its longitudinal plasmon excitation in polarized light.

Poster 180

Surface Modification of Gold/Iron-oxide Composite Nanoparticles by Phosphorylcholine Groups

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Composite nanoparticles consisting of gold and magnetic iron oxide are expected as a new type of magnetic nanocarrier for biomedical applications by using gold part as a general tag for thiolated functional biomolecules. For the applications in the presence of contaminating proteins, suppression of nonspecific adsorption onto the iron oxide part is indispensable. For this purpose, the surface composite nanoparticles were modified with phosphorylcholine group, which is biocompatible and widely used to prevent protein adsorption. Protein purification systems using these composite nanoparticles were also demonstrated. The composite nanoparticles were synthesized in aqueous solution systems by radiochemical process[1]. Surface modification by the phosphorylcholine groups were performed by silane coupling reaction onto iron oxide part[2]. Figure 1 shows the TEM micrograph of the surface modified composite nanoparticles. Small gold particles of 3-5 nm were kept immobilized on the surface of iron oxide particles of 20-30 nm even after the silane coupling reaction. Characterization of the composite nanoparticles by the techniques of FTIR, ICP and zeta potential measurement showed the existence of phosphorylcholine group. Adsorption of thiol functionalized polyethylene glycol showed that Au-S bonding activity is maintained in the surface modified composite nanoparticles. The protein adsorption experiment indicated that nonspecific adsorption of contaminating proteins were significantly suppressed by the phosphorylcholine group modification. The composite nanoparticles modified with phosphorylcholine group are very promising for protein related applications.

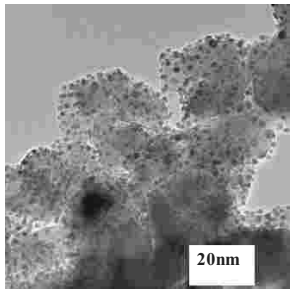

Fig.1 TEM of the Au/Fe_3O_4 NPs modified with phosphorylcholine group

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Poster 181

SYNTHESIS AND APPLICATION OF MANGANESE DIOXIDE COATED MAGNETITE FOR REMOVAL OF As(III) FROM CONTAMINATED WATERS

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The increasing demand for water supply for human consumption and industrial development, combined with more stringent environmental legislation have stimulated the development of new materials and methods for the treatment of contaminated aqueous solutions. Our group has been working at the production of high performance adsorbent materials. However, from the point of view of industrial scale applications, one challenge deserves attention: the improvement of the solid-liquid separation of the loaded material (with the contaminant) from clarified solutions or, ideally, from pulps. This challenge can be approached with the introduction of magnetic properties in the adsorbent material. It is important to stress that magnetic separations are rapid, easy to be applied in a large scale, easily automated and, therefore, have a great potential for a wide range of applications in different areas such as mineral processing, water treatment and biomedicine. A magnetic composite have been developed by precipitating manganese dioxide in presence of magnetite, a magnetic iron oxide (Figure), resulting in manganese dioxide coated magnetite, which was utilized to remove As(III) from contaminated aqueous solutions. Results obtained at the preparation of the magnetic composite show that the composite is chemically stable. Characterization revealed a surface area about 60 m^2/g. The sorption isotherm data led to a maximum loading of 50 mg_{As}/g_{solid} at pH 7.0. The magnetic property of magnetite, which is attached to the active MnO_2, allows an easy removal of the particles from the solution.

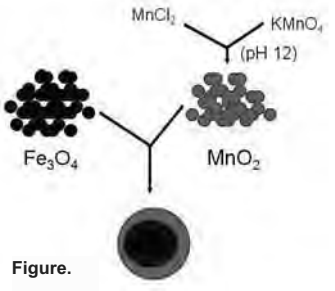

Figure.

Poster 182

Fabrication and Characterization of Morphologically and Magnetically Uniform Janus Microspheres

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The quality and reproducibility of biomedical techniques relying on magnetic beads depend not only on bead monodispersity, but also on their uniformity in magnetic composition and morphology. Numerous commercial magnetic beads were characterized previously and the sphere-to-sphere magnetic responsiveness was found to vary from 30% to 80%.[1] We demonstrate a scalable technique of thermally evaporating Nickel onto monodisperse polystyrene microspheres to produce magnetic beads that are more morphologically and magnetically uniform than those previously reported. Furthermore, the magnetic moment of the particles can be adjusted by varying the thickness of the magnetic deposition layer, magnetization parameters, and deposition material.

The magnetic properties and uniformity of these particles were characterized with microscopy (Figure 1), DC SQUID, and by measuring the particle's asynchronous frequency response in a rotating magnetic field.[2] The coefficient of variation (CV) in particle size and morphology after deposition was determined to be approximately 1%, which is comparable to the reported 1% CV in the polystyrene spheres used. This indicates that the size variability observed did not result from the deposition process. In addition, varying the nickel thickness alters the magnetic moment of the particle; 10 µm particles coated with 300 nm were found to have a magnetic moment of $3x10^{-12}$ Am^2 whereas 10 µm particle coated with 5 nm possessed a magnetic moment of $6x10^{-15}$ Am^2. Furthermore, the magnetic properties among the fabricated magnetic Janus beads were measured to have an 11% CV, compared to the 31% CV for commercial particles. The evaporation method was validated to produce magnetic beads with high magnetic and morphologic uniformity, allowing for more accurate and precise quantitative analysis techniques in biomedical applications.

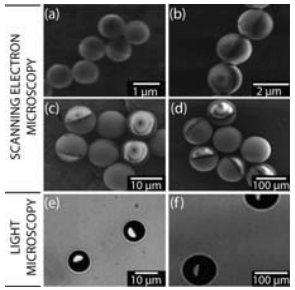

Figure 1: Janus magnetic particle characterization: SEM images of (a) 1 µm, (b) 2 µm, (c) 10 µm and (d) 100 µm particles and light microscopy images of (e) 10 µm and (f) 100 µm particles.

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Stable suspensions of Fe-filled carbon nanotubes

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Biocompatible Fe-filled carbon nanotubes (Fe-CNTs) with tailored functionalities are foreseen as a promising tool for investigation of biological materials (cells, tissue, human body). This type of application requires to achieve stable suspensions of Fe-CNTs preventing clustering and sedimentation due to their ferromagnetic filling. In this study, Fe-filled multiwalled carbon nanotubes (Fe-MWNTs) are functionalized via the non covalent approach using various biocompatible surfactants (CMC, Polylysine (Lys:Phe, 1:1) and PL-PEG-NH_2). A comparison of these different surfactants is shown and the stability of Fe-MWNTs suspensions over a significant period of time is demonstrated.

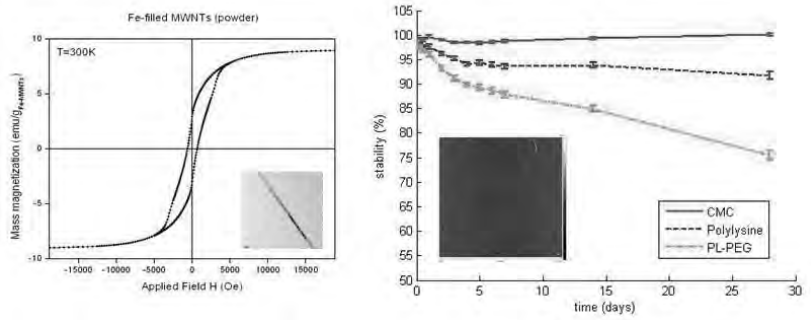

Figure 1: AFM image of Fe-MWNTs functionalized with CMC (right). Stability of Fe-MWNTs suspensions at 50 µg/mL (left)

In the second part of our work, we show ferromagnetic properties of Fe-MWNTs suspensions. Inherent high aspect ratio of Fe-MWNTs leads to a highly non linear anisotropic and hysteretic magnetisation curve. Individual Fe-MWNTs having a preferred direction along their axis. These unique properties of Fe-MWNTs open a perspective of entirely new detection techniques in biomedical applications. In particular the magnetic moment with its preferred magnetic orientation is advantageous for the development of economical detection techniques, which could be combined with medical procedures as minimal-invasive interventions.

Biocompatible water based magnetic nanofluids: colloidal stability investigations by light scattering methods

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Most of biomedical applications of magnetic fluids [1,2] require magnetic nanoparticles to be non-toxic, chemically stable, uniform in size and well-dispersed in aqueous media to ensure the colloidal stability of magnetic fluids under physiological conditions [3,4]. Significant particle aggregation must be excluded in applications where the risk of blood clots in blood vessel exists [1, 2]. For other applications, such as high gradient magnetic separation in biotechnology, magnetic nanoparticle clusters of controlled size and shape [5] with high magnetic moment in applied magnetic field are of particular interest.

Nanosized magnetite particles, with mean physical diameter of about 7 nm, obtained by chemical coprecipitation procedure were dispersed in water carrier by applying sterical stabilization of particles in order to prevent their aggregation and to ensure colloidal stability of the systems [3, 4]. Different chain length (C12-C18) carboxylic acids (lauric (LA), myristic (MA), palmitic (PA), stearic (SA) and oleic (OA)) were used for double layer coating of magnetite nanoparticles. Structural and magnetic properties of the magnetic fluids were investigated by electron microscopy (TEM), dynamical and static light scattering (DLS, SLS) and magnetometry (VSM) to evaluate the role of chain length and of the saturated/unsaturated nature of surfactant layers.

Results of comparative analyses concerning the efficiency of surfactant covering of particles to prevent agglomeration processes [6, 7] or to tailor the size of agglomerates, as well as magnetic, solid and hydrodynamic size distributions of particle clusters, are discussed with respect to the influence of the surfactant chain length and dispersed particle volume fraction. Depending on surfactant and particle concentration, the particle cluster hydrodynamic diameter was found to range from 30 nm to 130 nm (see Fig.1 below).

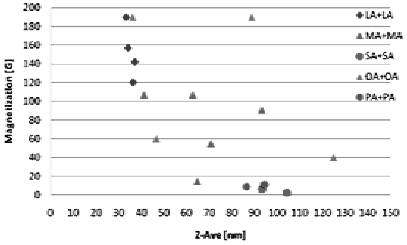

Fig.1 Particle cluster hydrodynamic diameter (Z-Ave) vs. sample saturation magnetization (i.e. concentration) for five different double layer coatings of particles.

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Estimation of the Oblongness of Aggregates of Magnetic Particles Formed in Static Magnetic Field Using ESR spectroscopy

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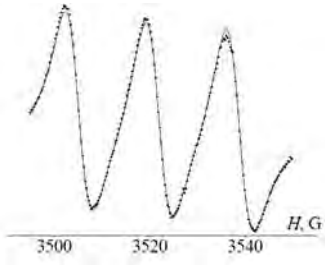
Magnetic particles in static field tend to order along the field lines and form linear structures like chains and needles. There is the problem of how to define the length or oblongness of the formed aggregates. The abilities of the optical methods are quite limited because of the non-transparency of the objects under study (ferrofluids and ferrofilms). In this case the magnetic resonance methods (EMR) like ferromagnetic resonance (FMR) and electron spin resonance (ESR) are proved to be more informative. FMR spectroscopy is based on the observation and analysis of the spectra of the magnetic particles. Changes of the particles ordering lead to the FMR spectrum transformation due to the local magnetic field that one particle produce on the adjacent once. To use the ESR spectroscopy to study magnetic liquids the low molecular paramagnets, so called paramagnetic sensors (PS), are introduced into the system. These paramagnets are sensitive to the conformation of the magnetic fields from magnetic particles and their spectra as well as FMR transform when the particles assemble in chains or needles. Despite the fact that FMR spectroscopy is based on the direct observation of the spectra of the magnetic particles its utilization is limited by some individual cases because of the absence of the precise theory of the FMR line shape. Meanwhile the theory of resonance line shape for paramagnets is well developed. In our work we have introduced stable nitroxide radical TEMPOL as PS into magnetite hydrosol and look after the radical spectra. The PS spectrum lines have shifted broadened and changed their shape. To analyze PS line shape a new model has been suggested where the long needles consisted of magnetic particles have been assigned as the sources of the local magnetic fields. The main aspects of the theoretical description are presented in the work [1]. In order to calculate the experimental line shape the free induction decay (FID) has been calculated. Fourier transformation of the FID results in the ESR spectrum. The final relation for FID is constructed in terms of two linewidths – monopole D_m and dipole D_A. The relationship between these characteristic parameters provides information on the oblongness of the linear aggregates (N_{obl}): $\varphi N_{obl}^2 = \frac{81\sqrt{3}}{50\pi^2}\left(\frac{D_A}{D_m}\right)^3$, where $N_{obl}^2 = \frac{n_l^3}{N_n}$.

Here φ is the volume fraction of the particles, $n_l = L/(2R)$, L is length of the aggregate, R is the average particles radius, and N_n is the total number of particles in aggregate. In case when aggregate consists of one chain the $N_{obl} = n_l$ and the length of chain can be estimated.



The results of the experimental approbation of the approach are shown in the figure where points represent the experimental spectrum and the line is the theoretical fitting. Theoretical spectrum demonstrates a good agreement with the experimental one with the main parameters: D_m = 4.6(1) G, D_A = 3.88(66) G, N_{obl} = 6.0(3).

We may conclude that the suggested model and developed in its framework approach expands the abilities of electron magnetic resonance to study the system containing magnetic particles and their prolate aggregates.

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Fluorescent magnetic nanoparticles for the delivery of biomolecules

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Magnetic nanoparticles can be used to deliver biomolecules to living cells. Under the influence of a magnetic field, the contact between the nanoparticles and the target cells is enforced. This method is especially useful to deliver biomolecules to cells that are difficult to transfect by other means. In this study we report on the synthesis of fluorescent core-shell fluorescent magnetic particles that allow tracing the particles and monitoring the transfection procedure or delivery of other biomolecules in vivo.
The nanoparticles consist of a magnetite/maghemite core prepared by wet chemical precipitation. They are functionalized with carboxymethyl-dextran (CMD) to which rhodamine B isothiocyanate (RBITC) was attached. The particles were further coated with a poly (vinyl alcohol) (PVA) shell, which mediates uptake into the cells without the use of transfection agents (Figure 2).

Key Words: Magnetic particle, Polymeric shell, Fluorescence

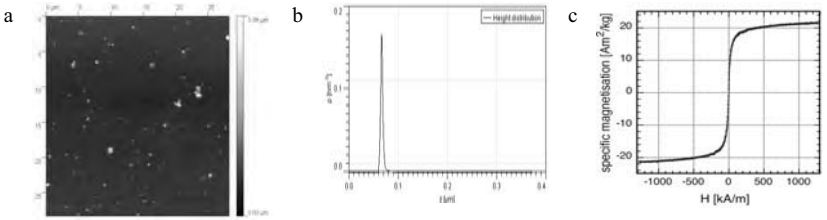

Figure 1. Physical characterization of the fluorescent nanoconjugates a) AFM images of the particles immobilized on mica and dried in air. b) Height distribution over the whole area shown in (a). c) VSM magnetisation loop of the fluorescent nanoconjugates after magnetic washing.

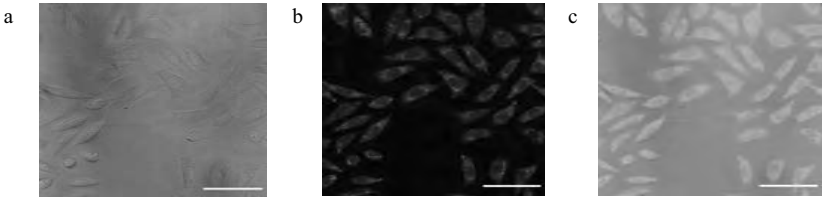

Figure 2. Particle uptake. Confocal Laser Scanning Microscopy image of CHO cells incubated with fluorescent nanoconjugates. a) Transmission image b) RBITC fluorescence c) Overlay of transmission image and RBITC fluorescence (Scale bar 50 µm).

