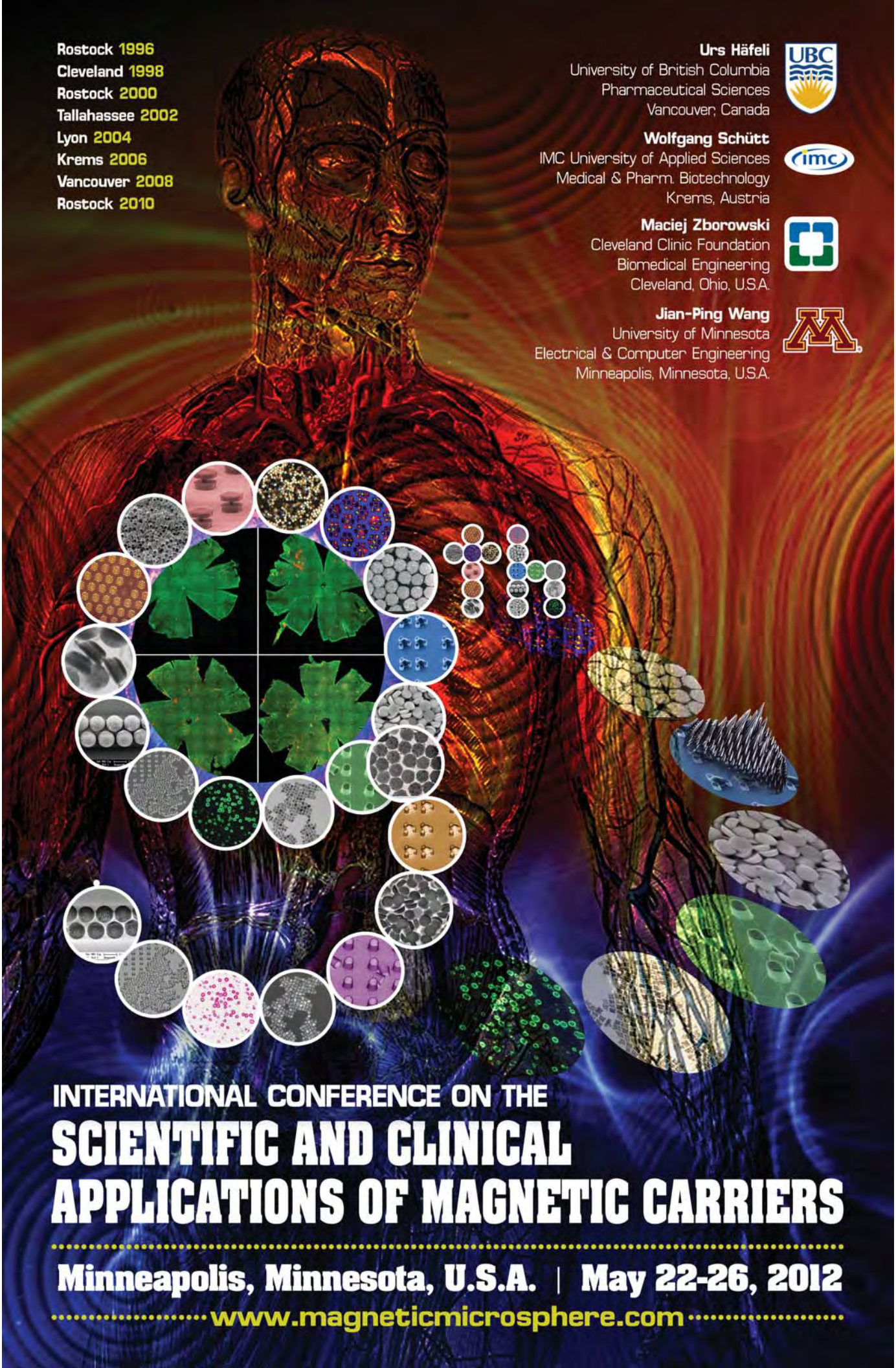

Rostock 1996
Cleveland 1998
Rostock 2000
Tallahassee 2002
Lyon 2004
Krems 2006
Vancouver 2008
Rostock 2010
Urs Häfeli
University of British Columbia
Pharmaceutical Sciences
Vancouver; Canada
Wolfgang Schütt
IMC University of Applied Sciences
Medical & Pharm. Biotechnology
Krems, Austria
Maciej Zborowski
Cleveland Clinic Foundation
Biomedical Engineering
Cleveland, Ohio, U.S.A.
Jian-Ping Wang
University of Minnesota
Electrical & Computer Engineering
Minneapolis, Minnesota, U.S.A.
INTERNATIONAL CONFERENCE ON THE
SCIENTIFIC AND CLINICAL
APPLICATIONS OF MAGNETIC CARRIERS
Minneapolis, Minnesota, U.S.A. | May 22-26, 2012
www.magneticmicrosphere.com

Coffee cup design by Guillaume Amouroux and Sam Gilchrist © 2012

Welcome Message

It is our great pleasure to welcome you all to the now already 9th 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. One of the main goals of this conference is to think about your research and that of your colleagues here, and get ready to start new collaborations. This will advance our field even more!

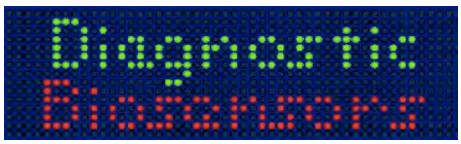
We wish you all a great conference, lots of new "aaah" moments, and just a wonderful time.

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.
Jian Ping Wang, University of Minnesota, Minneapolis, U.S.A.

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 May 23 and 25 in the McNamara Centre and May 24 in TCF Bank Stadium.



Attractions Around Minneapolis

Minneapolis Sculpture Garden: this free museum in a park filled with 40 large-scale works of art, including the iconic Spoonbridge and Cherry.

Bell Museum of Natural History: explore the biodiversity of the world at this museum at UMN

Mill City Museum: this museum is built on the ruins of the formerly world's largest flour mill. The Mill is across the river from the UMN

Grand Rounds: Hike or bike through 51-miles of urban scenic trails through the 7 districts of the city.

Mall of America: visit the 2nd largest mall in North America

Metrodome: check out the home of sports teams such as the Twins, Vikings and Gophers!

Guthrie Theater: catch a performance in this 2-stage theater located just across from the university campus.

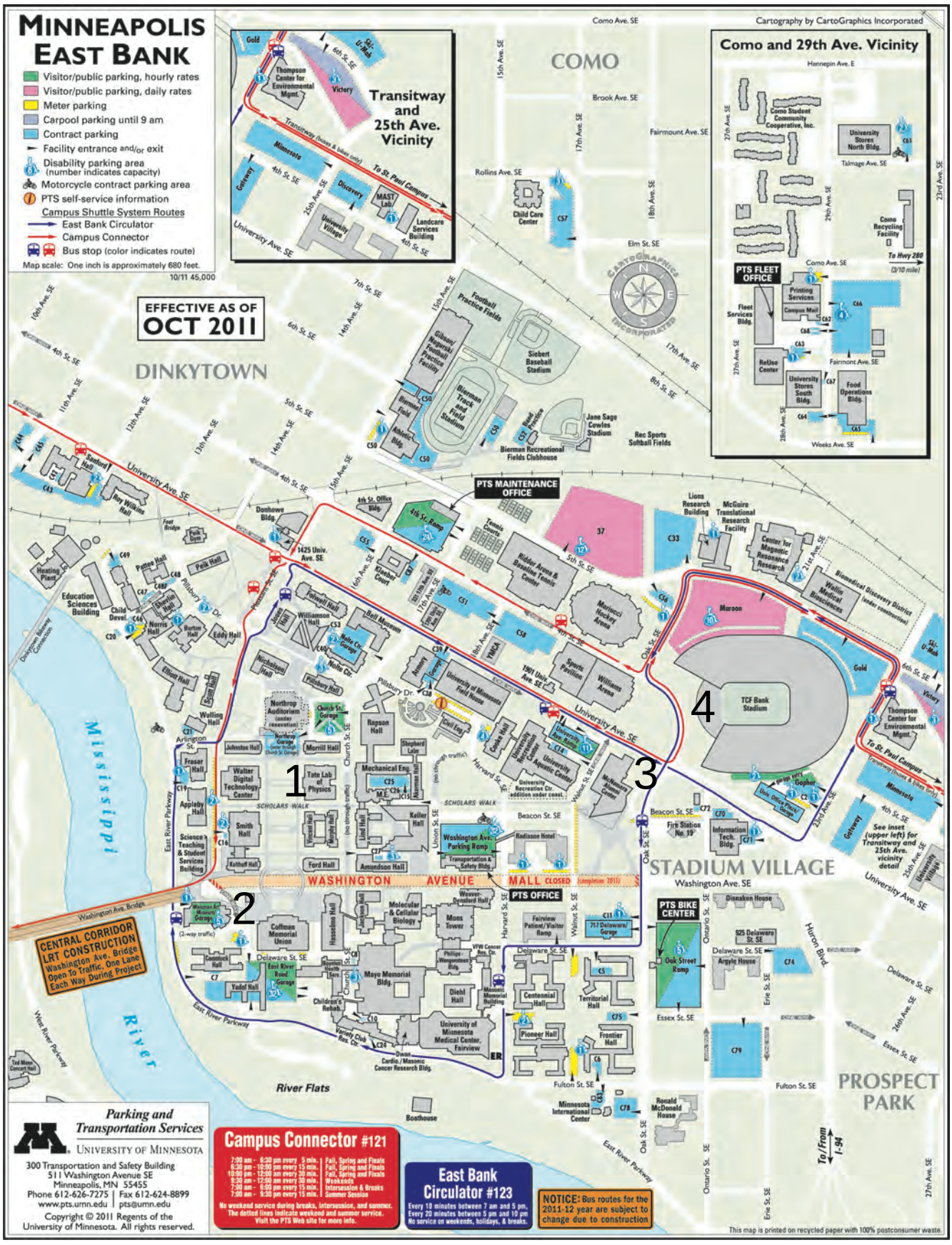
Walker Art Centre: one of the best contemporary art museums in the U.S. This museum is adjacent to UMN