Poster 187

Directed assembly of magnetic nanoparticles for biomedical applications

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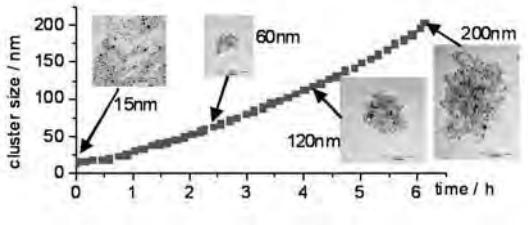
Our research focus in recent years has been on the development of methods to control the size and properties of stable suspensions of magnetic nanoparticles and nanoparticle assemblies. For instance we have introduced a novel bottom-up approach 'competitive stabiliser desorption' to activate nanoparticles towards self-assembly in a controlled manner. [1] This method provides temporal control over the size of the resulting assembly. We are currently studying methods to control composition, primary nanoparticle size and inter-particle interactions within these magnetic colloids, as these factors determine the emergent magnetic and magnetic resonance properties. To this end we have applied field-cycling NMR relaxometry, [2-5] a technique which is sensitive the magnetic properties of the colloids, as probed by the ^{1}H nuclei of the surrounding solvent molecules. The work presented describes these experiments and some recent research where magnetic nanoparticle assemblies were stabilised by surface active agents including lipids and polyelectrolytes. [5-6] Interest in these materials arises due to their potential biomedical applications in MRI and as drug delivery vehicles, for which control over size and magnetic properties is critical.



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Poster 188

Field-controlled deformations and volume changes of magnetopolymeric microcapsules

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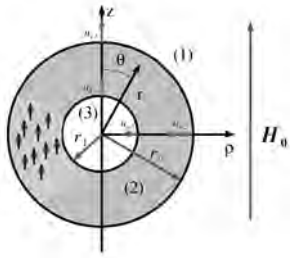
Magnetopolymeric hollow (MH) microspheres like magnetopolymerosomes described in [1], have some unique qualities lacking in their completely solid (microbeads) or completely liquid (vesicles) analogues. Due to the elasticity of its walls, such a capsule preserves its equilibrium shape in the absence of the magnetic field and changes it from a sphere to a prolate ellipsoid being subjected to a field. Moreover, being hollow, such an object deforms much easier than a solid sphere made of the same magnetic polymer or ferrogel [2]. These remotely controlled variations of the cross-section could be used for dragging MH capsules through narrow channels (under field) and then blocking the channels with such stopples just by switching off the field. Another specific property of an MH container is its ability for field-induced changes of the inner cavity volume. Provided there is a hole or pore in the magnetopolymeric shell, a capsule, in response to an applied field, could let in the fluid from its environment or let out a part of its content. Upon removing of the field, the action is reversed.



In [3,4] we have set out a study of shape and volume changes of a MH sphere in the framework of the magnetoelasticity theory. The model scheme is shown in Fig.1: a hollow sphere made of a magnetic polymer or ferrogel (2) is embedded in a fluid (1) and contains another fluid (3) inside. The sphere wall contains embedded magnetic nanoparticles and thus is capable of magnetic deformations. The applied field is assumed to be uniform. (A non-uniform case could be accounted for as well thus providing not only the capsule deformations but the net force to drag it.)

In the present work the finite-element modeling of magnetic deformations of a MH capsule is refined by taking into account magnetic striction of the material ignored in [3,4]. The point is that the magnetostriction effect, in fact, quite strongly affects the magnetomechanics of ferrogel objects. It turns out [5] that, depending on the presence of particle chains, albeit quite short, the magnetodeformational ability of a MH sphere enhances / reduces several times in comparison with the non-magnetostictive model [3,4], this refers both to the overall elongation u_{zz} of the object and to the volume changes of its inner cavity.

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Metabolites signal changes induced by magnetosomes during MR experiment

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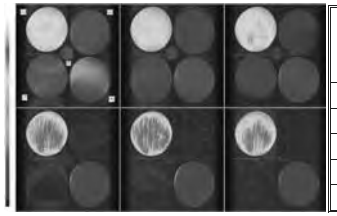
Magnetic resonance results in clinical practice can be influenced by a lot of direct and indirect factors. Especially in Magnetic Resonance Spectroscopy Imaging (MRSI), the final metabolite concentration map is affected by wide range of parameters – diet, physical and condition, age, drugs, environment etc[1]. This can lead to incorrect clinical conclusions. Moreover, magnetite nanoparticles produced by human body or used as contrast agents in MRI[2], can also alter metabolite signal in MR experiment. However, this can bring much more inaccuracy to final results. Therefore it is necessary to clarify this issue. We performed preliminary in vitro MRI/MRS experiments of magnetosomes in various solutions – saline, glucose, myo-inositol and choline. Concentrations were chosen to model real physiological conditions. Magnetosomes were prepared from magnetotactic bacteria strain *Magnetotacticum Magnetospirillum* with mean size 34 nm for one magnetosomes[3]. Magnetosomes are bacterial magnetic nanoparticles enclosed natural organic membrane and they are generating chains inside bacterial body. To observe signal changes in magnetosome solutions with molecules of interests, we measured T_2 relaxation times. Images were acquired using "Spin echo" sequence with repetition time TR = 1000 ms and different echo times (TE = 18 – 150 ms). In glucose and myo-inositol solutions, the relaxation properties of protons showed similar decrease of signal as in water solution. On the other hand the 6 times increase of signal was observed in choline solution compared to water. Huge T_2 increase (75 times) was found in solutions with NaCl molecules (Figure 1). Moreover, the signal intensity was almost constant for all magnetite particles concentrations, up to TE ≈ 100 ms. To clarify this anomaly, series of experiments with magnetite nanoparticles in saline solutions with different concentrations were performed. Surprisingly our results depend on whether the samples were prepared from distilled water or 'normal' (tap) water. Result confirmed our previous findings. The reason is unclear at present and calls for the next study. However, these findings can be important in clinical in-vivo applications, especially in hypointensive artefacts mapping and metabolite MRSI quantitation.



SAMPLE (c_{mag}=17.1µg/ml, c_{MOI}=10g/l)	T_2 [ms]	s.d. [ms]
0 (water)	5.90	0.257
1 (NaCl)	449.38	28.329
2 (glucose)	7.28	0.231
3 (myo-inositol)	9.76	0.235
4 (choline)	34.22	1.855

Figure 1 : T_2 relaxation times for each magnetosome solution in water (0) , NaCl (1), glucose (2), myo-inositol (3) and choline (4) solution. To emphasize the visual effect of relaxation mechanism we used solutions with molecules concentration 10g/l. The magnetosome concentration was17.1 µg/ml.

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Formulation development and *In-vitro* characterization of chitosan magnetic nanoparticles containing artesunate for targeted delivery to breast cancer

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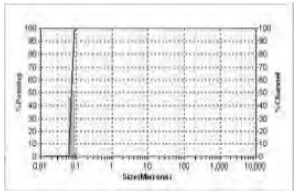
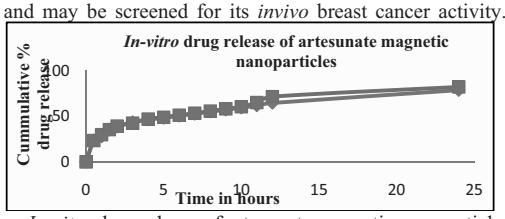
Artesunate is a semi-synthetic derivative of artemisinin, the active principle extracted from the herbal plant Artemisia annuna, which is traditionally used for the treatment of malaria in china. It has been very recently reported that, artesunate having proficient anticancer activities with very low toxicity to normal healthy cells. Artesunate has been analysed for its anticancer activity against 55 cell lines in the Developmental Therapeutics Program of the National Cancer institute, USA and reported that it have assured activity. It was also reported that artesunate having inhibitory effects on angiogenesis and on expressions of vascular endothelial growth factor. The drawbacks of conventional chemotherapeutic agents are the lack of site specificity and high toxicity to the healthy cells. In the present study, the artesunate magnetic nanoparticle was successfully formulated for the targeted delivery of drug to breast cancer cells guided by the externally applied magnetic field. The different forms of chitosan was used for the formulation and evalauted for its effect on drug release and surface morphology. The magnetite was prepared by using ferrous chloride tetrahydrate and ammonia solution by simple precipitation method. The chitosan magnetic nanoparticle was formulated by gelation method with the help of water soluble and acetic acid soluble chitosan and sodium tripoly phosphate. Six formulations were prepared by varying the chitosan concentration of water soluble and acetic acid soluble chitosan. Drug was encapsulated on the blank magnetic nanoparticles through spontaneous emulsification and solvent evaporation method. The prepared magnetite and artesunate magnetic nanoparticle was characterized for its particle size and particle size distribution using particle size analyser. The particle sizes are varying in the range of 73-110 nm. The zeta potential of the formulation was evaluated using zetasizer. The surface morphology of formulated artesunate magnetic nanoparticles was examined under Scanning Electron Microscopy (SEM). The encapsulation efficiency and loading capacity of artesunate magnetic nanoparticles was found to be 76% to 88% and 27% to 31% respectively. The magnetic susceptability of the magnetite and formulation were evaluated using Fugro magnetic susceptibility meter. Magnetic susceptibility of magnetite was found to be 90×10^{-5}). The *in-vitro* drug release from the magnetic nanoparticle in phosphate buffer solution pH 7.4 was studied and found to be 90 % in 48 hours.

Hence we conclude that the developed chitosan magnetic nanoparticles of artesunate shown better release characteristics and may be screened for its *invivo* breast cancer activity.



Particle size of magnetite



In-vitro drug release of artesunate magnetic nanoparticle

Poster 191

Hard Magnetic Barcode nanowires for biosensing applications

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Barcode nanowires with several segments can generate intense research interest in recent times as they can facilitate a novel platform for multiplexed bio analysis. Multi-functional digital hard magnetic barcode nanowires with a large number of readily distinguishable segments have been investigated for multiplexed bio analysis. In this work, we have employed a template-assisted electrochemical synthesis in fabricating multi-segmented barcode nanowires by three-electrode configuration potentiostatic method. Different magnetic (CoNiP) and non-magnetic (Au) barcode segments in a given single nanowire are presented with various barcode combinations. In order to develop multiplexed diagnostic system using digital hard magnetic barcode nanowires, decoding technique is necessary. In this time, we are considering decoding by TMR (tunneling magnetoresistance) or GMR (giant magnetoresistance) sensors under incorporation with flow cytometry technology. Furthermore, a facile route has been demonstrated to selective immobilization of proteins on the Au segments. Finally, the study proposed a novel scheme for decoding the magnetic barcodes using magnetoresistive sensors.

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Poster 192

Biocompatibility of bacterial magnetic particles in mice

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The use of particle reduction in size from micro to nanoscale not only provides benefits to diverse scientific fields but also poses potential risks to human and the environment. For the successful application of nanomaterials in bioscience, it is essential to understand the biological fate and potential toxicity of nanoparticles. As BMPs are nano-scale, the aim of this study was to evaluate the biological distribution as well as the potential toxicity of BMPs to enable their diverse applications in life science such as drug development, protein detection, and gene delivery.

Biocompatibility, biodistribution, biodegradation, inflammation and interference with cells and normal functioning of organs, among other factors, will determine the toxicity of BMPs, and therefore the extent of their use. In this study, the BMPs-treated mice were injected with 48.2 mg/kg BMPs and the mice were separated to 6 groups for sacrificed and detected digestive organs at 2, 6, 10, 14, 22, 30 days respectively.

The appearance of all of the mice was normal and the body weight of the mice increased in the course of nature after injecting BMPs in each group. The organ coefficients of heart, liver and kidney were in the normal arrange in each group, but the organ coefficients of spleen and lung were higher and beyond the normal arrange 2, 6 and 10 d after injecting BMPs, which indicating that BMPs might accumulate in lung and spleen largely so that the immunoresponse and inflammation were stimulated leading to edemas of these organs. The blood routine examination results of all of the group were in the normal arrange which consisted with previous results indicating that serious anaemia and inflammation were not stimulated by BMPs. TEM examination of ultrathin sections from heart, kidney, spleen, lung and liver indicated that the BMPs existed in lung, spleen and liver cells. Abundance of endocytosis vesicles were present in the cells containing BMPs and the BMPs-contained endocytosis vesicles in lung, spleen and liver were merged with lysosomes, suggesting that the BMPs may be ingested into lung, spleen and liver cell via endocytosis and then in face of decomposition by lysosomes. Histological examination of the heart, liver, spleen, lung and kidney indicated serious inflammation was not stimulated by BMPs but monocytosis had been present in liver, spleen, lung and kidney at incipient stage and diminished 22 d after injection. The immunogenicities of BMPs was 250, 500, 4000, 6000, 12000 and 12000 on day 2, 6, 10, 14, 22 and 30 d after injection respectively. As BMPs can stimulate relatively drastic immunoreaction, it might be necessary to modify the surface of BMPs before clinical application.

BMPs mainly accumulated in some organs (lungs, spleen and liver) but have no acute toxicity. BMPs can also stimulate animals' immunoresponse so that they could be used for immunoassay in vitro and should be prudently modified before injecting.

Key words: bacterial magnetic particles; biocompatibility; biotoxicity; immune

Acknowledgements

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Poster 193

Utilization of AC and DC Magnetic Fields for Focused Magnetic Fluid Hyperthermia and Magnetic Particle Fractionation

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Introduction: In this study, AC and DC Magnetic fields were used effectively to implement a focused magnetic fluid hyperthermia(MFH) system and a microfluidic magnetic nanoparticle fractionation system. Focused Hyperthermia system was tested by both in vitro and in vivo experiments and it was shown that focused heating of the tissues can be achieved without damaging the healthy tissues. By using magnetic field gradients similar to the ones implemented in the hyperthermia system, we showed that size separation of magnetic nanoparticles can be achieved.

Focused Hyperthermia System:

Background: In a typical MFH treatment, firstly, magnetic fluids are dispersed into target tissue and secondly, by the application of AC magnetic fields, heating of the fluids and correspondingly damaging of the tumors is achieved [1]. But, as a result of the magnetic fluid diffusion from tumor to surrounding tissues or incorrect localization of the fluids in the target tumor area, unwanted heating of the healthy tissues can be seen [2]. To overcome the normal tissue damage drawback of MFH, a focused hyperthermia system should be generated, to heat very small regions without destroying the normal healthy tissue.

Methods: By depositing appropriate DC magnetic field gradients on the AC magnetic fields one can generate AC field dominant regions and achieve focused heating of the magnetic particles at these regions. Figure 1 shows a system that accomplishes this task. Here two solenoids on the right and left of the figure are excited with equal but opposite DC currents. The static field vectors generated by the solenoids cancel each other at the center of the system and a region with a very small DC magnetic field is formed around the center which can be named as the field free region (FFR). If an alternating magnetic field is applied to the space between the solenoids, alternating field will be dominant in the FFR and only the magnetic particles inside the FFR will be heated. The field free region explained above can be reduced further (i.e., more intense focus can be obtained) by increasing the current magnitudes flowing through the DC solenoids. In addition to that, the position of the focus can also be changed via giving different amplitudes of currents to the DC solenoids. By adding one more solenoid between the lateral DC solenoids of Figure 1, implementation of focused heating applicator can be completed as in Figure 2a. The solenoid in the middle (AC Solenoid) generates the alternating magnetic field responsible for the heating of the magnetic particles and other lateral solenoids (DC solenoids) generate a static magnetic field gradient such that a field free region is generated in the middle of them.

Experiments: To validate the focused heating ability of the hyperthermia system, several in vitro and in vivo experiments were made [3]. During the experiments three different DC magnetic field conditions were tested by applying static currents(IDC) of 0.5A, 1A and 1.8 A to both DC solenoids. In vitro experiment setup can be seen in Figure 2a. Inside the AC solenoid, three spherical plastic cups of diameter 0.4cm were placed, which were filled with a magnetic fluid (Liquids Research Ltd.). An AC magnetic field with strength of 4.5kA/m at 80 kHz was applied and corresponding temperature increases of the cups were recorded at different DC magnetic field conditions (Figure 3). In vivo experiments were done on the tails of 200g adult rats. Ferrofluids were injected percutaneously to the tails of the anesthetized rats. After that, rat tail was placed along the axis of the solenoids (Fig. 2b). An AC field (7.6kA/m) at 80 kHz was applied and the temperature was recorded at different DC field conditions.

Results: As shown in Figure 4, the temperature rise of the central cup doesn't change while the temperature rise of the lateral cups decreases as the amplitude of the DC solenoid current increases. This shows us that focusing is achieved successfully by increasing the DC solenoid currents. Also it can be seen that, by giving different amounts of currents to the DC solenoids, the position of the focus can be changed (rightmost plot in Fig.3). In in-vivo experiments, AC field exposure lasted nearly 25 minutes and temperatures above 46ºC were obtained for all three experiments. Histological examination showed that, as we increased the dc solenoid current from 0.5 to 1.8 A, the total burned tissue volume decreased from 1.6 to 0.2 cm^3, verifying the focusing capability of the system.

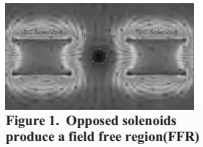

Figure 1. Opposed solenoids produce a field free region(FFR) in the middle of the solenoids.

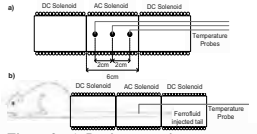

Figure 2. a) In vitro experiment setup, b) in vivo experiment setup.

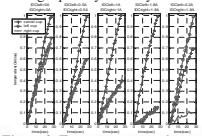

Figure 3. Temperature vs. time graphs of the in vitro experiments.

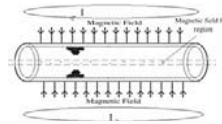

Figure 4. Basic Design, z-component of the magnetic field vectors cancelled along the channel to produce Field Free Region.

Magnetic Particle Fractionation System:

Theory: Basic design of the particle fractionation system can be seen in Figure 4. Equal but opposite DC Magnetic fields are applied from the sides of the fluidic channel and they cancel each other along the channel axis. Similar to the hyperthermia system, a region having approximately zero magnetic field(H_z) is produced in the middle of the channel (Field Free Region). As a result of this magnetic field gradient, bigger particles are attracted to the side walls, and the smaller particles reside in the middle of the channel. The pumping of the nanoparticles(ferrofluid) from inlet to outlet is achieved by applying additional sinusoidal currents to the coils where Icoil = Ioffset + |cos(2*pi*f*t)|. By this way, particles residing in the field free region will be pushed from inlet to outlet. **Simulation:** 2D axial simulation (made by Comsol Multiphysics 3.5) of the system can be seen in Figure 5. As a result of the axially symmetric geometry, two coils produce circular loops. Between these loops, a microfluidic channel with 200um width and 0.9mm length is placed. Channel inlet is placed at r=0; r-component of the magnetic field is positive for r.>0, and this produces a positive flow from inlet to outlet. Figure 6 shows the z-velocity profile along the width of the channel. This figure states that the smaller particles inside the FFR (around the channel axis) do not move in ±z direction. However, bigger particles have non zero z-velocities and they will be attracted to the channel walls. Figure7 shows the r velocity along the width of the channel, and this states that smaller particles(the particles inside the FFR) will have the highest r-velocity and they will reach the outlet first, while the bigger particles will be remaining attached to the channel walls. After first elution is taken at the outlet, the next step will be reducing the amount of the offset current (DC current). This will allow the comparably larger particles to disengage from the walls and reach the channel axis, and they will be collected at the outlet similarly by the help of sinusoidally changing magnetic field. Here, the gradient produced by the DC current determines the dispersity of the collected particles(this gradient was determining the intensity of the heating focus in the hyperthermia system). By applying higher magnetic field gradients (i.e., high DC current) elutions closer to the monodisperse distribution will be achieved. As a next step, microfluidic channel will be fabricated by soft lithography with PDMS.

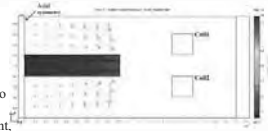

Figure 5. Velocity field magnitude profile of the ferrofluid. Arrows show the magnetic field vectors.

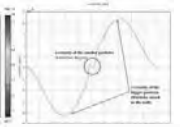

Figure 6. z-velocity, in the middle of the channel. (x axis: channel width)

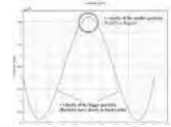

Figure 7. r-velocity, in the middle of the channel. (x axis: channel width)

Conclusion: In this work, by the effective usage of alternating and static magnetic field gradients, both a focused hyperthermia system and a magnetic particle fractionator system were designed. By using the magnetic particle fractionator system, narrow sized distributions can be collected and these particles can be effectively used along with the implemented focused hyperthermia system. We believe that by the help of both designs, Magnetic Fluid Hyperthermia will be a much more effective modality in the treatment cancer tissue. **References**: [1] 1993 *Int. J. Hyperthermia* 9 51–68, [2] J. Mater. Chem., 2004, 14, 2161-2175, [3] Med. Phys. Volume 36, Issue 5, pp. 1906-1912, 2009.

Poster 194

Synthesis and Characterization of Magnetic Nanoalloys from Bimetallic Carbonyl Clusters

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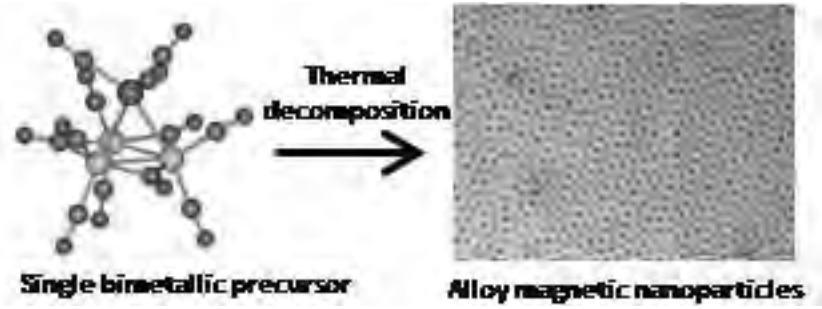
Magnetic nanoparticles are potentially useful for biomedical research because their unique chemical and physical properties. In particular, magnetic alloy nanoparticles are of interest due to their magnetic properties and chemical stability. However, controlling the composition of magnetic alloy nanoparticles can be difficult, when they are produced from two or more precursors. This could be overcome by using a single precursor of bimetallic carbonyl cluster in a thermal decomposition process. We have used this novel synthesis method to produce $FeCo_3$, $FeNi_4$, FePt and Fe_4Pt alloy magnetic nanoparticles, with average diameters of 7.0 nm, 4.4 nm, 2.6 nm and 3.2 nm. The chemical and physical properties of the synthesized nanoparticles were examined by elemental analysis, transmission electron microscopy, powder X-ray diffractometry and SQUID magnetometry. The chemical composition of the synthesized nanoparticles reflected that of the bimetallic carbonyl cluster used for their synthesis. Different reaction conditions, such as ligand concentration, ligand type and reaction temperature had very little effect upon the chemical and physical properties of the synthesized nanoparticles. This work represents a versatile method for the synthesis of magnetic alloy nanoparticles and can be applied to a variety of other elements.



Tracking transplanted neural progenitor cells in spinal cord slices by MRI using CoPt nanoparticles as a contrast agent

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Neural progenitor cells (NPCs) exhibit features that make them suitable candidates for spinal cord reconstruction and MRI offers the potential to track cells *in vivo* using innovative approaches to cell labeling and image acquisition. In this study experiments were carried out to optimize the loading condition of nanoparticles and to define an appropriate MRI imaging technique. A combination of cell-counting experiments, immunocytochemistry and time-lapse imaging analysis showed that CoPt hollow nanoparticles (CoPt NPs) at a concentration 16 μg/ml reduce T_2 relaxation times in labeled rat NPCs giving greater contrast on spin echo MRI acquisitions at 4.7 T, yet do not affect cell viability, *in vitro* differentiation potential and directed migration characteristics in electric fields compared to controls. After optimizing nanoparticle concentrations and labeled cell numbers, we transplanted CoPt-loaded NPCs into spinal cord slices and confirmed that MRI can efficiently detect low numbers of CoPt-labeled NPCs, with the enhanced image contrast depending on the numbers of labeled cells. Our studies demonstrate that MRI of grafted NPCs labeled with CoPt NPs is a useful method for evaluating cellular migration in organotypic spinal cord slices and further applying on *in vivo* cell tracking.

Preliminary investigations on labelling of the vaccine adjuvant $Al(OH)_3$ with Resovist® for magnetic resonance tracking

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Already in 1926 Glenny et al. reported the use of aluminium compounds as an adjuvant in vaccines. Since that time it is most commonly used as aluminium hydroxide ($Al(OH)_3$) [1]. Nevertheless, the mode of action could not be ascertained in detail. For a long time a depot effect was accepted as an explanation, but in recent years it became clear that the effect is not only caused by this [2]. A novel method to investigate the fate of $Al(OH)_3$ *in vivo* could be magnetic resonance imaging (MRI). As $Al(OH)_3$ is not directly visible in MRI labelling with superparamagnetic iron oxide particles might be feasible. Here we used ferucarbotran particles (Resovist®, Bayer Schering AG, Germany) for the generation of complexes with $Al(OH)_3$. Resovist® is a commercially available MRI contrast agent for imaging of liver lesions consisting of carboxydextran coated iron oxide nanoparticles as an injectable solution. Since it is a FDA-approved contrast agent, the potential for clinical trials is given.

For labelling a colloidal suspension of $Al(OH)_3$ (Alhydrogel®, Sigma-Aldrich, Germany) was merged with ferucarbotran particles in varying ratios of iron and aluminium. The formed complexes were characterized regarding their size by dynamic light scattering measurements and their zeta potential by electrophoretic light scattering measurements. To investigate the stability of the aggregation the complexes were centrifuged after one, three and five days of incubation in different media. The pellet and the supernatant were analyzed concerning iron using atomic absorption spectrometry. Furthermore, ferucarbotran particles were linked with the fluorescence dye Texas Red-albumin (Invitrogen, Germany) by the periodate method [3] in order to demonstrate the composition of complexes by confocal laser scanning microscopy and fluorescence microscopy. Enhancing the amount of $Al(OH)_3$ in the mixture resulted in an obvious augmentation of the mean particle size of the complexes. When adding excessive adjuvant Resovist® was completely bound. The adsorption was stable for at least five days. Because of the gel character of $Al(OH)_3$ complexes were difficult to see using microscopy after fixation, even by utilization of liquid phases without any further preparation.

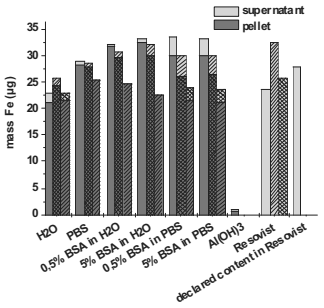

Figure 1. Mean iron amount (AAS) of Resovist®-$Al(OH)_3$ complexes centrifuged at day 1, day 3 and day 5 after aggregation in different media, n=5

The edges of the complexes were not clearly visible and they showed a diffuse distribution of the ferucarbotran particles.

The first investigations reveal that $Al(OH)_3$ can be labelled with Resovist® simply by mixing. The adsorption probably results from electrostatic interactions due to opposing zeta potential of both components. The results of stability investigation are encouraging. The identification of suitable MRI parameters is ongoing.

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Distribution pattern of chemotherapeutics: Distinctions of systemic application versus magnetic nanoparticle guided delivery *in vivo*

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Introduction: In order to increase the dose of antineoplastic agents in the tumor area, the concept of Magnetic Drug Targeting has been developed. Magnetic nanoparticles (Ferrofluids = FF) consisting of iron oxide serve as carriers for chemotherapeutics and could be enriched by an external magnetic field after intraarterial application. An important question to be answered is how chemotherapeutic agents (i.e. mitoxantrone = MTO) will be distributed in the organism if it is bound to ironoxide nanoparticles during application. A quantitative determination of the biodistribution of magnetic nanoparticles can be achieved with Magnetorelaxometry using SQUID (Superconducting Quantum Interference Devices). Compared to these results there is evidence, that MTO distribution is different and so we performed both, HPLC measurements and SQUID analysis for the same liver and kidney specimen where high MTO enrichment and nanoparticle accumulation are expected.

Materials and Methods: We focused on the different biodistribution of nanoparticle bound MTO plus respective FF enrichment and pure MTO after intravenous (i.v.) application. For the animal experiments New Zealand White Rabbits were used. The animals, weighing about 4.5 - 5.2 kg, were treated (i.v.) with ferrofluid-bound MTO containing a drug amount of 10% compared to the regular systemic dose which requires 10 mg MTO/m^2 body surface (n=5). The other study group (n=6) received the regular systemic dose of pure MTO. After 24h hours the animals were sacrificed and the organs harvested. Complete organs of liver and kidneys were processed and MTO was extracted for HPLC-analysis. Tissue homogenates have also been measured by SQUID.

Results and Discussion: MTO can be extracted and analyzed reliable from liver and kidneys (**Figure 1**). HPLC-measurements show a high enrichment of MTO in kidneys concerning both application modes (**Figure 2**).

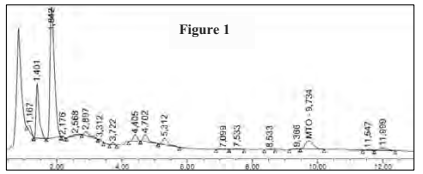

Figure 1: HPLC analysis of MTO. HPLC-chromatogram of processed liver tissue containing MTO bound to ferrofluids.

Figure 2: HPLC monitored MTO amounts in liver and kidneys. **a)** i.v. application of 2.4-2.9 mg pure MTO. HPLC analysis show high enrichment of MTO in kidneys. **b)** Application of ironoxide nanoparticle suspensions containing 0.26-0.28 mg MTO. An increased percentage of MTO relating to the overall dose is now detectable in the liver.

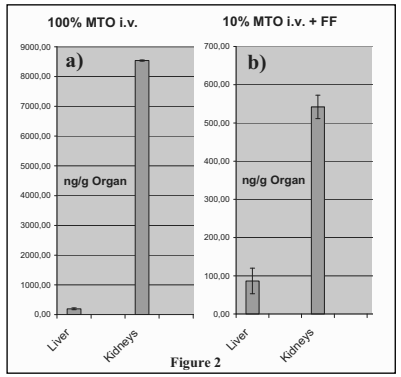

Figure 2

Nanoparticle mediated drug application dramatically influences the biodistribution of MTO after i.v. injection. Application of pure MTO leads to 190 ng/g in liver tissue and 8500 ng/g in kidneys. In contrast to that nanoparticle guided administration leads to a MTO concentration of 86 ng/g in liver and 540 ng/g in kidneys. With this application mode, MTO enrichment in kidneys is only 6-fold higher than in liver compared to a 44-fold increased accumulation after pure i.v. injection. The shift in the relation of MTO enrichment between liver and kidneys is due to the reticulo-endothelial-system (RES) in liver trapping unsolved particles from the blood stream. The RES leads to an enrichment of nanoparticles in liver, confirmed by SQUID analysis of ironoxide. The amount of MTO, however, is not proportional to the detected iron in the liver, which leads to the suspicion, that MTO is released from the particles.

Conclusion: As a consequence, to avoid the wash out of nanoparticles in suspensions via the RES, alternative application ways have to be developed for an effective access to the target region.

Acknowledgement: Else Kröner-Fresenius Foundation and Deutsche Forschungsgemeinschaft DFG (AL552/3-1).