For more things to do, visit Minneapolis Tourism (www.minneapolis.org)

Wireless Internet at the University of Minnesota *for guests*

1. Look for wireless networks and select **UofM Guest**.
2. Open a web browser and try to browse to any address.
3. When prompted, enter your email address and agree to UofM acceptable use policies
4. You will be connected to the internet!

UofM ECE Information Technology
612-625-5013
support@ece.umn.edu



1- Van Vleck Auditorium in the Tate Lab of Physics (Conference Talks)

2- Weisman Art Museum

3- McNamara Alumni Center

4- TCF Bank Stadium

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 18, 2012.**

We will once again 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 18, 2012** 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/).

Social Program

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

Tuesday Evening, May 22, 2012

A welcome reception will be held at the Weisman Art Museum, **open to all participants of the conference.** It will start at 6:30 PM and go till 11 PM. Chemicell and TurboBeads generously sponsored this reception. The Museum will remain open to explore during our reception. Conference registration will be available beginning outside the Dolly Fitterman Room at 5:00 PM till the end of the reception.

Wednesday Evening, May 23, 2012

After the talks, there will be a poster session with Beer and Pretzels at the McNamara Alumni Centre generously sponsored by Diagnostic Biosensors. This is followed by a banquet and social evening, all included in the registration!

Thursday, May 24, 2012

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 at TCF Bank Stadium with Beer and Pretzels generously sponsored by Stemcell Technologies, Inc. The rest of the evening is free to explore Minneapolis!

Friday Evening, May 25, 2012

On this evening, we will have our traditional boat cruise down the Mississippi.

Saturday, May 26, 2012

The meeting will end at 4 PM.

9th International Conference on the Scientific and Clinical Applications of Magnetic Carriers - Minneapolis, U.S.A.

Tuesday, May 22, 2012 *Program as of 16/5/2012*

12:00	**Registration desk opens in the Van Vleck auditorium** until the end of the BioMax satellite symposium			
13:30	**BioMax satellite symposium** "Novel diagnostic bio-assays based on magnetic particles" - Part 1 and 2 - free to all participants!			
15:10	**Coffee break**			
15:30	**BioMax satellite symposium** "Novel diagnostic bio-assays based on magnetic particles" - Part 3 and 4 - free to all participants!			
17:30	End of BioMax satellite symposium			
18:30	Registration desk opens in the Dolly Fiterman Riverview Gallery until the end of the reception / welcome cocktail			
18:30	**Informal reception and welcome cocktail (Apero) in the Dolly Fiterman Riverview Gallery** - *generously sponsored by **TurboBeads** and **Chemicell***			
22:00	End of reception			

Wednesday, May 23, 2012

7:30	**Registration desk opens / Posters with odd numbers can be put up** today during the lunch break (pins will be provided)			
	Opening Session			
9:00	Hafeli/Schuett/Zborowski/Ping	Opening of the conference / Welcome		
9:10	Hafeli, Urs	Short review of the last 2 years of magnetic carriers research	Vancouver, Canada	Talk 0
9:30	**Sooryakumar, Ratnasingham**	**Mobile Magnetic Tweezers: From Research Tool to Engineering Applications and Biomedical Diagnostics**	Columbus, USA	Invited talk 1
10:15	**Coffee break** *sponsored by **Strem Chemicals***			
	Session 1: Nanotechnology			
10:45	Ivkov, Robert	Assessing murine hepatic and splenic injury from inadvertent heating of systemically delivered non-targeted magnetic nanoparticles	Baltimore, USA	Talk 1
11:00	Jing, Ying	Design and Demonstration of Biocompatible Fe-Si Nanoparticles for Cancer Therapy	Minneapolis, USA	Talk 2
11:15	Lu, Yi-Ching	Tea Catechins Enhance Nanoparticle Uptake by Glioma Cells	Tao-Yuan, Taiwan	Talk 3
11:30	Mitroova, Zuzana	Magnetoferritin in aqueous suspensions: effect of loading factor	Kosice, Slovakia	Talk 4
11:45	Winter, Jessica	Magnetic Nanoconveyer Belts for Cell and Molecular Separation	Columbus, USA	Talk 5
12:00	Sandre, Olivier	Droplet microfluidics to prepare magnetic polymer vesicles and to confine the heat in hyperthermia	Bordeaux, France	Talk 6
12:15	**Lunch at McNamara Center / Poster session / Exhibitors**			
	Session 2: Biosensors			
13:45	Dias, Tomás	Assessment of cell-free DNA integrity using a Magnetoresistive Chip-based platform for cancer diagnostic purposes	Lisbon, Portugal	Talk 7
14:00	Hein, Matt	Magnetic Nanocilia Sensors and Actuators	Minneapolis, USA	Talk 8
14:15	Lee, Hakho	Magnetic nanoparticle and NMR-based sensor for medical diagnosis	Boston, USA	Talk 9
14:30	Østerberg, Frederik	On-chip measurements of Brownian relaxation vs. concentration of 40 nm magnetic beads	Lyngby, Denmark	Talk 10
14:45	Love, David	Hetero-coated magnetic microcarriers for point of care diagnostics	Cambridge, UK	Talk 11
15:00	van Ijzendoorn, Leo	Frequency-selective rotation of magnetic nanoparticles for rapid and sensitive solution based detection of biomolecules	Eindhoven, Netherlands	Talk 12
15:15	Wang, Shan	A New Tool for Cancer Biomarker Detection: Multiple Samples and Multiple Parameters on a Single Magnetic Biochip	Stanford, USA	Talk 13
15:30	**Coffee break**			
	Session 3: Analytical Techniques			
16:00	**St. Pierre, Tim**	**Development of FerriScan: A Personal Story from the Lab Bench to Marketing a Product**	Perth, Australia	Invited Talk 2
16:45	Odenbach, Stefan	Rheology of biocompatible ferrofluids	Dresden, Germany	Talk 14
17:00	Thünemann, Andreas	Fast and Reliable in situ Analysis of Dispersions of Magnetic Nanoparticles	Berlin, Germany	Talk 15
17:15	Usselman, Robert	Modeling Nanoparticle Properties By Electron Magnetic Resonance	Boulder, USA	Talk 16
17:30	Anker, Jeffrey	Magnetic Luminescent Core-Shell Particles for Drug Delivery and Imaging	Clemson, USA	Talk 17

17:45	**Poster session (odd numbers) with beer and pretzels - PLEASE RATE POSTERS** - *sponsored by* ***Diagnostic Biosensors***			
19:45	**Banquet and social evening in the McNamara Center, included in registration fee** - *sponsored by* ***micromod*** **- please remove posters by end of banquet**			
22:00				
Thursday, May 24, 2012				
8:00	**Registration desk opens / Posters with even numbers can be put up** today during the lunch break (pins will be provided)			
8:30	**Hawkins, Peter**	**Tutorial I - Magnetic Particles in Immunoassays**	Bristol, UK	Tutorial 1
	Session 4: Magnetic Nanoparticle Synthesis			
9:00	Andrew, Jennifer	Bi-phasic Magnetic Materials: Towards Multifunctional Contrast Agents	Gainesville, USA	Talk 18
9:15	Becker, Sören	Using nanotechnology for the formation of multimodal marker for in vivo ß-cell labeling	Hamburg, Germany	Talk 19
9:30	Jain, Nirmesh	Penetration of Solid Tumors by Sterically Stabilized Magnetic Nanoparticles	Sydney, Australia	Talk 20
9:45	Huber, Dale	Synthesis of Iron Oxide Nanoparticles by the in situ Generation of Iron Oleate	Albuquerque, USA	Talk 21
10:00	Lak, Aidin	Large single-core iron oxide nanoparticles: a tracer for magnetorelaxometry immunoassays	Braunschweig, Germany	Talk 22
10:15	**Coffee break**			
10:45	Marquina, Clara	MgO-coated magnetite nanoparticles for detection and treatment of Fusarium spp. fungi	Zaragoza, Spain	Talk 23
11:00	Niehaus, Jan	Adjusting the MRI contrast by formation of defined SPIO clusters	Hamburg, Germany	Talk 24
11:15	Peng, Mingli	A novel approach of transferring oleic acid capped iron oxide nanoparticles to water phase	Xi'an, China	Talk 25
11:30	Roig, Anna	Magnetically Labeled Endothelial Progenitor Cells for Cellular Therapy in Brain Ischemia Treatment	Bellaterra, Spain	Talk 26
11:45	Thanh, Nguyen T.	One-pot synthesis of hybrid magnetic and fluorescent core-shell structure of FePt@CdSe Nanoparticles	London, UK	Talk 27
12:00	**Guo, Haibo**	**Morphological Mapping of Iron Oxide Nanoparticles Using Thermodynamic Modeling**	Melbourne, Australia	Invited talk 3
12:45	**Lunch in the TCF Bank football stadium, DQ Clubroom / Exhibitors**			
	Session 5: Magnetic Hyperthermia			
14:30	**Rinaldi, Carlos / Ivkov, Robert**	**Debate: Do We Really Know How Magnetic Hyperthermia Works?** ***Moderated by Kannan Krishnan***	Baltimore/Mayaguez, USA	Invited talk 4
15:15	Borca-Tasciuc, Diana-Andra	Potential sources of errors in measuring and evaluating the specific absorption rate of magnetic nanoparticles in alternating magnetic field	Troy, USA	Talk 28
15:30	Creixell, Mar	EGFR-Targeted Magnetic Nanoparticles Kill Cancer Cells Under the Application of a Magnetic Field Without a Perceptible Rise in Temperature	Mayagüez, USA	Talk 29
15:45	Dennis, Cindi	Effect of Internal Magnetic Structure of Iron Oxide Magnetic Nanoparticles on the Field Dependence of the SLP for Hyperthermia	Gaithersburg, USA	Talk 30
16:00	Dutz, Silvio	Magnetic hyperthermia - Immobilisation state of magnetic multicore nanoparticles injected into living tumors	Vancouver, Canada	Talk 31
16:15	Goya, Gerardo	Is the magnetic hyperthermia mechanism a universal one? The case of dendritic cells.	Zaragoza, Spain	Talk 32
16:30	Hadjipanayis, Constantinos	Thermotherapy of Experimental Glioblastoma with Laponite-Embedded Magnetic Iron-Oxide Nanoparticles	Atlanta, USA	Talk 33
16:45	Bernau, Vianney	Injectable Nanocomposite for local Hyperthermia	Lausanne, Switzerland	Talk 34
17:00	Southern, Paul	Inter-Particle Interactions in the Static and Dynamic Magnetic Properties of Ferucarbotran Colloids	London, UK	Talk 35
17:15	**Group photograph**			
17:30	**Poster session (even numbers) with beer and pretzels - PLEASE RATE POSTERS** - *sponsored by* ***StemCell Technologies*** **/** ***Exhibitors***			
20:00	**Free evening - use it to meet old friends, discuss new collaborations, and enjoy Minneapolis on your own! - please remove posters by end of the evening!**			
Friday, May 25, 2012				
8:00	**Registration desk opens** / No poster sessions today!			
8:30	**Hawkins, Peter**	**Tutorial II - Magnetic Particles in Immunoassays**	Bristol, UK	Tutorial 2
	Session 6: Magnetic Imaging / MPI			
9:00	Bales, Brian	GEH121333: A New Iron Oxide Nanoparticle for Magnetic Resonance Imaging in Inflammation and Oncology	Niskayuna, USA	Talk 36
9:15	Begin-Colin, Sylvie	Why a dendritic approach to biocompatible iron oxide nanoparticles for bioimaging ?	Strasbourg, France	Talk 37
9:30	Kim, Jongsik	High Speed Magnetomotive Optical Coherence Tomography	Urbana, USA	Talk 38

9:45	Liebl, Maik	In-Vivo Quantification of Magnetic Nanoparticle Distributions after Magnetic Drug Targeting in a Rabbit Carcinoma Model using Magnetorelaxometry	Berlin, Germany	Talk 39
10:00	**Coffee break**			
10:30	Saville, Steven	The Influence of Linear Magnetic Chain Formation on Transverse Relaxation Rates in Magnetic Resonance Imaging	Clemson, USA	Talk 40
10:45	Rahn, Helene	Quantitative Tomographic Examination of Magnetic Nanoparticles	Dresden, Germany	Talk 41
11:00	Riffle, Judy	Magnetic Block Ionomer Clusters (MBIClusters) with Ultrahigh Transverse NMR Relaxivities for Dual Imaging and Therapeutic Agents	Blacksburg, USA	Talk 42
11:15	Trekker, Jesse	Optimizing Superparamagnetic Nanoparticles towards Contrast Enhanced MRI for sensitive in vivo cell detection	Leuven, Belgium	Talk 43
11:30	**Krishnan, Kannan**	**Developing Tracers for Enhanced Resolution and Sensitivity in MPI**	Seattle, USA	Invited talk 5
12:15	**Lunch at McNamara Center / Exhibitors**			
12:30	**POSTER PRIZE** - *Presented by Cordula Gruettner during the lunch*			
	Session 7: Magnetic Separation			
13:30	Herrmann, Inge	Cleaning Blood: Applications of Ultra-strong Metal Nanomagnets in Nanomedicine	Zurich, Switzerland	Talk 44
13:45	Benelmekki, Maria	Magnetophoretic transport of non-magnetic latex colloidal particles across a magnetic fluid volume under a uniform magnetic field gradient	Braga, Portugal	Talk 45
14:00	Dapprich, Johannes	Identification of New Sequence Variants by Targeted Enrichment and Next-Generation Sequencing	Lawrenceville, USA	Talk 46
14:15	Dutz, Silvio	A Microfluidic Chip for Size Dependent Fractionation of Magnetic Microspheres	Vancouver, Canada	Talk 47
14:30	Ijiri, Yumi	Novel method for magnetic field and size based purification of magnetic nanoparticles	Oberlin, USA	Talk 48
14:45	Ooi, Chin Chun	Effect of Magnetic Field and Magnetic Field Gradient on Effectiveness of the Magnetic Sifter for Cell Purification	Stanford, USA	Talk 49
15:00	Plouffe, Brian	Magnetic Particle-Based Microfluidic Circulating Tumor Cell Separation for Oncological Therapeutic Monitoring	Boston, USA	Talk 50
15:15	Samia, Anna Cristina	pH-Controlled Adsorption of Cd ions on Carboxyl-Terminated Superparamagnetic Iron Oxide Nanoparticles	Cleveland, USA	Talk 51
15:30	Sharma, Anirudh	Magnetic barcode nanowires for osteosarcoma cell control, detection and separation	Minneapolis, USA	Talk 52
15:45	Yang, Liangrong	A novel separation technique: gas-assisted superparamagnetic extraction for scale-up of protein isolation (bovine serum albumin BSA)	Beijing, China	Talk 53
16:00	**Coffee break**			
	Session 8: Magnetic Gene Deliver / Interesting Magnet Systems			
16:30	Schuerle, Simone	An Electromagnetic Manipulation System for Pre-clinical Testing of Targeted Drug Delivery	Zurich, Switzerland	Talk 54
16:45	Mykhaylyk, Olga	Silica-Iron Oxide Magnetic Nanoparticles for Viral Gene Delivery	Munich Germany	Talk 55
17:00	Zimmermann, Katrin	Optimization of magnetic nanoparticles assisted lentiviral gene transfer and cell positioning	Bonn, Germany	Talk 56
17:15	Shapiro, Benjamin	Treating Tinnitus by Magnetic Pushing of Therapy: Rat Experiments	College Park, USA	Talk 57
17:30	Dumas-Bouchiat, Frederic	Magnetic tool for in-vivo magnetic capture: Applications in blood filtering and proteomic diagnostics	Grenoble, France	Talk 58
17:45	Nacev, Alek	Improving the Treatment of Hypoxic Breast Cancer Liver Metastases by using Dynamic Magnetic Shift	College Park, USA	Talk 59
18:00	**Traditional boat cruise on the Mississippi** - **Buses leave in front of the University Hotel ;** dress appropriately, it gets windy on the boat!			
Saturday, May 26, 2012				
8:00	**Registration desk opens**			
8:30	**Hawkins, Peter**	**Tutorial III - Magnetic Particles in Immunoassays**	Bristol, UK	Tutorial 3
	Session 9: Magnetic Drug Delivery			
9:00	Bahadur, Dhirendra	Hyperthermia and other stimulants for drug release: in vitro and in vivo investigations through nano particulates and hybrids	Mumbai, India	Talk 60
9:15	Brazel, Christopher	Magnetite Nanoparticle-Loaded Poly(caprolactone-b-ethylene glycol) Micelles as Magnetic-Heating Activated Carriers for Anticancer Drugs	Tuscaloosa, USA	Talk 61
9:30	Gabbasov, Raul	Biodegradation of Magnetic Nanoparticles in Mouse Liver from Combined Analysis of Mössbauer and Magnetization Data	Moscow, Russia	Talk 62
9:45	Kempe, Maria	Magnetic Nanoparticles for Implant-Assisted Magnetic Drug Targeting in Cardiovascular Medicine	Lund, Sweden	Talk 63
10:00	Klostergaard, Jim	Anti-Tumor Activity of Drug-Loaded Magnetically Responsive Nanoparticles	Houston, USA	Talk 64