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Radiation stability of the PEG stabilized biocompatibile magnetic fluid

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The radiation stability of magnetic fluids was studied very rarely [1]. However, the combined hyperthermia and radiation with 20Gy was shown to be significantly more effective than radiation with 20Gy alone [2]. So, the aim of the presented work was to investigate the stability of biocompatibile magnetic fluid, i.e. water-based magnetic fluid containing magnetite nanoparticles (MNs) stabilized by two surfactants, natrium oleate as a first surfactant and Poly(ethylene glycol) (PEG) as a second surfactant after electron irradiation. The magnetic fluid with MNs stabilised only with one surfactant (natrium oleate), which was used as precursor for preparation of the biocompatibile magnetic fluid was studied too. The obtained results showed that the 8MeV electron irradiation with dose 1000Gy caused 50% reduction of the saturation magnetization in the case of the magnetic fluid with only one surfactant while in the case of the biocompatibile magnetic fluid (with two surfactants) only 25% reduction of the saturation magnetization was observed. However, the highest reduction of the saturation magnetisation was observed up to 20Gy and for higher irradiation the reduction of the magnetisation had saturating behaviour. This reduction of the magnetisation is due to the visible aggregation of the MNs. The results of the size distribution analysis of the MNs before and after irradiation showed no changes, that means that MNs of all size contribute to the aggregation by the same way. As possible mechanisms of the degradation in magnetisation after irradiation can be considered nuclear reactions, ionization processes or degradation of the surfactant molecules. However, there are no nuclear reactions at the used electron energy 8MeV. The only possible processes at the used electron energy are ionization and surfactant molecules destroying that could lead to the aggregation of the particles. All samples were also checked by infrared spectroscopy and no changes in the structure of the molecules of natrium oleate, PEG and MNs were observed. We can conclude, that the ionization can be mainly responsible for aggregation of the magnetic particles.

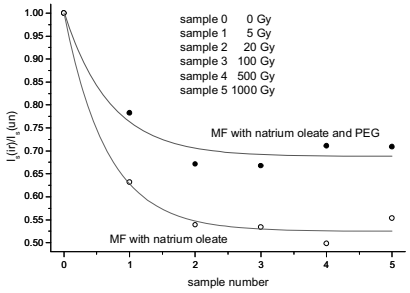

Figure 1. The reduced saturation magnetization. The solid lines are for eyes.

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Magnetic core-shell nanoparticles as MRI contrast agents: biodistribution in an *in vivo* animal model

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Magnetic Resonance Imaging (MRI) is one of the most powerful diagnostic tools in medicine, due to its non-invasive nature and high spatial resolution. Although enormous progress has been achieved in the improvement of the technique itself, the development of MRI contrast agents is still a wide research field. Magnetic core-shell nanoparticles are very promising materials to synthesize biocompatible magnetic fluids, able to modify the longitudinal T1 and transversal T2 proton relaxation of water in body tissues. Moreover, the coating not only helps to make the particles biocompatible but also it can be functionalized in order to link the nanoparticle to a biomolecule of interest (antibody, tumor marker receptor, chemotherapeutic drug, etc.) improving the performance of the MRI contrast agent [1].

In this work the viability of three different biocompatible magnetic fluids, containing three different sets of nanoparticles (arc-discharge synthesized Fe@C [2] and dextran-coated Fe_3O_4), as MRI contrast agents has been studied. The experiments have been carried out in *phantoms* as well as in an *in-vivo* preclinical animal model (New Zeeland rabbits). T1 as well as T2-weighted MR coronal and sagittal images of the rabbit abdomen were taken 15 minutes after administration of the dispersion, and periodically repeated along twenty months post-injection. The nanoparticles content has been each time evaluated in liver, kidney and muscle tissues. The analysis of the phantoms allowed us to quantify the concentration of nanoparticles in each organ. By means of these experiments the biodistribution of the nanoparticles after injection and its time evolution have been studied. Although in all cases most of the nanoparticles accumulated in the liver, different time-evolution has been observed depending on the type of particle. It has been found that Fe@C particles have longer residence time than the Fe_3O_4 ones.

Our results suggest that the synthesized suspensions can be used as positive as well as negative MRI contrast enhancer agents, mainly for liver MRI.

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Influence of the average size and polydispersion on the Specific Power Absorption in $CoFe_2O_4$ Nanoparticles.

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The synthesis of novel magnetic materials, mainly magnetic nanoparticles (MNPs) for heating purposes in biomedical applications has gained interest along the last years. It has been proved that magnetic intracellular hyperthermia can effectively induce cell apoptosis [1], with the additional benefits related to work in a safer electromagnetic frequency region ($f = 10^2$ - 10^3 kHz) that has no adverse effects on living tissues. However, power delivery from MNPs is still on the low-range (1-10 W per gram of tissue) as compared to traditional microwave or conduction-based hyperthermia methods, which can deliver up to 2-3x10^3 Watts into the target region.[2] Most of the experimental work reported so far has been focused on iron-oxide-based (maghemite and magnetite) nanoparticles because of biocompatibility reasons. However, since optimum power absorption for this material occurs in particles of 20-25 nm, colloidal stability of such nanoparticles is not easily achieved. Thus, an alternative for reducing the optimum particle sizes is to work with materials having large magnetocrystalline anisotropy. In order to explore the influence of small particle sizes on power absorption, we have synthesized cobalt ferrite particles with average sizes $5 < d < 25$ nm, using high-temperature decomposition of $Fe(acac)_3$ and $Co(acac)_2$ in the presence of a long-chain alcohol as reported by Sun et al. [3] with a modification developed to control the final size by changing the molar ratio between the metallic precursor and the surfactant [4]. Larger particles (i.e., d > 12 nm) were prepared by a re-growth method using previously synthesized particles as seed for the next ones. The resulting nanoparticles were very stable against agglomeration because of the surfactant molecules attached to the surface of the magnetic cores.

Transmission Electron Microscopy (TEM) images and X-ray diffraction (XRD) data confirmed that the average particle size increased with increasing molar precursor/surfactant ratio. Magnetization and ac susceptibility measurements showed that the saturation magnetization M_S at room temperature of all samples were similar to the expected ≈65/70 emu/g of bulk material, but decreases as the average particle size decreases due to surface effects. Heating experiments were conducted in an adiabatic system at frequency f = 260 kHz and amplitude B = 16 mT. Temperature vs. time curves were taken as a function of particle size revealed a strong increase in the specific power absorption (SPA) values for particles with <d> around 12 nm. The largest SPA value obtained for this average size was 98 W/g, and fast drop of SPA values were observed both below and above this <d> value.

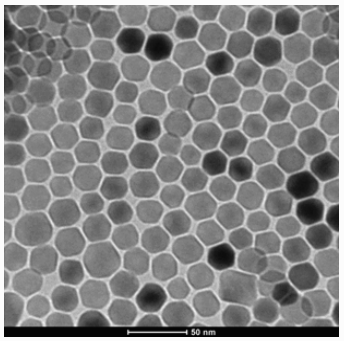

Figure 1. TEM image of $CoFe_2O_4$ nanoparticles of average size 22 nm.

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Poster 201

Water Dispersible Magnetic Nanoparticle Clusters and their Application as Contrast Agent

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The efficient phase transfer of the magnetic nanoparticles from non-hydrolytic to hydrolytic medium is of growing importance in numerous biological and biomedical applications such as magnetic resonance imaging, magnetic separation, magnetic drug targeting or delivery and magnetic hyperthermia treatment. It is well-established that high quality magnetic nanoparticles in terms of size, crystallization and magnetization can be obtained through high temperature organic phase synthesis in non-polar organic solvent. Since the biological and biomedical applications must be required aqueous magnetic nanoparticle dispersion, a key challenge to this point is the phase transfer of the magnetic nanoparticles to aqueous medium while maintaining size, monodispersibility, crystallinity and magnetization.

Our objective in this project is to develop a versatile and reliable method for preparing size-controlled, stable and biocompatible magnetic nanoparticle clusters in water and their potential application as contrast agent in MRI. Magnetic nanoparticle clusters[1] with controlled size in the range of 60-130 nm were prepared from oleic acid stabilized primary nanoparticles with a size of 6 nm [2]. Subsequently, the oleic acid coated magnetite nanoparticle clusters were encapsulated with biocompatible copolymer Pluronic PF127 as a result of hydrophobic Van der Waals force between aliphatic chains of the oleic acid and polypropylene oxide of the Pluronic copolymer. The formation of stable and dense nanoparticle clusters with a mean size of 100 nm in water was demonstrated with TEM and PCS studies and further work is in progress to evaluate the efficiency of the magnetic nanoparticle clusters as contrast agent.

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2. Sun, S., and Zeng, H., *J.Am.Chem.Soc*., **2002**, 124, 8204.

Poster 202

In vivo Nanoparticle Toxicity Trials in *Drosophila*

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Dramatic advances in nanomaterials synthesis and characterization provide promising possibilities for biomedical applications. As demand increases nanoparticle contact with living organisms beyond the laboratory is becoming an ever-increasing concern. The growing demand for well-characterized, low cost toxicity assays is drawing considerable interest in the use of small model organisms. The fruit fly *Drosophila melanogaster* offers a well-characterized repertoire of genetic tools, a relatively short lifespan, a rapid reproduction rate, a panel of efficient molecular techniques and a completely sequenced and mapped genome. We use micromanipulation and microinjection techniques to study the effect of coated magnetite (Fe_3O_4) nanoparticles in *Drosophila* embryos. This approach allows for a controlled, local microimplantation into target areas within the intact embryo. We microinjected (a) deionized water as a control, (b) a 46nm magnetite coated with aminopropylsilane (APS), (c) a 76nm co-precipitated magnetite coated with carboxymethyldextran (CMDx), (d) a 40nm magnetite coated with CMDx synthesized by thermal decomposition and (e) a 100nm commercially available glucoronic acid, in segments A5 and A6 at developmental stage 15 in the postero-dorsal end of the embryo. Light microscopy examination show that nanoparticles coated glucoronic acid nanoparticles had the lowest mortality rates when compared to all other groups. In contrast APS-coated nanoparticles exhibits higher mortality rates compared to control injections and had a marked tendency to aggregate around the visceral muscles. Our study represents the first target-specific toxicity trials in a model organism. This, together with targeted transgenic analysis should establish a cell mechanistic toxicological assay in the context of a living organism.

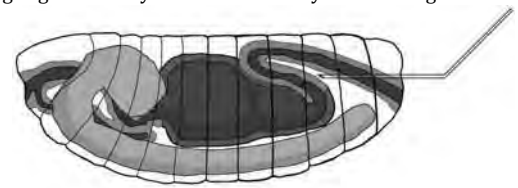

Figure 1: *Drosophila* stage 15 embryo local microinjection in abdominal segments 5/6.

8th International Conference on the Scientific and Clinical Applications of Magnetic Carriers, Rostock, Germany from May 25-29, 2010.

PRODUCTION OF IRON OXIDE CARBON NANOCOMPOSITES BY LASER PYROLYSIS: APPLICATION AS MRI CONTRASTS

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Abstract

Carbon encapsulated iron-oxide nanoparticles were obtained by using laser pyrolysis method. The synthesis method consisted in the laser decomposition of an aerosol of ferrocene solution in toluene in an ethylene/oxygen mixture. As a difference from previous works where a complex mixture of iron and iron carbide phases were formed [1], the new experimental conditions employed generated 7±3 nm magnetic iron oxide cores dispersed in a particulate carbon matrix (Fig 1). In order to reduce the carbon content from the initial 85 % to 60% keeping the magnetization, the samples were carefully heated in air. The samples were characterized by standard techniques as XRD, TEM, TG and elemental analysis as well as for their magnetic properties. The samples were treated with nitric acid and iron nitrate in a similar way as in [1] to produce stable colloidal aqueous dispersions.

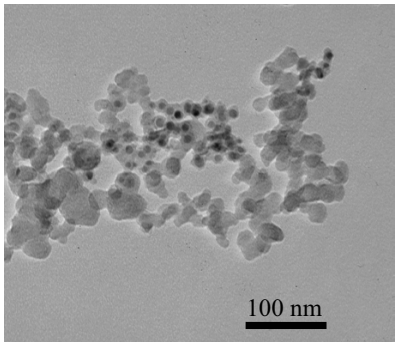

Figure 1: TEM micrograph of a Fe_2O_3@C nanocomposite

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Magnetic heating experiments with nano-crystalline $Co_{0.4}Zn_{0.6}Fe_2O_4$

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Magnetic fluid hyperthermia is a method of cancer therapy performed at temperature range 42 – 44 ℃ based on increased the rmal sensitivity of the tumour cells in comparison with the health tissue.

In this work we study the heating effect of nanoparticles of the cobalt zinc ferrite of the composition $Co_{0.4}Zn_{0.6}Fe_2O_4$ as the heat mediator for magnetic fluid hyperthermia especially with the respect to possibility of the self-controlled mechanism of the heating.

The particles were prepared by a co-precipitation method followed by a thermal treatment in the range 400 – 600 ℃ to gain dimensi on variability of the sizes 5 – 40 nm. The XRD analysis revealed single-phase composition of the samples with cubic spinel structure. Particles of the Co-Zn ferrite were successfully covered by the silica shell with average width of the shell about 20 nm evidenced by TEM and IR spectral analysis. The colloidal stability was confirmed by the method DLS including the hydrodynamic size measurement with the value of diameter of the specimen from the range of 150 – 200 nm.

Particles of the mean core diameter ~ 22 nm and Curie temperature in the vicinity of 45 ℃ were selected for the heating experiments. The investigation was performed on the home made apparatus in AC electromagnetic field of the maximal amplitudes of 13.8, 11.2 and 6.0 $kA \cdot m^{-1}$ and the frequencies of 107 kHz, 480 kHz and 960 kHz, respectively, employing silica coated particles suspended in water in the concentration of 1.77 $mg_{(Co+Fe)} \cdot ml^{-1}$. The heating curves measured in the AC field with maximal amplitudes of 8.8, 7.5 and 6.0 $kA \cdot m^{-1}$ and frequency of 480 kHz showed the usually observed character with decreasing slope and a tendency to saturation with T_{max}: 53.2, 47.2 and 39.5 ℃, respectively. The specific absorp tion rate (SAR) at temperature 37 ℃ measured in the range of the magnetic field parameters displayed the values of 36.5, 19.4 and 3.6 $W \cdot g^{-1}_{(Co+Fe)}$, respectively.

The support by the projects ASCR KAN200200651 and GACR P204/10/0035 and Research Program AVOZ10100521 is gratefully acknowledged.

Keywords: *Magnetic nanoparticles, Cobalt Zinc ferrite, Magnetic heating measurement, Specific absorption rate*

Extracellular and intracellular magnetic heating by core-shell $La_{0.75}Sr_{0.25}MnO_3@SiO_2$ nanoparticles

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Keywords: Manganese perovskite, Core-shell nanoparticles, Magnetic heating, In-vitro examinations

Stable water suspensions of core-shell $La_{0.75}Sr_{0.25}MnO_3@SiO_2$ nanoparticles were prepared in a sequence of the sol-gel procedure in the presence of citric acid and ethylene glycol, thermal and mechanical treatments, the silica encapsulation employing tetraethoxysilane and final size fractionation by the centrifugation[1,2], see Figure.

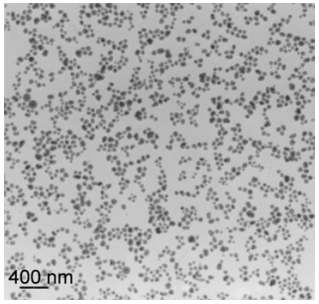

Figure. TEM of $La_{0.75}Sr_{0.25}MnO_3@SiO_2$

Rat mesenchymal stem cells (rMSCs) were used for the *in-vitro* experiments carried out under an alternating field in the range of 6.1 - 8.9 kAm^{-1} and frequency of 480 kHz. The aim was to distinguish two types of heating namely extracellular by nanoparticles surrounding the cells and intracellular by internalized nanoparticles.

The extracellular samples, suspensions of $La_{0.75}Sr_{0.25}MnO_3@SiO_2$ nanoparticles in the concentration of 0.9 mg_{Mn}/ml and 400 000 cells were subjected to the magnetic heating from 26 to 45 °C. An interplay of the gradual decrease of the magnetization due to an increase of the temperature approaching the Curie temperature of 62 °C and thermal losses allowed to achieve the required low terminal temperature of ~ 45 °C. It led to a substantial drop in the viabilities down to 10 – 20 % from the initial values of ~ 96 %.