10:15	**Coffee break**			
11:00	Prina-Mello, Adriele	Multiparametric toxicity evaluation of SPIONs as identification for multifunctional nanoparticles for theranostic applications	Dublin, UK	Talk 65
11:15	Tietze, Rainer	Mitoxantrone delivery via Magnetic Drug Targeting for tumor therapy-Characterisations and biological outcome	Erlangen, Germany	Talk 66
11:30	**Gregory-Evans, Kevin**	**Focused Magnetic Stem Cell Targeting to the Retina Using Magnetic Nanoparticles**	Vancouver, Canada	Invited talk 6
12:15	**Lunch boxes at Van Vleck Auditorium**			
	Session 10 : Biological Applications			
13:15	Riegler, Johannes	Magnetic delivery of mesenchymal stem cells after arterial injury	London, UK	Talk 67
13:30	Tefft, Brandon	Magnetizable Duplex Steel Stents Enable Endothelial Cell Capture	Rochester, USA	Talk 68
13:45	Bonnaud, Cecile	Magnetic Janus Liposomes: Design of magnetic thermosensitive biomembranes for MRI and drug delivery	Marly, Switzerland	Talk 69
14:00	Marten, Gernot	Modular Construction of Tailor-Made Bioactive Hybrid Nanoparticles	Vancouver, Canada	Talk 70
14:15	Gazova, Zuzana	BSA-modified magnetic fluids as therapeutic agents targeting insulin-associating amyloidosis	Kosice, Slovakia	Talk 71
14:30	Guan, Yueping	Peroxidase-like activity of magnetic nanoparticles and their applications in immunoassay	Beijing, China	Talk 72
14:45	Gutierrez, Lucia	Renal iron load in sickle cell disease determined by magnetic resonance imaging measurements	Perth, Australia	Talk 73
15:00	Hight Walker, Angela	Bismuth-doped Cobalt Ferrite Nanoparticles for MRI and CT Contrast Enhancement	Gaithersburg, USA	Talk 74
15:15	Horak, Daniel	Anti-EpCAM-Immobilized Albumin-Coated Monodisperse Magnetic Poly(Glycidyl Methacrylate) Microspheres for Detection of Circulating Tumor Cells	Prague, Czech Rep.	Talk 75
15:30	Lee, Hakho	Novel core-shell magnetic nanoparticles as highly efficient contrasting agents for magnetic resonance detection	Boston, USA	Talk 76
15:45	**Closing Comments and Announcement of the NEXT MEETING: Urs Hafeli / Stefan Odenbach**			
16:00	**Meeting ends**			

Talk and Poster Abstracts

Tuesday, May 22, 2012 at 1:30 PM

BioMax Satellite Symposium "Magnetic Particle Biosensors"

Van Vleck Auditorium, University of Minnesota, Minneapolis, MN, USA

13:30 – 13:40	Welcome and introduction to BioMax: "Novel diagnostic Bio-Assays based on Magnetic Particles" Jeroen Lammertyn (Katholieke Universiteit Leuven, Belgium)
First topic:	**Molecular architectures for magnetic particle biosensing**
13:40 – 14:10	"Solid-Phase Proximity Ligation Assay a Sensitive Molecular Tool for Protein Detection" Masood Kamali-Moghaddam (Uppsala University, Sweden)
Second topic:	**Homogeneous assays with magnetic particles**
14:10 – 14:40	"Experiments on self assemblies of magnetic colloids" Jean Baudry (Universite Pierre et Marie Curie, ESPCI, France)
14:40 – 15:10	"Magnetic particle based capturing and detection for sensitive immunoassays" Arthur de Jong (Eindhoven University of Technology, The Netherlands)
15:10 – 15:30	Coffee break
Third topic:	**Microfluidic technologies for magnetic particle assays**
15:30 – 16:00	"Applications of magnetic particle-based labs-on-a-chip" Martin Gijs (Ecole Polytechnique Fédérale de Lausanne, Switzerland)
16:00 – 16:30	"Manipulating magnetic beads on a digital microfluidic platform for bio-assay development" Jeroen Lammertyn (Katholieke Universiteit Leuven, Belgium)
Fourth topic:	**Scientific challenges for industrial magnetic particle biosensors**
16:30 – 17:00	"Science and technology for magnetic particle biosensing" Ron van Lieshout (Philips Research, The Netherlands)
17:00 – 17:30	"Present and future applications of magnetic particles in in-vitro diagnostics applications" Mike Martens (Future Diagnostics, The Netherlands)

MARIE CURIE ACTIONS

BioMaX

SEVENTH FRAMEWORK PROGRAMME

Wednesday, May 23, 2012

7:30	**Registration desk opens / Posters with odd numbers can be put up** today during the lunch break (pins will be provided)			
	Opening Session			
9:00	Hafeli/Schuett/Zborowski/Ping	Opening of the conference / Welcome		
9:10	Hafeli, Urs	Short review of the last 2 years of magnetic carriers research	Vancouver, Canada	Talk 0
9:30	**Sooryakumar, Ratnasingham**	**Mobile Magnetic Tweezers: From Research Tool to Engineering Applications and Biomedical Diagnostics**	Columbus, USA	Invited talk 1
10:15	**Coffee break** *sponsored by* ***Strem Chemicals***			
	Session 1: Nanotechnology			
10:45	Ivkov, Robert	Assessing murine hepatic and splenic injury from inadvertent heating of systemically delivered non-targeted magnetic nanoparticles	Baltimore, USA	Talk 1
11:00	Jing, Ying	Design and Demonstration of Biocompatible Fe-Si Nanoparticles for Cancer Therapy	Minneapolis, USA	Talk 2
11:15	Lu, Yi-Ching	Tea Catechins Enhance Nanoparticle Uptake by Glioma Cells	Tao-Yuan, Taiwan	Talk 3
11:30	Mitroova, Zuzana	Magnetoferritin in aqueous suspensions: effect of loading factor	Kosice, Slovakia	Talk 4
11:45	Winter, Jessica	Magnetic Nanoconveyer Belts for Cell and Molecular Separation	Columbus, USA	Talk 5
12:00	Sandre, Olivier	Droplet microfluidics to prepare magnetic polymer vesicles and to confine the heat in hyperthermia	Bordeaux, France	Talk 6
12:15	**Lunch at McNamara Center / Poster session / Exhibitors**			
	Session 2: Biosensors			
13:45	Dias, Tomás	Assessment of cell-free DNA integrity using a Magnetoresistive Chip-based platform for cancer diagnostic purposes	Lisbon, Portugal	Talk 7
14:00	Hein, Matt	Magnetic Nanocilia Sensors and Actuators	Minneapolis, USA	Talk 8
14:15	Lee, Hakho	Magnetic nanoparticle and NMR-based sensor for medical diagnosis	Boston, USA	Talk 9
14:30	Østerberg, Frederik	On-chip measurements of Brownian relaxation vs. concentration of 40 nm magnetic beads	Lyngby, Denmark	Talk 10
14:45	Love, David	Hetero-coated magnetic microcarriers for point of care diagnostics	Cambridge, UK	Talk 11
15:00	van Ijzendoorn, Leo	Frequency-selective rotation of magnetic nanoparticles for rapid and sensitive solution based detection of biomolecules	Eindhoven, Netherlands	Talk 12
15:15	Wang, Shan	A New Tool for Cancer Biomarker Detection: Multiple Samples and Multiple Parameters on a Single Magnetic Biochip	Stanford, USA	Talk 13
15:30	**Coffee break**			
	Session 3: Analytical Techniques			
16:00	**St. Pierre, Tim**	**Development of FerriScan: A Personal Story from the Lab Bench to Marketing a Product**	Perth, Australia	Invited Talk 2
16:45	Odenbach, Stefan	Rheology of biocompatible ferrofluids	Dresden, Germany	Talk 14
17:00	Thünemann, Andreas	Fast and Reliable in situ Analysis of Dispersions of Magnetic Nanoparticles	Berlin, Germany	Talk 15
17:15	Usselman, Robert	Modeling Nanoparticle Properties By Electron Magnetic Resonance	Boulder, USA	Talk 16
17:30	Anker, Jeffrey	Magnetic Luminescent Core-Shell Particles for Drug Delivery and Imaging	Clemson, USA	Talk 17
17:45	**Poster session (odd numbers) with beer and pretzels - PLEASE RATE POSTERS** - *sponsored by* ***Diagnostic Biosensors***			
19:45	**Banquet and social evening in the McNamara Center, included in registration fee** - *sponsored by* ***micromod*** **- please remove posters by end of banquet**			
22:00				

Mobile Magnetic Tweezers: From Research Tool to Engineering Applications and Biomedical Diagnostics

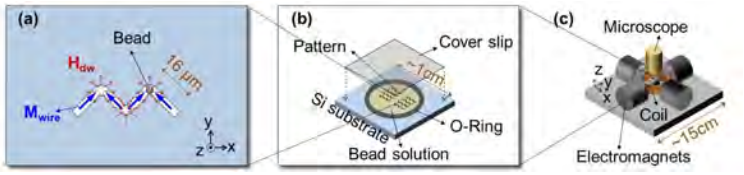
R. Sooryakumar

Department of Physics
The Ohio State University
Columbus, OH 43210.
e-mail: sooryakumar.1@osu.edu

One of the major challenges in nanoscience, and the advancement of nanotechnology in general, is the development of precision tools for the manipulation and transport of nanoparticles and biological entities with directed forces. The difficulty of such manipulation becomes even more pronounced in a native fluid environment when stochastic Brownian motion disrupts targeted activities.