Intracellular experiments were carried out on the cells labeled in advance by $La_{0.75}Sr_{0.25}MnO_3@SiO_2$ particles in the content of 0.17 pg_{Mn}/cell, as determined by MR relaxivity measurements. A small increase of the temperature from ~ 26 °C up to ~ 34 – 35 °C during the experiment can be attributed to a slight transfer of heat from the exciting coil and from the particles incorporated in the cells. In spite of that a decrease of the viabilities from the initial values of ~ 75 % to ~ 50 % was observed. The acting of the internalized nanoparticles was confirmed by the comparative "blind" experiments and study of the cells morphology. The cells after heat treatment were swollen when compared to cells in control samples and some of them had burst.

The support by the projects ASCR KAN200200651 and Research Plan AV0Z10100521 is gratefully acknowledged.

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Optimization of Magnetic Anisotropy and Applied Fields for Hyperthermia Applications

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Magnetic nanocrystalline particles with high anisotropy energy barriers may be useful in biomedical applications such as magnetic fluid hyperthermia. Such particles show good heating efficiencies in alternating magnetic fields. Recently non-oxide, high magnetic moment materials were produced experimentally for a hyperthermia application [1]. The $Fe_{70}Co_{30}$ nanoparticles had a cubic shape with side length 12 nm, which included a 2 nm nonmagnetic shell, and a core magnetization of 1845 emu/cm^3. We use micromagnetics to explore these particles, which occupy the interesting region where thermal energies and the energy barriers due to magnetic anisotropy are comparable. The micromagnetic simulation was based on the stochastic Landau-Lifshitz-Gilbert (LLG) using the thermal fluctuation formalism developed by Brown [2]. The calculation included magnetostatic interactions between 10 particles with random easy axes and was repeated 1000 times to generate adequate statistics. We find the optimized anisotropy energy of these nanocrystalline iron cobalt particles to be 3.142×10^{-13} ergs which corresponds to an energy barrier of 7.6 k_BT at room temperature. Interestingly, the calculated attempt frequency is 2×10^7 Hz, which may be compared to the oscillating frequency of 500 KHz. Figure 1 (a) shows the total energy per unit volume for different anisotropy constants. The highest peak shows approximately 1873 Oe·emu/cm^3 hysteresis loss energy density per particle at an anisotropy of 6×10^5 ergs/cm^3. We have explored the effects of varying the applied field and find that the addition of a 20 Oe static field applied perpendicular to the oscillating field approximately doubles the energy loss for a given applied power. This is an important benefit for magnetic hyperthermia. Figure 1 (b) shows the total energy per unit volume with the static applied field for different anisotropy constants. The highest peak shows approximately 3504 Oe·emu/cm^3 per particle at the same anisotropy energy density. This is apparently caused by a reduction in the distribution of energy barriers. We further find that use of a square wave produces greater heating than the normal sinusoid, even when normalized by its greater incident power. These proposed changes to the applied field should increase the applicability of hyperthermia.

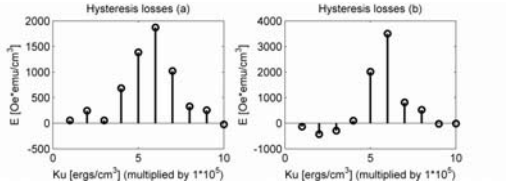

Fig. 1. Hysteresis losses of randomly oriented iron cobalt nanoparticles for different anisotropy energy densities, K_u from 1×10^5 to 1×10^6 ergs/cm^3, (a) without the static magnetic field and (b) with the static magnetic field.

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Poster 207

Design and synthesis of magnetite- and antibiotic-loaded Poly(3-hydroxybutyric acid-co-hydroxyvaleric acid) (PHBV) super paramagnetic nanoparticles.

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Magnetite nanoparticles are biocompatible because they are efficiently cleared from the body and do not cause oxidative stress or long-term effects on the liver. Magnetite assembled into 2-to-15 nm particles exhibit super paramagnetism, that make them suitable as contrast agent for MRI. PHBV has been described as carrier for drug delivery into the body. This leads us to load magnetite 2-to-15 nm and ceftiofur into PHBV nanoparticles (200 nm) to examine its biochemical properties as a putative double agent as both MRI marker and drug delivery agent.

Superparamagnetic magnetite was synthesized by the co-precipitation method. Magnetite and ceftiofur loaded PHBV nanoparticles were synthesized by the double-emulsion/solvent evaporation method.

The super paramagnetism of magnetite is maintained after loading into PHBV nanoparticles. The presence ot ceftiofur in the polymer did not alter magnetite magnetic properties either. The double-loaded super paramagnetic nanoparticles exhibit negative zeta potential, diameter < 300 nm, 90% efficiency of the antibiotic loading and 20% efficiency of magnetite loading.

Results suggest that the super paramagnetic and antibiotic-loaded polymeric nanoparticle is a promising tool for both simultaneous imaging and local drug delivery.

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Poster 208

A Parametric Study of Specific Absorption Rates in Magnetic Nanoparticles for Magnetic Fluid Hyperthermia

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The specific absorption rate (SAR) values of suspensions of thermally blocked magnetic nanoparticles (cobalt ferrite – $CoFe_2O_4$) in different solvents were investigated by measuring their temperature increase under an applied magnetic field at different frequencies and magnetic field intensity values. Cobalt ferrite nanoparticles were used because they can be made to relax by a single, well-characterized relaxation mechanism and with a single measureable relaxation time constant. As such, this can be an idealized model system to test expressions for the energy dissipation rate of magnetic nanoparticles in oscillating magnetic fields, with applications in hyperthermia and triggered drug release. A custom experimental induction heater setup was designed and built to operate in frequencies ranging from 196 to 618 kHz and with adjustable magnetic field intensity ($H_{max\ @618kHz}$ = 6 kA/m). Cobalt ferrite particles of 8.6 nm mean diameter were suspended in heptane, mineral oil, octadecene, and combinations of the latter two, at a concentration of 2.5% w/w and their rate of energy dissipation was measured using the custom setup and quantified as Specific Absorption Rate (SAR). Temperature measurements were obtained using fluoroptic immersion probes (Luxtron). The suspension in heptane exhibited a significant thermal response to an applied magnetic field when compared to the samples suspended in the other solvents, with a maximum SAR value of 9.025 W/g at 292 kHz and magnetic field intensity of 9kA/m. For all frequencies tested, a quadratic increase in SAR value with increasing magnetic field intensity was observed, in agreement with commonly used models. On the other hand, with respect to frequency dependence, an increase in SAR values was seen with increasing frequency of the magnetic field, up to a peak value of 5.277 W/g at 6kA/m and f=506kHz, after which, SAR values decreased with increasing frequency.

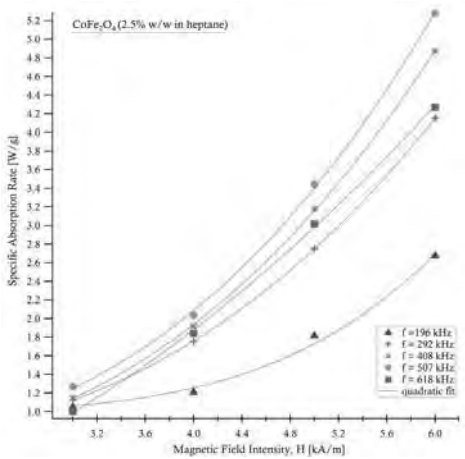

Figure 1. SAR vs Magnetic Field Intensity for cobalt ferrite suspended in heptane.

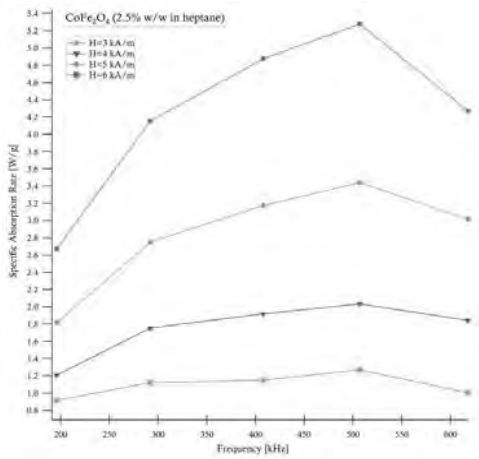

Figure 2. SAR vs Frequency for cobalt ferrite suspended in heptane.

Poster 209

Magnetic microbubbles as mediators of gene delivery through Ultrasound-activation.

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In recent years microbubbles technology has gained lot of interest in the field of gene and drug delivery. Based on the "Magnetofection" concept [1], besides the development of magnetic acoustically active lipospheres [2, 3], we have been able to prepare lipid monolayer shelled microbubbles loaded with highly positively charged naked magnetic nanoparticles (Fig. 1) (composed of iron oxide) through electrostatic and matrix affinity interactions. These magnetic microbubbles show strong ultrasound contrast.
Treatment of cancer cells with these microbubbles using ultrasound exhibited strong dose-dependent cytotoxic effects, although ultrasound alone, lipid microbubbles alone, magnetic nanoparticles or magnetic microbubbles alone at the corresponding concentrations did not affect the cell viability. On the other hand, when these magnetic microbubbles were mixed with plasmid DNA encoding a reporter gene, we achieved gene delivery to cultured adherent cells only when ultrasound was applied. Gene transfer efficiency was strongly dependent on the application of a gradient magnetic field to sediment the microbubbles on the target cell membranes.
From the preliminary experiments we conclude that magnetic microbubbles could be used as magnetically targeted diagnostic agents for real-time ultrasound as well as magnetic resonance imaging. At the same time, such magnetic microbubbles may be useful for therapeutic purposes such as in cancer therapy, vascular thrombolysis and gene therapy. However, further improvements are required to control their cytotoxicity.

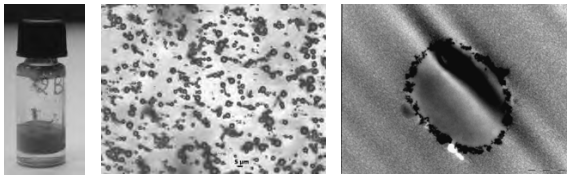

Figure 1: Magnetic microbubble preparation; a) Macroscopic view b) Phase contrast image and c) Transmission electron microscopic image

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Poster 210

DNA hybridisation on magnetic particles in continuous flow

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DNA hybridisation is an important tool for environmental, forensic and clinical screening. Conventional methods often require long incubation times and several washing steps, thus rendering the whole procedure time consuming and labour intensive. We have developed a microfluidic platform for continuous flow DNA hybridisation on the surface of magnetic particles with total analysis times under five minutes. Particles are pulled through laminar flow streams containing reagents and washing buffer. The principle of this method is as follows: Commercially available magnetic particles modified with streptavidin were reacted off-chip with a biotinylated oligonucleotide capture probe. These particles were then introduced into a microfluidic chip and deflected by an external magnet across streams containing sample DNA, buffer, marker and buffer respectively (Figure 1).

Initially, DNA hybridisation with one reaction step was investigated, whereby sample DNA was fluorescently labelled with AlexaFluor555 and therefore only three laminar flow streams were used. The sample concentration was varied, and a level as low as 20 nmol L^{-1} was detected, using a fluorescence microscope and CCD camera. The residence time of a particle within the sample stream was also varied and its effect on the hybridisation was investigated. At low concentrations, increasing residence time provided higher signal, at higher concentrations of sample the effect was smaller due to the signal becoming saturated.

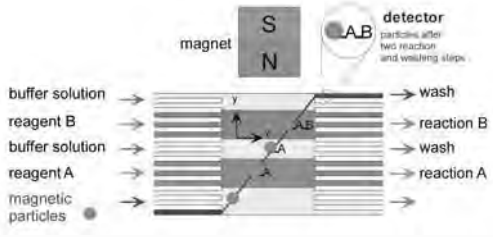

Figure 1: Principle of multistep reaction in continuous flow on particle surface.

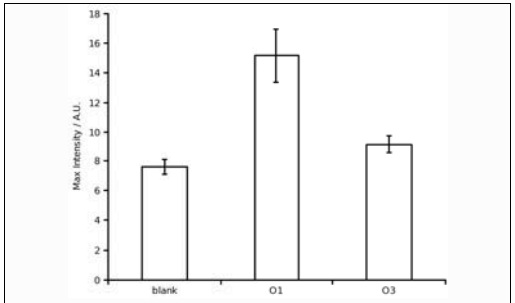

Figure 2: Discrimination between complementary and non-complementary sequence. O1 – complementary, O3 – non-complementary.

Following from this, two-step, label-free DNA hybridisation and detection was performed in a similar way, initially using PicoGreen for visualising the resulting double stranded DNA. An increase in signal was found, but due to the short probe length (24 bp) the signal intensity was rather poor. An alternative format, a sandwich DNA assays with an 18 mer capture oligonucleotide, a 61 mer sample and an 18 mer fluorescently labelled marker was therefore employed. Discrimination between non-complementary and fully complementary sequences of the sample to the marker can be easily achieved, as shown in Figure 2. We are currently investigating three-base mismatch sequences.

With this method, easy and fast DNA hybridisation can be achieved in continuous flow, using magnetic particles as a solid support for the reaction. It provides a significant reduction of procedural time compared to conventional methods, but also a potential for automation of the process.

Improving the detection sensitivity of magnetic micro beads by spin valve sensors

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Detection of magnetic fields generated by magnetic particles which are coated with chemical or biological species of interest can be made using giant magnetoresistive (GMR) or Planar Hall effect (PHE) spin-valve structures. The magnetic beads polarized by a dc or ac magnetic field can affect the magnetization state of the spin-valve sensor, leading to a detectable signal. Results from theoretical modelling, as well as laboratory tests, show that spin-valve sensors can resolve single micrometer-sized magnetic beads. Because both systems, microbeads and spin-valve sensors, are made-up from magnetic materials, there is a magnetostatic interaction between them. The external magnetic field can been applied parallel to the sensor surface or perpendicular on the sensor's surface. The spin-valve devices are sensitive only for in plane magnetic fields. For micromagnetic simulations each bead is assumed to be a sphere with a diameter of about 0.2 μm and the distance between the bead and the GMR sensor's surface is 0.2 μm. The saturation magnetization of the magnetic micro-bead is assumed to be 400 emu/cm^3. The sensor is a multilayer structure FeMn/$Ni_{80}Fe_{20}$(10 nm)/Cu(4 nm)/$Ni_{80}Fe_{20}$(10 nm). The design of the spin-valve structure used for micromagnetic simulations is shown in Fig. 1(a). If the magnetic field is applied perpendicular to the sensor surface, the microbeads will produce horizontal components of the stray field, Fig. 1(b), which can change the magnetic state of the sensor and hence can generate a GMR response. For a large number of particles, located above the centre of the sensor's surface, the horizontal components of the stray fields, in the film plane, will cancel each other and the GMR response will be very weak, comparable with the sensor response without magnetic beads, Fig. 1c. Micromagnetic simulations regarding the GMR response of the spin-valve structure for different positions of the magnetic beads have been made. Some results are presented in Fig. 1(c); Δy denotes the displacement of the particles relative to the sensor surface.

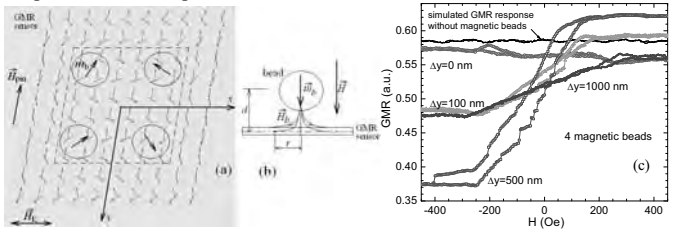

Fig. 1(a) The structure of the spin-valve sensor used for micromagnetic simulations and the magnetic particles located above the free layer. The orientations of the anisotropy, H_K, and pinning fields, H_{pin}, are presented; m_b denotes the magnetic moment of the bead; (b) the illustration of the stray fields produced by the beads under the action of an external magnetic field perpendicular to the sensor surface; (c) The field dependence of the GMR effect for different positions, Δy, of the particles above the sensor surface.