We have developed new approaches, based on programmable magnetic signatures patterned on a surface, to create microscopic transporters whose trajectories and functionalities are remotely controlled. Requiring only five tiny electromagnets, a game controller to direct the motion and the power equivalent to a 60W light bulb, tunable femto- to pico-Newton range forces guide, assemble and manipulate magnetic nano-particles, as well as labeled and unlabeled biological cells in a fluid environment.

Highlights of these joystick- and voice-activated approaches for fundamental nanoscience, engineering and medicine will be discussed as we move towards realizing new micro- /nano-scale devices and intracellular probes.



Schematic representation of: (a) Zigzag wires on Si platform with magnetizations $\mathbf{M}_{wire}$ pointing towards and away from vertices to create fields $\mathbf{H}_{dw}$ to attract magnetic beads to vertices. (b) Si platform with coverslip and O-ring to prevent fluid flow and evaporation. (c) Electromagnets and coil to generate fields in x, y and z directions. The platform is observed by a microscope.

Invited Talk 1

Assessing murine hepatic and splenic injury from inadvertent heating of systemically delivered non-targeted magnetic nanoparticles

CARMEN KUT[1], YONGGANG ZHANG[1], MOHAMMAD HEDAYATI[1], HAOMING ZHOU[1], CHRISTINE CORNEJO[1], DAVID BORDELON[1], JANA MIHALIC[2], MICHELE WABLER[1], ELIZABETH BURGHARDT[1], CORDULA GRUETTNER[3], ALISON GEYH[2], CORY BRAYTON[4], THEODORE L. DEWEESE[1], ROBERT IVKOV[1,*]

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Magnetic iron oxide nanoparticles are being considered for therapeutic delivery of cell-specific heat for cancer following systemic administration. To achieve the goal of a minimally-invasive theranostic platform, the magnetic nanoparticles are often labeled with cancer-cell specific ligands, i.e. monoclonal antibodies. Much of the attention to date has focused on development of nanoparticle formulations that efficiently produce heat with alternating magnetic fields, and on the targeting molecules. Less attention has been devoted to the fact that a significant portion of an injected dose of these nanoparticulate formulations is typically sequestered by the organs of the reticuloendothelial system, a result of their physico-chemical properties. Inadvertently heating the particles located in these unintended sites during the course of cancer therapy has the potential to profoundly harm the patient – a consequence receiving little attention.

This study provides preliminary data for the potential to injure normal tissues in mice from heating magnetic nanoparticles deposited in the liver and spleen. On days 1-3, twenty-three male nude mice were administered daily intravenous injections of nanomag-D-spion (micromod Partikeltechnologie, GmbH) superparamagnetic iron oxide nanoparticles (0 mg, 1.65 and 7.2 mg). On day 6 they were exposed to an alternating magnetic field (0, 24, and 60 kA/m). On day 7, blood, liver, and spleen were harvested and analyzed for total iron content.

Mice that received high-dose and high-field exposure experienced increased mortality, elevated blood chemistry and significant liver and spleen necrosis. Mice treated with low-dose and low-field survived but had elevated LDH levels and local coagulative necrosis in the spleen.

Potential clinical use of magnetic nanoparticle hyperthermia following systemic delivery requires careful consideration and a more comprehensive toxicity study. Further, a significant amount of careful attention must be directed toward developing more refined targeting of the nanoparticles in addition to improving their heating rates.

Talk 1

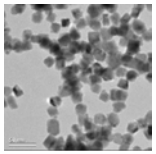
Design and Demonstration of Biocompatible Fe-Si Nanoparticles for Cancer Therapy

Ying Jing and Jian-Ping Wang

Department of Electrical Engineering, University of Minnesota

There has been extensive research on making use of the heat generated from magnetic nanoparticles under AC magnetic field for cancer treatment. In this technique, magnetic nanoparticles undergo magnetization switching and generate hysteresis loss due to different mechanisms. It is the advantage of delivering local heat that makes this technique promising for a therapy with small side effects. There is exploration of various magnetic materials in the field for candidate heating media. It's desirable to have nanoparticles made of low toxic elements and with suitable magnetic properties.

Here we study Fe-Si nanoparticles for the application of magnetic cancer therapy. This system contains only benign elements Fe and Si suitable for biomedical use. The saturation magnetization is relatively high compared to iron oxide particles. Synthesis of Fe-Si nanoparticles was accomplished by using a novel physical gas condensation method. Morphology and structure of nanoparticles were characterized by TEM. Exploration of magnetic property was conducted by VSM. AC magnetic field heating has been carried out based on those particles and showed a promising trend. This work provides a route to develop biocompatible magnetic nanoparticles for magnetic cancer therapy.



TEM image of Fe-Si Nanoparticles

Talk 2

Tea Catechins Enhance Nanoparticle Uptake by Glioma Cells

Yi-Ching Lu and Yunn-Hwa Ma*

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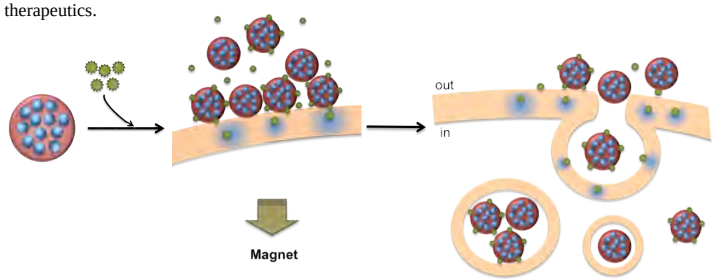
Nanoparticles may serve as a carrier for chemotherapeutic agents in target therapeutics for cancer treatment. Nanoparticle uptake by tumor cells may be critical in determining amount of the drug getting to its intracellular target. It has been demonstrated that epigallocatechin-3-gallate (EGCG), a major component of tea catechins, has been shown to bind to some membrane structures and increase membrane fluidity. We hypothesize that tea catechins may enhance nanoparticle uptake by tumor cells. In this study, uptake of dextran-coated magnetic nanoparticle (MNP) by glioma cell (LN-229) in culture was studied. EGCG administered with fluorophore-labelled MNP appeared to enhance cellular uptake of MNP within 2 hr, as observed by confocal microscopy and flowcytometry. Quantitative MNP uptake was measured with a potassium thiocyanate method. EGCG (1-20 μM) enhanced MNP uptake in a concentration-dependent manner. After 24 hr incubation with 6 μM of EGCG, cellular uptake of MNP increased by 14 fold. The effect of EGCG was further enhanced by 1.8 fold by placement of an NdFeB magnet under the culture plate, which may be due to an enhanced sedimentation induced by the magnetic field. Similar effects were observed with MNP modified with polyethylene glycol. However, EGCG did not enhance MNP uptake by human umbilical vein endothelial cells without magnetic influence, suggesting the specificity of the effects of EGCG. Although EGCG at concentrations studied exerts strong anti-oxidant activity; another anti-oxidant, curcumin did not enhance MNP uptake, suggesting the effect of EGCG may not be mediated by its anti-oxidant activity. The results suggested that EGCG may act on the plasma membrane of glioma cells or the surface of nanoparticle to enhance particle uptake. The enhancement effects of EGCG may be applicable in areas such as magnetofection, cell labeling/tracing, or target therapeutics.



Talk 3

Magnetoferritin in aqueous suspensions: effect of loading factor

Z. Mitroova[1], P. Kopcansky[1], L. Melnikova[1], M. Timko[1], M. Koralewski[2], J. W. Klos[2], M. Baranowski[2], M. Okuda[3], W. Schwarzacher[3], M.V.Avdeev[4], V.I.Petrenko[4,5], V.M.Garamus[6], L. Almasy[7]

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The aqueous suspensions of magnetoferritin (magnetic nanoparticles encased in a spherical protein shell of apoferritin, with internal and outer diameters of 8 and 12 nm) are studied regarding their structural and magnetic properties as a function of the loading factor (LF = amount of iron atom per protein molecule). They are compared with the aqueous suspensions of horse spleen ferritin (HSF) and nanoscale magnetite. The Faraday rotation effect (light wavelength range 300–680 nm) and the magnetization of the studied materials reveal similar magnetic field dependencies (typical graphs are in Fig.1) and characterize magnetoferritin suspensions as superparamagnetic system in contrast to horse spleen feritin HSF. They are described well by Langevin function with the log-normal distribution of the particle size, which allows to determine the diameter of magnetic core. For the low field region, specific Verdet constant shows a linear dependence with LF (see inset to Fig.1). These results give some evidence for the hollow structure in the case of not fully loaded core of the ferritins. The correlation of the magnetic properties with the structure of the suspensions as revealed by small-angle X-ray and neutron scattering is discussed. It is concluded that the colloidal stability of the magnetoferritin suspensions depends on the LF factor. Differences in Faraday rotation spectra allow to discriminate between maghemite or magnetite core of magnetoferritin which can be very powerful method in determination of the oxidation state of iron oxide especially useful in biomedicine.

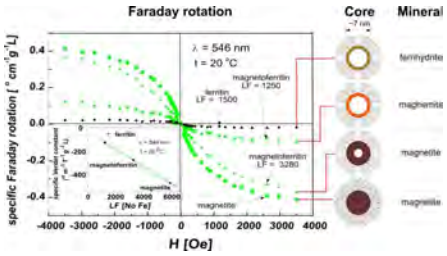

Fig. 1: Comparison of specific Faraday rotation for λ = 546 nm as a function of the applied magnetic field for ferritin, magnetoferritins and magnetite aqueous suspension. The insert shows the dependence of specific Verdet constant on loading factor.

Acknowledgements

This research was supported from the Ministry of Science and Higher Education, within grant No N N202 124535, and partially from the European Community's Seventh Framework Programme (FP7/2007-2013), Grant Agreement n° 228673 (MAGNONICS), the Projects No 26220120033 and 26110230061 in the frame of Structural Funds of European Union, Centre of Excellence of SAS Nanofluid and VEGA 0077 as well as APVV 0171-10.

Talk 4

Magnetic Nanoconveyer Belts for Cell and Molecular Separation

Kalpesh D. Mahajan[1], Greg Vieira[2], Gang Ruan[1], Jeffrey J. Chalmers[1], R. Sooryakumar[2], Jessica O. Winter[1,3*]

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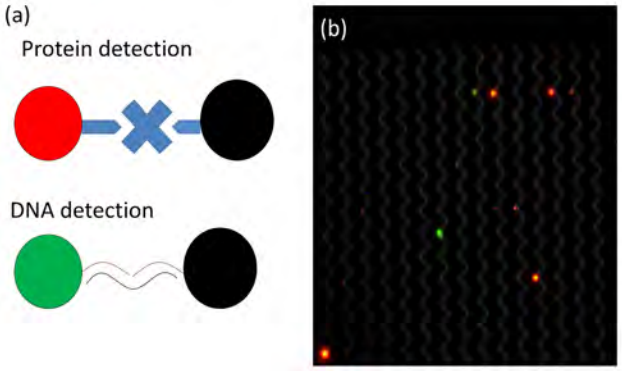
Biomarkers analysis is important not only in diagnostics, but also in monitoring disease progression and response to therapy. Because of their special physical properties and size comparable to that of biomolecules, nanomaterials can enable ultrasensitive detection of biomarker molecules. However, the majority of these schemes focus on detection only, rather than isolation of target biomolecules, which would permit further analysis and manipulation.

Here, we describe a nanoconveyer belt platform to detect and separate cells and biomolecules on the same microchip. The platform consists of magnetic quantum dot nanoparticles coupled with magnetic disc or nanowire arrays and electromagnets that apply an external magnetic field. This technology can isolate labeled cells with *in situ* surface protein quantification, and with small variations can also be used for detection and separation of molecules in very low concentration solutions. Moreover both cell separation and molecular detection of genetic markers are carried out with ultra-small sample volumes (~5 µl). With this novel nanoconveyor belt technology, we have manipulated human leucocytes on a microchip and characterized CD45 receptor expression on the cell surface *in situ*. We have also detected and separated avidin and short p53 single strand DNA from a 10^{-10} M solution either separately or multiplexed in combination. These technologies could ultimately be combined in a lab on chip design incorporating mixing, detection, magnetic separation, and characterization. This technology thus has the potential to impact a number of fields, including diagnostics, chemical synthesis, and chromatography.



Talk 5

Droplet microfluidics to prepare magnetic polymer vesicles and to confine the heat in hyperthermia

Olivier Sandre*, Damien Habault, Alexandre Déry, Jean-François Le Meins, Sébastien Lecommandoux

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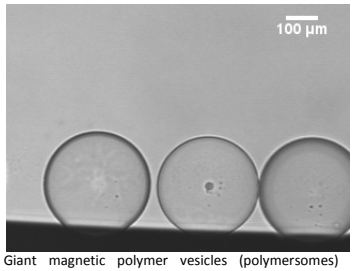
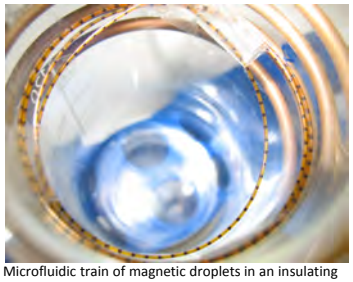


Giant magnetic polymer vesicles (polymersomes) prepared in a double-emulsion co-flows micro-chip.



Microfluidic train of magnetic droplets in an insulating oil to confine the heat produced by RF hyperthermia.

In this work, we present two types of microfluidic chips involving magnetic nanoparticles. In the first case, the nanoparticles are self assembled with an amphiphilic diblock copolymer by a double-emulsion process in order to prepare, in one step, giant magnetic vesicles (polymersomes) at a high throughput.[1,2,3] Special attention has been paid to the drying step by which the double emulsion droplets evolve to polymersomes with a thin (nanometric) magnetic membrane. In the second example, a simple emulsion chip is used in order to obtain regular trains of magnetic droplets which circulate inside an inductor coil. The heat produced by absorption of a radio-frequency magnetic field (magnetic hyperthermia) is converted into temperature increase that is measured at a sub-millimetric scale. The results are compared to heat transfer models in two limiting cases (adiabatic or dissipative). The aim is to decipher the delicate puzzle about the minimum size required for a tumor "phantom" to be heated by radio-frequency hyperthermia in a general topic of anticancer therapy.[4,5]

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-2/ *Doxorubicin Loaded Magnetic Polymersomes: Theranostic Nanocarriers for MR Imaging and Magneto-Chemotherapy*, C. Sanson, O. Diou, J. Thévenot, E. Ibarboure, A. Soum, A. Brûlet, S. Miraux, E. Thiaudière, S. Tan, A. Brisson, V. Dupuis, O. Sandre, S. Lecommandoux, ***ACS Nano* 5** (2011) 1122, 10.1021/nn102762f
-3/ *Recent trends in the tuning of polymersomes' membrane properties (Colloquium paper),* J.-F. Le Meins, O. Sandre, S. Lecommandoux,. ***Eur. Phys. J.* E 34** (2011) 14, 10.1140/epje/i2011-11014-y
-4/ *Sensitive High Frequency AC Susceptometry in Magnetic Nanoparticle Applications*. F. Ahrentorp, A. Astalan, C. Jonasson, J. Blomgren, Q. Bin, O. Thompson Mefford, M. Yan, J. Courtois, J.-F. Berret, J. Fresnais, O. Sandre, S. Dutz, R. Müller, and C. Johansson. in **The 8th International Conference on the Scientific and Clinical Applications of Magnetic Carriers**. **2010**. Rostock, Germany: AIP Conference Proceedings Series, 1311: p. 213-223, http://scitation.aip.org/dbt/dbt.jsp?KEY=APCPCS&Volume=1311&Issue=1.
-5/ *Is intracellular hyperthermia superior to extracellular hyperthermia in the thermal sense?* Y. Rabin, ***Int. J. Hyperthermia* 18** (2002) 194 10.1080/02656730110116713

Assessment of cell-free DNA integrity using a Magnetoresistive Chip-based platform for cancer diagnostic purposes

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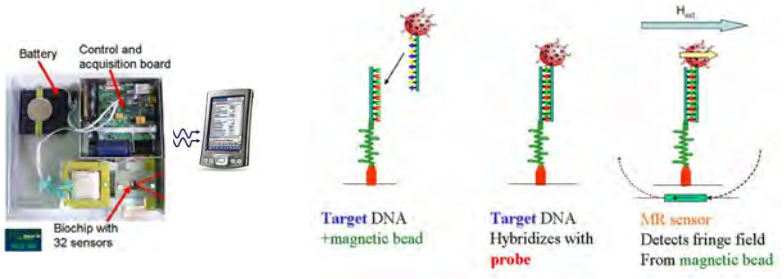
Measuring the levels of cell-free DNA (cfDNA) in blood circulation is a promising non-invasive methodology in the field of cancer diagnostics and therapy follow-up. The presence of high levels of cfDNA in blood and the production of longer cfDNA fragments are consistently verified in cancer patients when in comparison with cfDNA detected in healthy people. Thus, it has been suggested that the quantification of the cfDNA integrity in terms of the ratio between longer to shorter fragments may be valuable as a biomarker for cancer diagnostic purposes.