From these simulations the exact position of the particles for which the detection sensitivity of the GMR and PHE sensors achieves the maximum value can be obtained and a calibration curve can be build in order to perform quantitative measurements. Aspects regarding the field scanning method and the response of this system, according to proposed physical and electronic setup, are discussed in this paper.

Treatment of purulent wounds using suspensions of magnetite microparticles.

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Treatment of purulent wounds and chronic inflammations represent a series of significant challenges, and since ancient time people were looking for better methods of treatment. Colloidal magnetite was used for treatment of pyo-inflammatory diseases and poisonings in oriental (Avicenna) and Tibetan medicines. Later, after discoveries of sulfanilamides and antibiotics, the use of colloids becomes uncommon. However, the problem of stimulating purulent wounds healing (especially with complications) still is not solved completely. Our data suggest that in certain cases application of colloidal magnetite can facilitate healing of inflamed wounds which do not respond well to traditional treatment methods.

Healing of wounds, especially those complicated by inflammatory processes, is a complex multi-stage process. To expedite the healing, effective methods of bacterial biofilm destruction, immobilization and disintegration of pathogens and toxins, macrophages activation and formation of wound closures, which prevent exogenous re-infection, are highly desirable. We studied feasibility of treating chronic purulent wounds using colloids of magnetite nanoparticles as a supplement to traditional antibiotic-based methods, and investigated possible mechanisms of their activity.

Polydisperse iron nanoparticles were produced by co-precipitation of iron(II) and iron(III) salts by 25% ammonia solution. The suspensions were stabilized by albumin, lecithin, citric and ascorbic acids. The best results were achieved using ascorbic acid. Diluted (<1% of the bacterial phase) live cultures of Staphylococcus aureus were used to study the affinity of the nanoparticles to bacterial cells and their cytotoxicity. Electron microscopy (JEM-100C, Japan) studies suggest an affinity of the nanoparticles to the pathogen cells and cell membrane damage. Bacteria viability studies before and after application of magnetite ferricolloids suggest their antimicrobial activity against Staphylococcus aureus.

Preclinical trials of application of magnetite ferricolloids stabilized by the ascorbic acid for treating chronic purulent wounds and ulcers were conducted in Abkhazian hospitals. 23 patients with chronic ulcers of lower extremities were selected to participate in the trials. All patients were previously unsuccessfully treated using traditional antibiotics-based methods for an extended period of time. Magnetite ferricolloids were used as a supplemental method to the antibiotics therapy, as well as physical therapy. Ulcers of most patients improved after such combined treatment in a time shorter than previous unsuccessful antibiotic treatment (see Table). This method can be useful for treating drug-resistant infections and chronic inflammations.

Results of chronic ulcers treatment using ferricolloids of magnetite.

Patient (age)	Diagnosis	Period of unsuccessful treatment using other methods	Period of treatment with MK after which a full recovery was achieved
A., (47 y.o.)	Varicose ulcers	3 month	28 days
P., (76 y.o.)	Varicose ulcers	7 years	5 month
V., (55 y.o.)	Traumatic wound ulcer	30 days	12 days
G., (79 y.o.)	Ulcers of diabetic origin	26 days	25 days
M., (19 y.o.)	Chronic ulcer	4 month	16 days

Synthesis and investigation of cell interaction of magnetic nanoparticles with catechol-containing shells

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Magnetic nanoparticles used in biomedicine are commonly covered with an organic coating to enhance their biocompatibility and prevent rapid agglomeration of the particles. The stability of those core-shell particles is strongly influenced by the particle concentration and the surrounding medium. We report on a route to stabilize iron oxide-based magnetic nanoparticles by catecholic compounds known for their ability to form strong coordinative interactions with iron(III) ions.

Magnetic cores with mean diameters of 10 nm were synthesized by co-precipitation of iron(II) and iron(III) salts under basic conditions. Different monomeric catecholic ligands (levodopa, dopamine, hydrocaffeic acid) and a dopamine-containing dextran polymer (structures see Fig. 1) were used to coat the core particles. Defined conditions of coating, notably temperature and pH, had to be elaborated allowing the attachment of the catechols to the iron(III)-ions.

The prepared stable magnetic fluids were characterized with regard to their chemical composition (content of iron and shell material, Fe(II)/Fe(III) ratio) and their physical properties (size, surface charge, magnetic parameters).

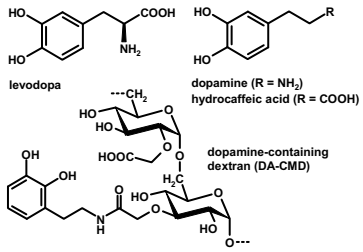

Fig. 1. Catecholic shell materials

The cytocompatibility of the core-shell particles was investigated by a live/dead viability assay using 3T3 fibroblast cells. No cytotoxicity were detected after 24 h of cell incubation. Preliminary experiments were performed to study the interaction of the prepared nanoparticles with tumor cells from breast cancer cell line MCF-7 and leukocytes. Cells were incubated with nanoparticles for different time periods and the magnetically labeled cells were separated by MACS using a SuperMACS and MS columns. We found an intense interaction of the MCF-7 cells with these particles whereas the leukocytes showed a lower tendency of interaction.

Overall, the used shell structures enables the formation of stable magnetic fluids, and in addition, they provide suitable functional groups for the immobilization of further molecules like drugs, markers or contrast agents. The described nanoparticle coating strategy therefore represents an interesting route to design tailored magnetic nanoparticles for biomedical applications.

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Iron-Boron Nanowires for Applications to Boron Neutron Capture Therapy

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Boron neutron capture therapy (BNCT) is an experimental cancer therapy, in which highly localized radiation is exploited to target tumor cells. ^{10}B converts to ^{7}Li upon absorbing a thermal neutron and emits highly energetic particles (2.31 MeV), which have a very local, highly destructive effect on nearby tissue.

The isotope ^{10}B has a more than one-thousand-fold higher thermal neutron capture cross section than the elements of the tissue. The particles emitted thereupon have track lengths in the range of cell radii ($5-9$ µm). Thus, ^{10}B that is delivered near tumor cells and irradiated with thermal neutrons is an auspicious adjuvant for the selective destruction of tumor cells.

Producing magnetic iron-boron thin films is possible by electrodeposition on conducting substrates [1]. In the present work electrodeposition is applied using a hexagonal pore array (formed by self-organization in anodic alumina) as a template system. In this way, magnetic nanowires with diameters $40-300$ nm and a widely tunable aspect ratio and chemical composition can be fabricated. The biocompatibility of these wires is ensured by a functional silica shell, which is realized by atomic layer deposition (ALD) [2] before electrodeposition. The anodic alumina template can subsequently be etched away selectively, leaving silica coated iron-boron nanowires.

Figure 1: Schematic representation of the impact of thermal neutrons on iron-boron nanowires with the resulting highly energetic α and ^{7}Li particles heading for a tumor cell

To determine the material's composition, iron-boron thin films are investigated by energy dispersive X-ray spectroscopy (EDX) and X-ray diffraction (XRD). The electrolyte used for electrodeposition of these thin films is then optimized for the fabrication of nanowires in order to achieve the highest B/Fe-ratio possible. The magnetic properties are investigated by magnetometry (superconducting quantum interference device (SQUID) and magneto-optic Kerr effect (MOKE) measurements).

These iron-boron particles hold the promise of production of magnetic suspensions for applications to BNCT. With the high amount of boron embedded in a ferromagnetic carrier and protected by a biocompatible silica shell, basic requirements for a boron delivery agent in BNCT are met. In the next steps, we will investigate the system's practical potential upon irradiation and behavior in biological medium.

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Fabrication of Functionalized Magnetic Mesoporous Silica Nanoparticles for Laccase Adsorption and Biocatalysis

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Large-pore magnetic mesoporous silica nanoparticles (MMSNPs) with wormhole framework structures were synthesized by using tetraethyl orthosilicate as the silica source and amine-terminated Jeffamine surfactants as template. Iminodiacerate was attached on these MMSNPs through a silane-coupling agent and chelated with Cu^{2+}. The Cu^{2+}-chelated MMSNPs (MMSNPs-CPTS-IDA-Cu^{2+}) showed higher adsorption capacity of 98.1 mg g^{-1}-particles and activity recovery of 92.5% for laccase via metal affinity adsorption in comparison with MMSNPs via physical adsorption (Fig. 1). The Michaelis constant (K_m) and catalytic constant (k_{cat}) of laccase adsorbed on the MMSNPs-CPTS-IDA-Cu^{2+} were 3.28 mM and 155.4 min^{-1}, respectively. Storage stability and temperature endurance of the adsorbed laccase on MMSNPs-CPTS-IDA-Cu^{2+} increased significantly, and the adsorbed laccase retained 86.6 % of its initial activity after 10 successive batch reactions operated with magnetic separation.

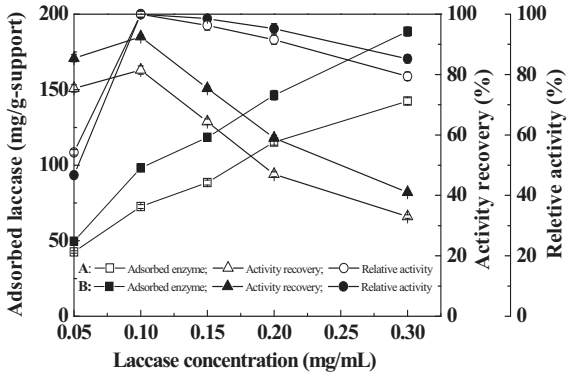

Figure 1: Laccase adsorption and activity recovery
On MMSNPs (A) and MSNPs-CPTS-IDA-Cu^{2+} (B)

Multifunctional Magnetic Polymeric Liposomes for the Delivery of Anticancer Drug Paclitaxel

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Tao Song and **Jin Chang***

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Purpose：To develop a novel kind of Multifunctional Magnetic Polymeric Liposomes(MMPL) for targeted therapy of the brain diseases by crossing blood-brain barrier (BBB).

Methods: MMPL formed from octadecyl quaternized carboxymethyl chitosan (OQCMC)/cholesterol, Folic acid, PEG, Tat and Paclitaxel, was assembled by Thin-Layer Evaporation (TLE) Method. The characterization of MMPL, such as morphology, size distributions, Zeta potential, magnetic properties, drug loading capacity, cytotoxicity, release behavior of paclitaxel in vitro and distribution in vivo, were also investigated in detail.

Results：From the results, MMPL had spherical shape and the mean particle size of OQCMC micelles was 106.7±0.7 nm with the poly dispersity index 0.357. Compared with traditional PC/Chol liposomes, MMPL had a little burst release and longer release time due to its high molar mass. The animal test showed that delivery of paclitaxel to penetrate the BBB into brain tissue can be improved by MMPL.

Conclusion: It was clear that MMPL having muilti-function of targeting, long circulating, BBB transferring and controlled releasing of drugs, may have more potential in the targeted therapy of brain diseases than traditional liposome.

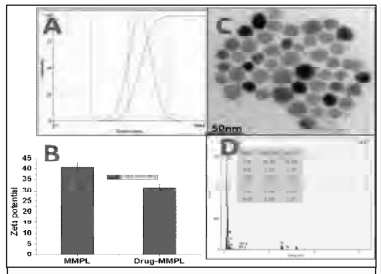

Figure 1. The Hydrodynamic Diameter of Multifunctional Magnetic Polymeric Liposomes (MMPL)(A); the Zeta potential of MMPL (B); the TEM images of MMPL(C).the EDX of MMPL(D)

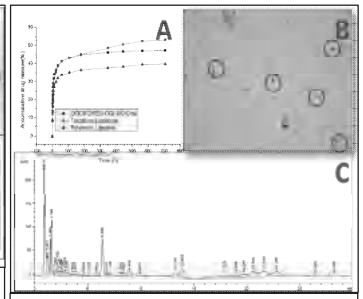

Figure 2. The in vitro release of paclitaxel (A), the section of the brain tissue(B), HPLC of the brain tissue(C)

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A novel approach of transferring oleic acid capped iron oxide nanoparticles to aqueous phase

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Some strategies have been developed to transfer oleic acid capped iron oxide nanoparticles to aqueous phase, such as ligand exchange and amphiphilic molecules encapsulating. However, these methods either had low exchange efficiency or the size of treated particles can be much larger than the starting nanoparticles [1] . Herein we present a novel method to transfer oleic acid-capped iron oxide nanoparticles to aqueous solution by oxidizing the oleic acid coated on the magnetic nanoparticle surface.

Firstly，the oleic acid-capped nanoparticles were synthesized using a thermolysis process and redispersed in hexane[2]. 80 mg nanoparticles was added into the 8 ml mixture of ethyl acetate/acetonitrile with volume ratio of 1:1, then sodium periodate aqueous solution (0.28 mol/L, 6 ml) was added under vigorous stirring at room temperature[3]. Two hours later, the top hexane layer was colorless and discarded, and the bottom aqueous layer was collected and magnetically separated. After washed with ethanol and distilled water for several times, the obtained iron oxide nanoparticles were dispersed in water and characterized.

TEM images and X-RD patterns show the phase transfer was successful performed without the size and crystalline change of iron oxide nanoparticles. The hydrodiameter measured by PCS is was 24±3 nm. The carboxyl groups on particle surface can be used to couple biomolecules such as protein and antibody.

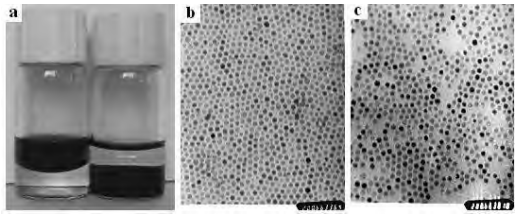

Figure 1. (a) Photographs of a two-phase mixture of iron oxide nanoparticles before (left) and after phase transfer (right). The top layers were hexane and bottom layers were distilled water; TEM images of nanoparticles (b) before and (c) after phase transfer. (magnification 200K×).

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MRI OF TUMOR CELL MIGRATION IN ANIMALS

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The process of metastasis formation involves the migration and 3-D invasion of tumor cells from a primary tumor to distant sites. Our aim was to monitor non-invasively the dynamics of cell migration and invasion in animals over prolonged time periods using MRI. Human breast carcinoma cells were labeled with superparamagnetic γFe2O3 iron oxide nanoparticles (mean diameter 17 nm) (Figure 1). Particles were stabilized and biofunctionalized with poly-l-lysine. Approximately 10,000 cells were incubated with 1 µg of particles for 24 h. Particles are readily taken up by cancer cells and stored in intracellular clusters (Figure 2). During cell division, the nanoparticle clusters are divided and split between daughter cells (Figure 3). Nanoparticles are not degraded by the cell and remain stable for at least 3 weeks. In-vitro collagen gel assays show that there is no difference between the spreading or invasion behavior of tumor cells with and without nanoparticles. MRI imaging (conventional multi-spin sequences with a repetition time of 1000 ms and 8 echo times between 11 and 165 ms) of cells suspended in 2% agar gave a detection limit of the R2 relaxation rate above agar background of 20 µM γFe2O3, equivalent to 70 cells/mm^3. The minimal detection volume of tumor cells in agar was 25 µl. Detection limit and minimal volume were verified by injecting labeled cancer cells in dead mice. 500 000 cancer cells labeled with 100 µg iron oxide nanoparticles were injected in the left rear leg. T2- and susceptibility-weighted MRI images show a rapid relaxation behavior (Figure 5.1) and pronounced phase shifts (Figure 5.2) in the vicinity of the injection area compared to control scans (Figure 4).
These data demonstrate the feasibility of the method for long-term observation of cancer cell migration in living animals with MRI.