In this work, two particular cfDNA fragments of different sizes (115-bp and 247-bp) from the ALU repeated sequence of the human genome were targeted using for the first time a magnetoresistive chip-based platform and magnetic nanoparticles as the reporter system. The 115-bp fragment (ALU115) is representative of the total cfDNA, while the 247-bp fragment (ALU247) represents the amount of tumorogenic cfDNA. The strategy adopted in this work is based on the use of thiol-modified recognition DNA probes, specific for each of the DNA target fragments, immobilized on top of magnetoresistive sensors by means of a thiol-gold interaction. ALU115 and ALU247 were generated in single-stranded configuration by asymmetric polymerase chain reaction (PCR). During the PCR, one biotin was incorporated per newly synthesized DNA fragment. By means of a streptavidin-biotin interaction, the DNA targets were labeled with streptavidin-coated superparamagnetic nanoparticles of 250 nm. The magnetically labeled targets were then interacted with the complementary probes attached at the sensors surface and the fringe field coming from the magnetic labels was detected and correlated with the presence of the cfDNA fragments. With this strategy, concentrations in the nanomolar range were detected. In future work, the limit of detection is expected to be lowered to pico/femtomolar range, which is the range at which cfDNA specimens can usually be found within the blood and, moreover, useful for cancer diagnosis purposes.



Prototype of the magnetoresistive chip-based platform & Biomolecular target-probe hybridization detection on-chip using magnetic labels as reporter system.

Magnetic Nanocilia Sensors and Actuators

Matt Hein[1*], Mazin Maqableh[1], Michael Delahunt[3], Peter Leeman[3], Prof. Carol shields[2], Prof. Beth Stadler(PI)[1], Dr. Mark Tondra[3]

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With the advent of micro fluidics, many exciting properties of fluids at the micro/nano have been uncovered and similarly many devices harnessing the uniqueness of fluids at these scales have been produced. As these devices get smaller and sample volumes get minimized, the need for greater control of these fluids increases in order to maintain the precision needed by users in industries such as biomedical devices and chemical analysis. As is typical, nature already has a solution though recreating this solution takes creative materials processing.

In this talk the fabrication of magnetic nanocilia (biological hair-like structures) sensors will be discussed with a focus on two different sensors. One sensor is designed to sense flow and manipulate it in micro channels and the other to sense vibrations with the potential to also harness the vibrational energy. The design is unique in that it overcomes the challenges of integrating traditional silicon based sensor with plastics while at the same time using fabrications techniques that can be easily scaled for large area production and for temperature sensitive biological applications. The sensors take advantage of magnetic field shape anisotropy of high aspect ratio (60nm diameter, 60,000nm length) HCP Co nanowires with their c-axis fabricated out of plane. The Co nanowires are suspended atop a GMR (Giant Magneto Resistance) sensor where the magnetic field of the nanowires directly interacts with the antiferromagnetic coupling of the GMR sensors as they "swing" back and forth across the sensor. For the vibration sensors, the anodized aluminum oxide(AAO) with electrodeposited wires were then integrated with off-the-shelf GMR SOIC chips and the wires were exposed through etching of the AAO. For the flow sensor and manipulator, the nanowires were placed inside of embossed PMMA fluidic channels designed to integrate into disposable PMMA microfluidic GMR sensor packages. These packages were designed with our industry partner(Diagnostic Biosensors).

From nanowire motion was then detected as mV signals from the GMR sensors in a bridge configuration. Manipulation of the flow was then induced by a eternal magnet if a time varying field. The vibration sensor, when tested at earthquake frequencies(0-10Hz) on a shake table, showed a single- and double-frequency signal. A control sensor was fabricated in the same way but without the wires and only a $1f$ signal was observed due to the GMR sensors motion in the Earth's field.

General diagram demonstrating the magnetic field from magnetic celia on GMR sensor(Left). Vibration sensor on GMR SOIC package(Middle). Flow sensor/ actuator exploded view(Right)

Magnetic nanoparticle and NMR-based sensor for medical diagnosis

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One of the major challenges in medicine is the rapid and accurate measurement of protein biomarkers, cells, and pathogens in biological samples. We have recently developed an NMR-based diagnostic platform for fast, quantitative and multi-channeled detection of biological targets. Termed as DMR for diagnostic magnetic resonance, the system measures the transverse relaxation rate (R_2) of water molecules in biological samples in which target molecules or cells of interest are labeled with magnetic nanoparticles (MNPs). Local magnetic fields created by MNPs accelerate the spin–spin relaxation of water protons, increasing the R_2 of samples and thus providing a sensing mechanism. As most biological objects have negligible magnetic susceptibilities, DMR measurements can be performed with few or no sample preparation steps, allowing for fast assays. Here we present the systematic development of DMR for clinical applications: we have (i) synthesized MNPs with high magnetic moments to enhance the detection sensitivity (Fig. 1a), (ii) miniaturized the entire NMR system to enable measurements on microliter sample volumes and in multiplexed format (Fig. 1b), and (iii) optimized the assay protocol for fast and sensitive detection of bacteria and cancer cells. With these and on-going advances in system design, the DMR technology holds great promise as a high-throughput, low-cost, and portable platform in clinical and point-of-care settings.

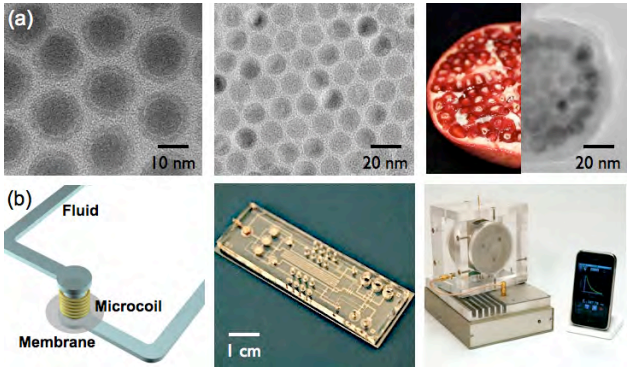

Figure 1. DMR (diagnostic magnetic resonance) platform. (a) Different types of magnetic nanoparticles developed for DMR assays. From left to right: Fe-based nanoparticles, Mn-doped ferrite, and multicore particles. **(b)** DMR system. From left to right: the schematic of an NMR probe, a microfluidic chip with integrated with NMR probes, and a portable NMR reader.

On-chip measurements of Brownian relaxation vs. concentration of 40 nm magnetic beads

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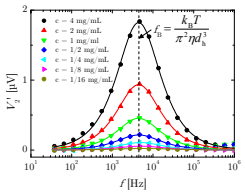
The binding of proteins or DNA to magnetic beads increase the hydrodynamic volume of the beads. Hence measurements of the bead rotation response in a liquid (Brownian relaxation) can be used for biomolecular detection in suspension[1-3]. Traditionally, these measurements are performed using commercial SQUIDs or inductive magnetometers[1-3]. We have previously shown that Brownian relaxation can also be measured in a simple lab-on-a-chip system using cross-shaped planar Hall effect sensors.[4] Decreasing the bead concentration will increase the sensitivity to the biomolecular target[3] and it is therefore important to determine the reliability of a sensor at low bead concentrations.

Here, we present Brownian relaxation measurements on a logarithmic dilution series of 40 nm beads (Ocean Nanotech) dispersed in water with bead concentrations c between 1/64 mg/mL and 4 mg/mL. The measurements are performed using a new sensor bridge geometry[5] (see inset in Fig. 2) by applying an AC bias voltage $V_{in}(t) = V_0\sin(2\pi ft)$ with $V_0 = 3.2$ V and measuring the bridge voltage V_{out} by use of lock-in technique. The field arising from the applied sensor bias is used to magnetize the beads, meaning that no external magnets are needed, and the dynamic magnetic bead response is measured as the 2nd harmonic sensor response as described in Ref. 4.

Fig. 1 shows the in-phase V_2' and out-of-phase V_2'' sensor signals, corrected for a background signal measured on a sample without beads, as function of f. These signals are proportional to the out-of-phase and in-phase magnetic susceptibility of the beads, respectively. The Brownian relaxation frequencies f_B can only be reliably extracted from fits to the Cole-Cole model for $c \geq 1/8$ mg/mL. For the six measurements with $c \geq 1/8$ mg/mL, we obtain f_B = (4.46±0.57) kHz corresponding to the hydrodynamic diameter d_h = (47.2±1.9) nm. This shows that the determination of f_B is independent of c in the investigated range. Fig. 2 shows values of V_2' vs. c obtained from 20 repeated measurements over a time interval of 7.3 min at f = 4667 Hz ($\approx f_B$) at each c. It is seen that the peak height is proportional to c and even bead concentrations down to 1/32 and 1/64 mg/mL are distinguishable. This shows that the bead concentration can be extracted from a single measurement at $f = f_B$. In summary, we have demonstrated that our bridge sensor design can be used for reliable measurements of f_B for 40 nm beads down to bead concentrations of 1/8 mg/mL with a Brownian relaxation response proportional to the bead concentration.