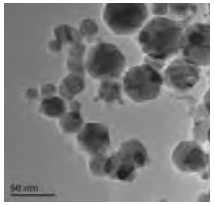

Fig 1: Fe2O3 particles

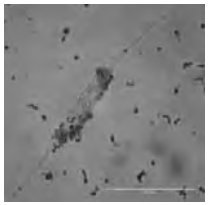

Fig 2: Cell with Fe2O3

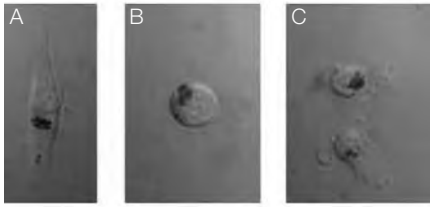

Fig 3: Fe2O3 distribution before (A), during (B) and after (C) cell divsion

Fig 4

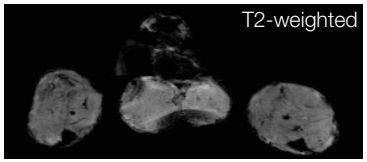

Fig 5.1

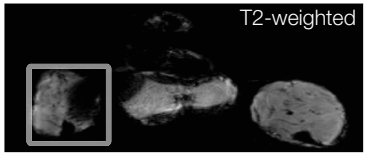

Fig 5.2

Poster 219

Refinement of Magnetic Nanoparticle Drug Carriers Using the Mechanism of Quadrupole Magnetic Field-Flow Fractionation

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Quadrupole magnetic field-flow fractionation (QMgFFF) is an analytical and characterization technique for magnetic nanoparticles such as those used for cell labeling and for targeted drug therapy [1-7]. These nanoparticles are generally composite materials consisting of magnetic cores composed of finely divided sub-single domain superparamagnetic material such as magnetite, with biocompatible coatings carrying antibodies or therapeutic drugs. The coatings stabilize the particles in suspension and also reduce the magnetic dipole-dipole interactions between the particles in a magnetic field. QMgFFF is capable of determining not only the mean magnetite mass per particle but also the mass distribution of magnetite among the particles.

Magnetic nanoparticle drug carriers continue to attract considerable interest for drug targeting in the treatment of cancers and other pathological conditions. The efficient delivery of therapeutic levels of drug to a relevant site while limiting nonspecific, systemic toxicity requires optimization of the drug delivery materials, the applied magnetic field, and the treatment protocol. Magnetic nanoparticle drug carriers are polydisperse in their magnetic properties and have widely varying response to a magnetic field. The consideration of the magnetic polydispersity of drug carriers is critically important for the development of effective magnetic targeting because the least magnetic particles in a formulation will contribute the most to systemic toxicity. The depletion of this fraction will therefore result in a more effective drug targeting reagent. A formulation that has a reduced systemic toxicity will allow higher doses of cytotoxic drugs to be delivered to the targeted site with reduced side effects. A method of refining a magnetic nanoparticle drug carrier to achieve this result is described. The method is based on the mechanism of QMgFFF with step-function reduction in field gradient. Quadrupole magnetic field-flow fractionation is used to characterize the refined and unrefined material, and the success of the approach is evident from the calculated equivalent spherical magnetic core diameter distributions [6].

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Poster 220

Short-term application of magnetic core-shell nanoparticles – Effect on immune cells

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Cancer is considered a major cause of death worldwide. The vast majority of cancer patients do not die from the primary tumor but from distant metastases which develop during the progression of the disease. Key events are the establishment of tumor vasculature and the invasion of tumor cells into the surrounding tissue and the circulation. These cells are suspected to be an origin of relapse and metastases. Therefore it seems to be a suitable approach to eliminate these cells from the circulation.
We could show previously, that the time-course of the labeling of tumor cells and peripheral blood leukocytes differs dramatically within the first 20 minutes with a maximum discrepancy between 8 and 12 minutes [1,2].
Here, we analysed the effect of magnetic core shell nanoparticles on the survival of leukocytes in general and especially the lymphocytes as important parts of the immune system during incubation and subsequent separation.
Leukocytes from tumor patients were inoculated with magnetic core/carboxy-methyl-dextran (CMD) nanoparticles with an average magnetite/maghemite core TEM-size varying between 3 and 15 nm for 4 min to 30 minutes. Magnetically labeled cells were separated by MACS. The separated cells were counted and analyzed by FACS. T-Lymphocytes were identified by CD3 and B-Lymphocytes by CD19 (Figure 1). For long-term cultivation and activation of T cells Dynabeads® CD3/CD28 T Cell Expander (Invitrogen) was used.
First, we analysed the labelling of leukocytes over a period of 30 minutes. The leukocytes accumulated in the positive fraction continuously from 10% after 4 minutes to 42% after 30 minutes. With regard to the subpopulations, granulocytes represented the majority of cells in both, the positive and negative fraction. Most of the lymphocytes as central components of the immune system remained in the negative fraction. The distribution of T-lymphocytes differs dramatically from B-Lymphocytes. Whereas the amount of B-Lymphocytes differed only slightly over an incubation time of 16 min, the number T-Lymphocytes in the positive fraction increased 2.5-fold from 4 minutes to 16 minutes. At 4 minutes 20% of total lymphocytes in the positive fraction were T-Lymphocytes. This portion rose to 70% after 16 minutes. A critical parameter is the integrity of the leukocytes especially in the negative fraction. During the incubation the loss of cells increased from 27% (4 min) up to 50% (30 min). T cells could be expanded after a 4 or 8 minute incubation with nanoparticles indicating full biological activity.
In conclusion, our enrichment procedure of tumor cells from peripheral blood preserves the integrity and biological activity of leukocytes in the negative fraction.

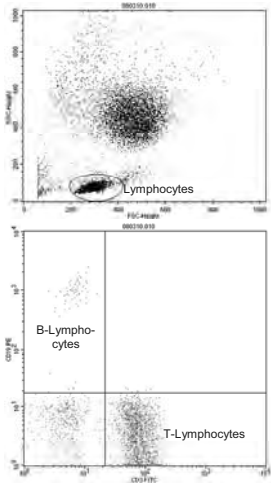

Figure 1: lymphocyte distribution from a negative fraction after incubation and separation with CMD nanoparticles.

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Immumagnetic assay of bio-targets using bio-functional magnetic nano-particles and high-T_c SQUID-detected nuclear magnetic resonance

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Herng-Er Horng (e-mail: phyfv001@scc.ntnu.edu.tw)

In this work the authors present the detection of the biotarget using bio-functional magnetic nanoparticles and high-T_c superconducting quantum interference devices (SQUIDs). To detect the biotarget, anti-bioprobe coated bio-functionalized magnetic nanoparticles was used as bio-markers. Magnetically labeled biotarget and bioprobe will interact and form immune complexes. The clustered immune complexes deteriorate the homogeneity of measuring field in NMR, which in turn causes the de-phasing of the proton's nuclear spin, and affect the effective longitudinal transverse relaxation time T_2*. As an example of human CRP was investigated and the time dependent effective T_2* and the spectral line width of NMR was characterized. The superior detection sensitivity in microtesla magnetic fields is demonstrated.

Immunomagnetic reduction assay on chloromaphenicol extracted from shrimp

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Drug residues in aquatic foods are strictly not permitted. Hence, it is necessary to qualitatively detect drug residues as exporting/importing aquatic foods. Currently, such technologies as chromatography/tandem mass (LC/MS/MS) spectrometry, high performance liquid chromatography, or competitive enzyme-linked immunosorbent assay are used for qualitatively detecting drug residues. However, these technologies can just be utilized at lab-based environment. It usually takes more than one week for delivering aquatic samples, extracting drug residues from samples, and doing assays. In practical, there is a demand to develop a technology for qualitatively detecting drug residues on fields. This developed assay technology should show merits of high sensitivity, low interference, and easy operation. In this work, the feasibility of immunomagnetic reduction assay on drug residues in aquatic foods is investigated. Chloromaphenicol (CAP) and shrimp are used as examples of drug residues and aquatic foods, respectively.

The reagent for assaying CAP is consisted of magnetic nanoparticles biofunctionalized antibodies against CAP (Ab35658-500, Abcam) and dispersed in pH-7.4 phosphate buffered saline solution. The mean diameter of magnetic nanoparticles is 63 nm. The magnetic immunoassay analyzer (XacPro-E101, MagQu) is utilized to record the mixed-frequency magnetic susceptibility χ_{ac} of reagent. Before magnetic nanoparticles associate with CAP molecules, each magnetic nanoparticle oscillates with applied magnetic ac fields. With the antibodies, magnetic nanoparticles specifically bind with CAP molecules. Portion of nanoparticles becomes larger, which particles show less χ_{ac}. Thus, χ_{ac} of reagent is reduced due to the association among magnetic nanoparticles and CAP molecules. This method is so-called immunomagnetic reduction (IMR). The reduction in χ_{ac} was found to be a function of the concentration of CAP. The low limit of detection is 0.1 ng/ml, which is significantly lower than the formal regulation 0.3 ng/ml. It was also evidenced that there is no observable interference from other kinds of drug residues as assaying CAP via IMR. Furthermore, the convenient processes for extracting CAP from shrimp are developed. The time taken for doing the extraction processes for IMR is just one third ofthat for LC/MS/MS. The extraction efficiency is higher than 80 %. According to the experimental data, the correlation coefficient R^2 between IMR and LC/MS/MS is 0.97. All the results show the possibility to utilize IMR for on-field qualitatively detection on CAP in aquatic foods.

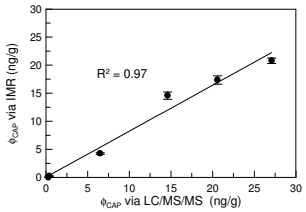

Figure. Correlation between the detected CAP concentrations via LC/MS/MS and IMR

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Poster 223

Nonlinear Behavior of Magnetic Fluid in Brownian Relaxation

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Magnetic nanoparticles in solution, i.e., magnetic fluids, have been widely studied for biomedical applications. One of the important properties of magnetic fluids is the Brownian rotational relaxation. We have been studying the nonlinear behaviors of Brownian relaxation in high fields for biomedical applications. Previously, we studied the nonlinear behavior by numerical simulations based on the Fokker-Planck equation, and clarified the nonlinear properties, such as decrease in the ac susceptibility, field-dependent Brownian relaxation time, and occurrence of higher harmonics[1].

In this study, we show the experimental results of the nonlinear properties of magnetic fluid. In Fig. 1, experimental results of the higher harmonics of the magnetization signal M are shown when an excitation field of $H_{ac}=H\sin(\omega t)$ was applied. The excitation frequency was chosen as $\omega\tau_B = 0.5$, where τ_B is the Brownian relaxation time. As shown in Fig. 1, amplitudes of the higher harmonics increased with the excitation filed strength H.

These experimental results were compared with numerical simulations. In the simulation, we took account of the size distribution of the magnetic nanoparticles existing in practical samples, which was estimated from the AC susceptibility measurement in weak fields by the singular value decomposition (SVD) method. We also took account of the size dependence of the magnetic moment m of the nanoparticles, which was obtained by combing the size distribution and the distribution of m estimated from M-H curve. In Fig. 2, the estimated size dependence of m is shown. As shown, m increased in proportion to the diameter d of the magnetic particle for small d, while the increase of m became slow for large d in the present sample. The solid lines in Fig. 1 represent the simulation results obtained with the m-d curve shown in Fig. 2. As shown, quantitative agreements were obtained between the experimental and simulated results.

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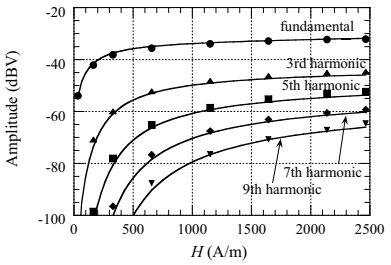

Fig. 1. Amplitude of fundamental and higher harmonics of the magnetization M as a function of H. Symbols and lines represent the experimental and simulation results, respectively. The normalized frequency of the excitation field was $\omega\tau_B = 0.5$.

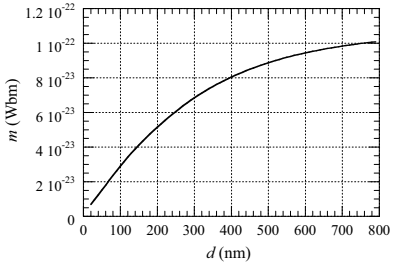

Fig. 2. Estimated size dependence of the magnetic moment m.

Poster 224

High-Field Gradient Permanent Micromagnets for Targeted Drug Delivery with Magnetic Nanoparticles

Vitalii Zablotskii, Martín Pastor Gutiérrez, Silvia Larumbe, Iñaki Pérez-Landazábal, Vicente Recarte, Cristina Gómez-Polo

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Controlled positioning of magnetic nanoparticles (MNPs) to certain body parts using external magnetic field is an actual problem of magnetic drug and gene delivery[1]. We present a review on the characteristics of external, inserted and implanted magnets and their ability to produce an alignment pose of MNPs in drug and gene delivery processes. The field strength diminishes rapidly as the target goes deeper into the body therefore one of the most profound limitations in the use of permanent and electro magnets is their poor ability to create the force necessary in order to retain the drug carrier MNPs and to focus them in the required spot of the organ being treated. Moreover, each organ requires its specific spatial distribution of MNPs in order to reach the therapeutic effect. This distribution is controlled by both spatial distributions of the magnetic field and its gradient. Thus, the latter should be optimized for the targeted organ. In this work we propose several new micromagnetic systems aimed at overcoming these hurdles and show their applicability to the controlled positioning of MNPs. Fig. 1 shows one of the proposed systems which consists of a square array of cylindrical micromagnets (microneedles) which can be inserted at the target site. We calculate distributions of the attractive force between a MNP and the micromagnets for this type of systems (example shown in fig.1).

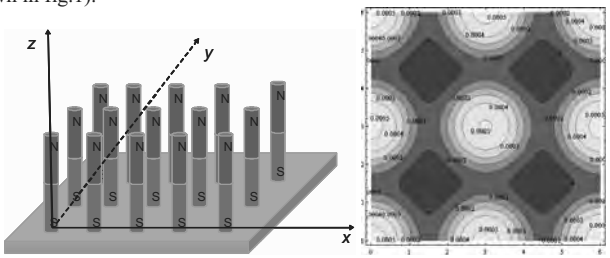

Figure 1: Tunable micromagnetic system for creation of many high-gradient areas (on the left) and the normalized force distribution in the plane z=*const* calculated using the model[2] (on the right). The light areas correspond to the highest forces.

In a magnetic needles array the force distribution and therefore the MNP distribution could be tuned by adjusting the needle diameter and period. Furthermore, magnets with periodical steps of alternative magnetization are proposed for high-peak field gradients creation in the target's specified regions. We demonstrate the benefits of the above proposed micromagnetic systems by analytical calculations of the force distributions and simulations with "Vector Field Opera 12" software, e.g.: i) an increase in the number of the captured MNPs due to one order of magnitude higher peak of the field gradient than that achieved in the commonly used systems with the same volume of magnetic material, ii) possibilities of controlled positioning of MNPs during a drug delivery process.

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Poster 225

Magnetic pressure as a scalar representation of field effects in magnetic suspensions

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Magnetic microsphere suspensions undergo complex motion when exposed to finite sources of the magnetic field, such as small permanent magnets. Understanding such motions is important in applications to cell separation, microfluidics, immunoassays, and magnetically targeted drug delivery. The computational complexity is compounded by a suitable choice of visualization as it often requires using all three components of the magnetic force vector field. A simpler approach is by showing the magnetic pressure distribution because it is a scalar quantity (p_m = **H**.**B**/2, a scalar product of the applied **H** and **B** fields, used in magnetohydrodynamics and ferrohydrodynamics) (1). Here the equilibrium distribution of the magnetic bead plug in aqueous suspension is calculated as an isosurface of the magnitude of the magnetic pressure, $p_m = const.$, in the field of two permanent magnet blocks calculated from closed formulas (2). The geometry was adapted from a publication on the magnetic bead suspensions in microsystems and the predicted bead plug distribution agrees with the experiment (3). The graph is easy to interpret in relation to the permanent magnet positions and their polarization orientation. The magnetic pressure also provides important information about expected field effects in applications to cell separation (4).

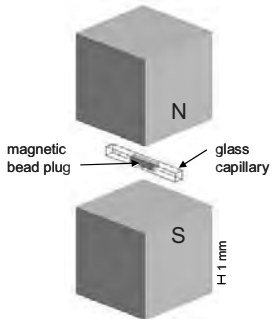

Figure The position and shape of the magnetic bead plug (ferrofluid) inside a glass capillary inserted between two permanent magnets polarized as indicated.

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Poster 226

Core-shell structured mesoporous /superparamagnetic composite spheres for targeted thrombolysis

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Urokinase plasminogen activator (uPA) is a well known enzyme and used widely for thrombolysis. In order to greatly reduce the administration doses and potential hemorrhage associated with uPA, targeted delivery is highly required. Extensive research has been carried out on encapsulation and delivery of thrombolytic enzymes based on series of carriers such as liposome[1] and polymer particles[2]. However, it was found that the overall targeting has not been substantially improved. Additionally, the accessibility of drugs decreased due to the shield effect of encapsulation structure. The employment of magnetic particles as a carrier for the targeted delivery of thrombolytic enzyme under magnetic guidance may overcome the formation of the mentioned effects by fast concentration of uPA on the pathologic site to cause targeted and effective thrombolysis[3]. Up to now, there have been several reports on the loading and release of uPA and other thrombolytic protein based on magnetic materials. However, these systems either show relatively complicated bio conjugation procedures[4] or poor magnetic response due to the difficulty in increase Fe3O4 fraction[5].

In this study, we design a new delivery system which composed of magnetic core particles and mesoporous shell structure. A facile procedure for synthesizing this novel carrier system has been verified. Utilizing MNPs synthesized by coprecipitation method as a starting material, oleic acid modified MNPs with a average diameter of 12 nm were firstly prepared. Then, using these MNPs, we successfully synthesized discrete and monodisperse silica coated submicron magnetic particles (SMPs, ~650 nm in diameter) with membrane emulsion procedure and Stöber coating method. Subsequently, a perpendicularly aligned mesoporous silica shell (~65nm in thickness) was coated on these large particles. Unlike traditional magnetic nanoparticles carrier, silica and mesoporous silica coated particles (MMS) possess high magnetic fraction and good magnetic response. More importantly, the surface area (200 m^2/g) and pore volume (0.23m^3/g) of MMS particles are relatively large. The uPA loading capacity of these materils indicated by adsorption curve is with the range of 100 U/mg to 250U/mg. Best results were obtainded when particles possess sufficiently magnetic property and mesoporous shell characteristics. High monodispersity, surface area and numerous accessible pore openings of MMS particles rendered high affinity, sorption capacity and activity maintenance of uPA. Sufficiently high magnetic response make these particles be easily targeted to local clot region with adsorbed uPA. Finally, they can reach a high clotlysis efficacy of ~90%. Further investigation need to be conducted to find out whether entrapment adsorption inside the mesopores can benefit uPA delivery while maintaining activity.

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Poster 227

Detecting Magnetically Modulated Fluorescent Probes In Turbid Media

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Imaging biochemical concentrations and biophysical properties in tissues is essential for understanding diseases and developing therapeutics. Fluorescent microparticle sensors are excellent tools for measuring local chemical concentrations. In tissue, however, background autofluorescence can obscure the probe signal, while scattering and absorption reduce the probe signal intensity. This talk discusses the use of magnetically modulated optical nanoprobes (MagMOONs) for chemical and mechanical measurements in tissue environments. MagMOONs are micro- and nanoparticles with orientation-dependent fluorescence and magnetically controlled orientation. These sensors blink when they rotate in response to rotating external magnetic fields. This blinking signal can be separated from backgrounds enabling spectrochemical sensing in highly autofluorescent media. The local viscosity can also be determined using the blinking rate. Figure 1 shows fluorescence signal intensity from MagMOONs embedded 6 mm into chicken breast tissue that intensely autofluoresces and also bleaches. The blinking MagMOON signal is reduced by a factor of 40 in the tissue due to scattering and absorption, but is easily visible over the background. Blinking was detectable through at least 9 mm of tissue, suggesting that whole mouse imaging would be feasible using red excitation.

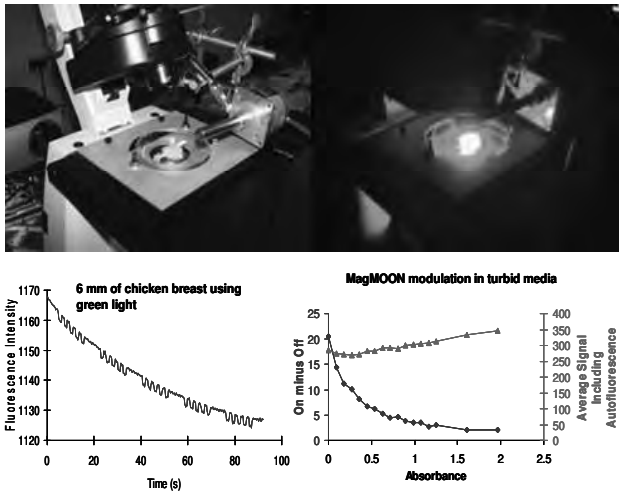

Figure 1. Fluorescent imaging in turbid chicken breast tissue. (a) Photo of the experimental setup for magnetic modulation and epifluorescence spectroscopy. The MagMOONs are in a glycerol-water solution in a capillary. This capillary is embedded in the chicken breast tissue. The MagMOONs are modulated by rotating a cylindrical NdFeB permanent magnet attached to a stepper motor or with electromagnet coils. (b) The setup seen with green excitation illumination. (c) Magnetically modulated fluorescence signal through 6 mm of chicken breast using green excitation and 565 nm long-pass emission. (d) effect of scattering (extinction) on modulated intensity (blue curve on left axis) as well as on background intensity (red curve on right axis).

Poster 228

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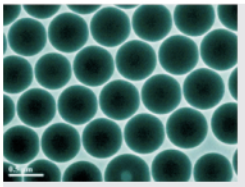
SM1-035			350nm
SM1-050			550nm
SM1-100			1200nm



AllMag® Superparamagnetic Monodisperse Silica beads possess uniform size and narrow size distribution and also high magnetic respond speed. The advantages of this type of beads favor its application in automated nucleic acids isolation and surface modification as bioconjugation carrier.

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Product Code	Beads Type	Functional Group	Mean Size
SD-100	Monodisperse	-COOH(by Silane Modification)	1200nm
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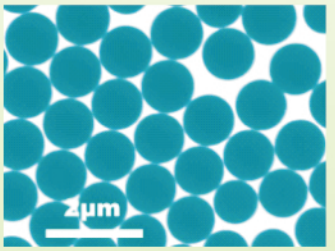

AllMag® Superparamagnetic Silica beads(-COOH) have high magnetite content, with highly active carboxyl functional group permitting the covalent attachment of proteins or ligands onto the surface of the beads, use for protein purification and separation, specific nucleic acid purification, affinity chromatography, cell isolation, and clinical diagnosis, etc.

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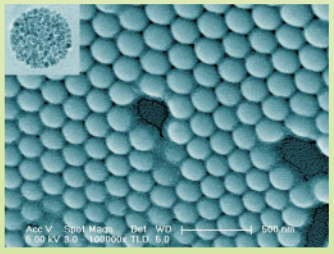

Product Code	Beads Type	Functional Group	Mean Size
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PM3-020		-COOH	180nm

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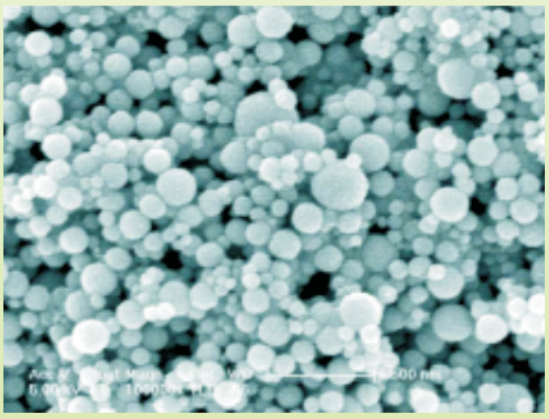

Product Code	Beads Type	Functional Group	Mean Size
SM1-015	Polydisperse	-OH	450nm
SM1-015B			>1000nm
SM1-015C			>1000nm
SM1-015D			>1000nm

AllMag® Superparamagnetic Polydispersed Silica Beads are designed for nucleic acid purification. These beads have a core-shell structure of magnetite-rich core and inorganic silica shell, with large numbers of silanol groups on the surface. This type of beads favor its application in Extraction of genomic DNA or Extraction of viral DNA/RNA, total RNA.

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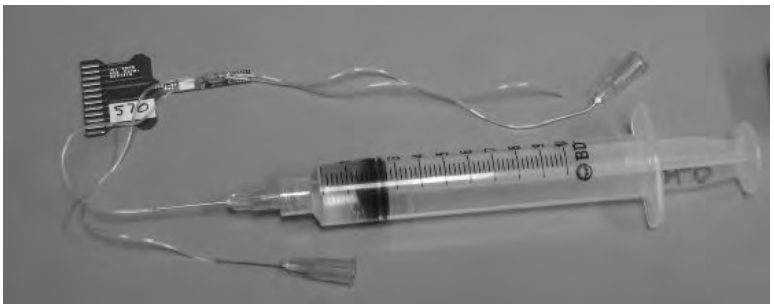

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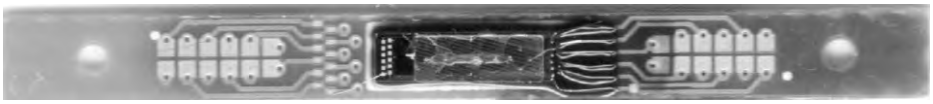

Prototype Magnetoresistive Biosensor chip with integrated microfluidic cover.

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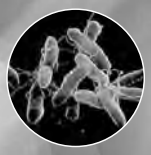
Pathogen detection

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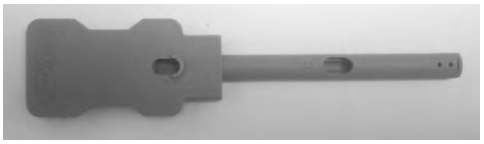

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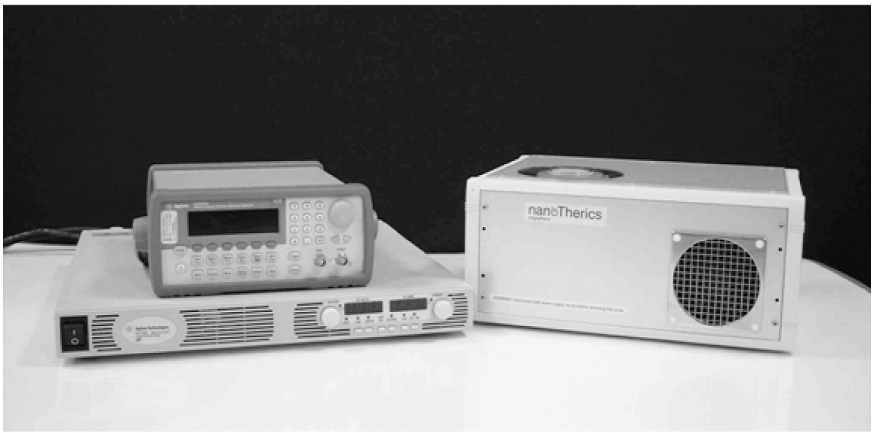
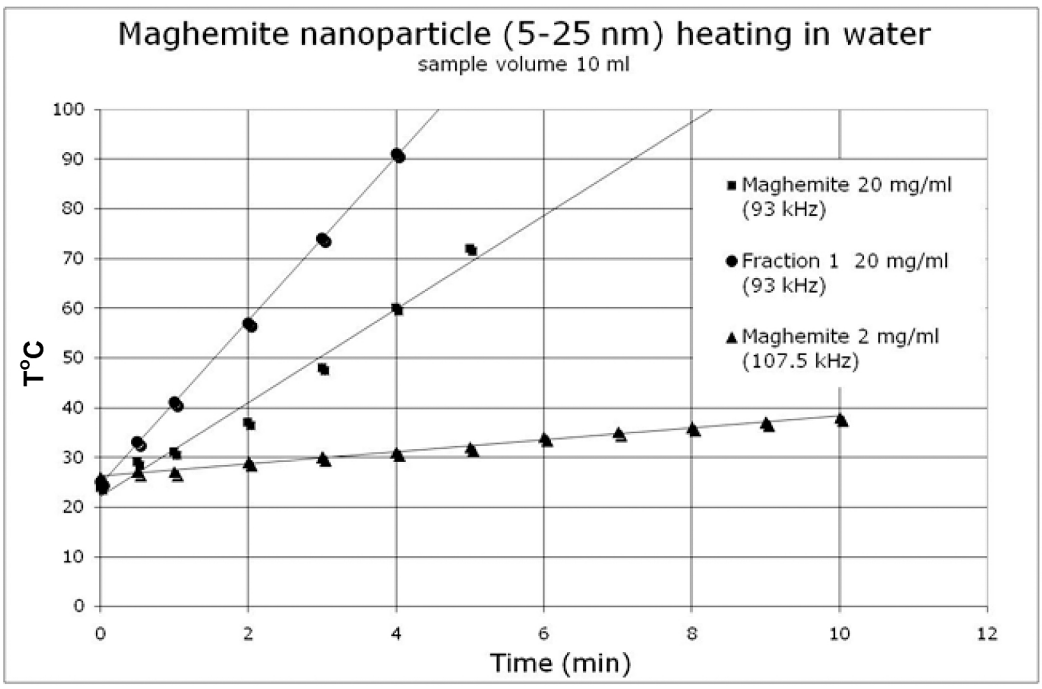


Parameters

Five different capacitor blocks and two coils (lengths 3 and 5 cm) provide 10 different frequencies nominally:
110 (25), 168 (17), 176 (23), 262 (23), 335 (17), 474 (11), 523 (20), 633 (9), 739 (16), 987 (12) kHz.

Particular frequencies and custom made coils can be supplied upon request. The sample aperture has a 44 mm diameter.

Results



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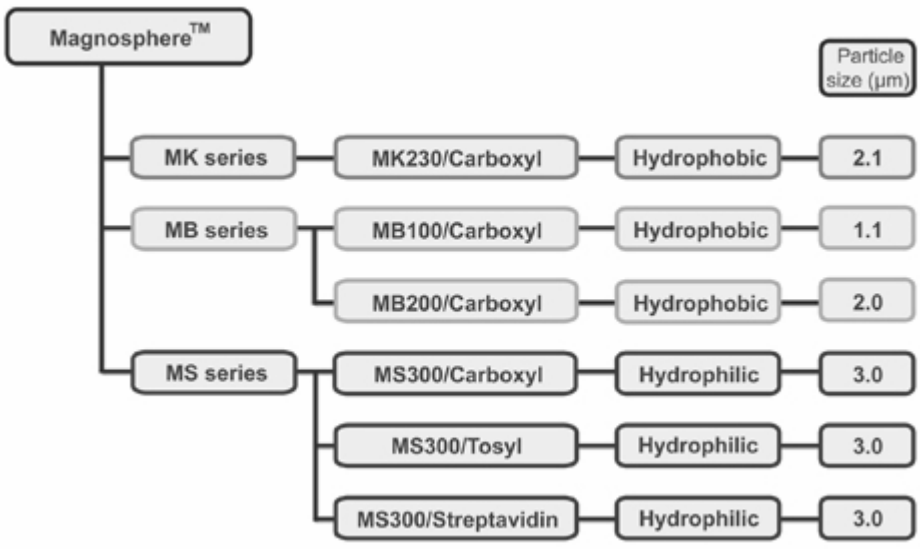

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