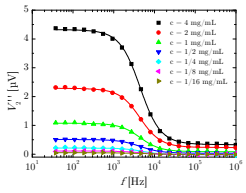


Fig 1: Second harmonic in-phase (left) and out-of-phase (right) sensor signals measured vs. frequency f for the indicated bead concentrations c. Signals are corrected using a background signal measured on a sample without beads. The solid lines are fits to the Cole-Cole model.

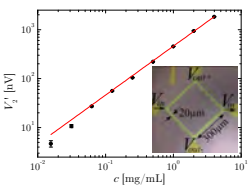


Fig 2: Second harmonic in-phase sensor response vs. bead concentration c measured at f=4667 Hz. The inset shows the geometry of the planar Hall effect bridge sensor.[5]

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Hetero-coated magnetic microcarriers for point-of-care diagnostics

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We report on the latest advances in the development of our magnetic encoded microcarriers [1]. Thin magnetic strips ('bits') are encapsulated in a biocompatible polymer backbone to form `tags'. The tags can be used to generate a large library of magnetically labelled bio-chemical analytes. Since the magnetic encoding can be applied post fabrication, all microcarriers are nominally identical, which makes them a cost effective micro-tagging strategy [2]. The number of unique codes doubles with every extra bit added, which also makes magnetic encoding extremely scalable. For instance a 7-bit tag offers 2^7=128 codes, but a 32-bit tag would offer over 4 billion unique codes. Applications range from DNA/protein analysis for genotyping and point-of-care diagnostics to drug development and combinatorial chemistry.

At the last meeting, we focussed on some novel aspects of SU8 surface chemistry and the effects of various linker molecules on binding efficiency [3]. Since then we have introduced a thin layer of gold on to one side of the microcarriers to provide a second functional coating. With this, we can now pursue two different chemical routes (carbodiimide chemistry and thiol containing self assembled monolayers) to add particular probe molecules to each side. While one probe remains specific to the analyte of interest, the other acts as a hybridisation control to interrogate the assay's binding conditions.

The complimentary target (pre-labelled with TAMRA) is added to the sample serum as a positive control. This eliminates the possibility of seeing no fluorescence and not being confident of whether the analyte was absent (true negative) or whether the binding conditions were insufficient (false negative). The target strand can be labelled with a different colour, e.g. with PicoGreen in an additional step, so that now a positive result requires the microcarrier to fluoresce red on one side and green on the other. As can be seen from the figure, there is a clear signature (peaks vs troughs) in the intensity profile corresponding to the microcarrier's orientation.

The microcarriers are read in-flow through a 50μm wide channel, which includes a TMR sensor able to detect the stray field (magnetic signature) of the passing microcarrier [4]. Thus, by combining the fluorescence data and the TMR data it is possible to conduct a multiplexed assay very quickly and cost-effectively on a small footprint device.

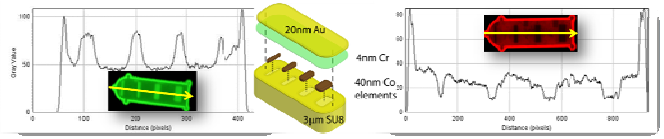

Figure: Exploded schematic of a hetero-functional magnetic microcarrier (centre) and line-intensity profiles showing microcarriers dual-labelled with fluorescein and TAMRA. The peaks and troughs are indicative of whether the fluorescence is on the top or bottom side respectively.

References:
[1] T. Mitrelias, T. Trypiniotis, C.H.W. Barnes *et al.*, JAP 105 (7) 07B301 (2009)
[2] B. Hong, T.J. Hayward, C.H.W. Barnes *et al.*, JAP, 105 (3) 034701 (2009)
[3] J.J. Palfreyman, P. Beldon, C.H.W. Barnes *et al.*, AIP Conf. Proc 1311 184-191 (2010)
[4] K.N. Vyas, J.J. Palfreyman, C.H.W. Barnes *et al.*, Lab-on-a-chip, submitted (2012)

Frequency-selective rotation of magnetic nanoparticles for rapid and sensitive solution-based detection of biomolecules

L.J. van IJzendoorn [2], A. Ranzoni [1,2], B. Saha [1], R.W.L. van Vliembergen [2], G. Sabatte [1], J.J.H.B. Schleipen [1], M.W.J. Prins [1,2]

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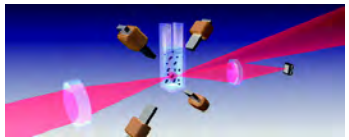
We describe an optomagnetic bionanotechnology for rapid and sensitive solution-based detection of biomolecules. Bio-active magnetic nanoparticles form clusters and undergo rotational motion in the volume of a fluid under frequency-controlled magnetic actuation. The nanoparticle clusters show a time-dependent cross-section to an incoming light beam (see Figure) [1]. We demonstrate that the scattered light signal relates to the number, the size and the magnetic properties of the nanoparticle clusters. We demonstrate dose-response curves for Prostate Specific Antigen (PSA) in buffer and in human blood plasma with a sub-picomolar detection limit, in a total assay time of 14 minutes [2]. We fit the dose response-curves using a model for the underlying processes, i.e. the capture of biomarkers and the subsequent biomarker-induced binding between nanoparticles. The sensitivity of the technology renders it of interest for applications in quantitative biology and medical diagnostics.



References

[1] Ranzoni et al., 'Frequency-selective rotation of two-particle nanoactuators for rapid and sensitive detection of biomolecules', **Nano Letters** 11, p. 2017 (2011).

[2] Ranzoni et al., "One-step homogeneous magnetic nanoparticle immunoassay for biomarker detection directly in blood plasma" ACS Nano (2012) doi:10.1021/nn204913f

A New Tool for Cancer Biomarker Detection: Multiple Samples and Multiple Parameters on a Single Magnetic Biochip

Jung-Rok Lee[1], Richard S. Gaster[2], Shan X. Wang[3,4,5]

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In the past decade, there have been considerable efforts in finding cancer biomarkers in terms of tumor antigens such as prostate-specific antigen (PSA), Carcino-Embryonic antigen (CEA), cancer antigen CA125, and so on as well as in utilizing them for the early detection. Some other proteins such as hormones and enzymes have been used as markers for cancer detection or monitoring. Recent studies show that detection of autoantibody could be a better diagnostic method with higher sensitivity and specificity for the early detection [1]. In addition, the needs for measuring multiple samples over the time course of a patient during treatment have been growing in order to understand cancer dynamics and pharmacodynamics better.

To meet these demands, we have developed a new technique using an array of GMR sensors to measure multiple targets in multiples samples, consuming small volumes of the samples. The technology is based on magneto-nanosensor chips with multiplexed real-time analyte-specific detection [2-4], which typically assays one patient sample per chip. With this new technique presented here, many different molecules such as cytokines, antibodies (**Fig. 1**), and oligonucleotides in multiple samples can be measured simultaneously with a single device. A panel of multiple types of biomarkers provides us with a better insight into the cancer, and higher sensitivity and specificity of diagnostics. Moreover, consumption of small volume of samples allows us to conduct the experiments where the volume of sample is limited or frequent bleedings are required in a single mouse, which cannot be measured with existing techniques. The unique features of this technique will enable scientists to gain more biological information with fewer devices and less sample volumes.

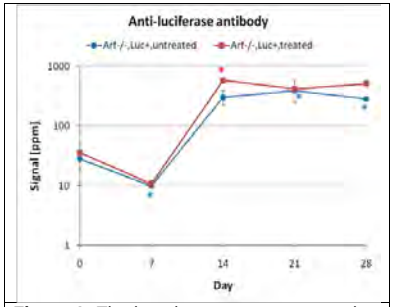

Figure 1: The host immune response against luciferase molecules was observed in lymphoma model as a function of time and drug treatment.

References:
1. Taguchi A, et al. Lung cancer signatures in plasma based on proteome profiling of mouse tumor models. Cancer Cell. 2011 Sep 13;20(3):289-99
2. Osterfeld SJ, Yu H, Gaster RS et al. Multiplex protein assays based on real-time magnetic nanotag sensing. PNAS 2008;105: 20637-20640.
3. Gaster RS, Hall DA, et al. Matrix-insensitive protein assays push the limits of biosensors in medicine. Nature Medicine, 2009; 15: 1327-1332.
4. Gaster RS, et al. Quantification of protein interactions and solution transport using high-density GMR sensor arrays. Nature Nanotechnology, 2011; 6: 314-320.

Development of FerriScan®: A Personal Story from the Lab Bench to Marketing a Product

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Occasionally, as scientists, we may uncover a method or discovery that has potential to be commercialised. It is usually not clear which ideas can be successfully taken from the lab bench to the market and which can not. Most of us are not well prepared for the journey to commercialisation. In this presentation, I will describe how, as a scientist, I managed to stumble my way along a journey from taking an idea from the lab bench to selling a product to customers.

FerriScan® is a magnetic resonance imaging based technology enabling radiologists to measure non-invasively the concentration of iron in human liver tissue. The technology is currently being used in about 125 clinics around the world and over 14,000 patient measurements have been made to date. The new technology has largely replaced the need for invasive liver biopsy procedures in these clinics for patients who are at risk of developing iron overload from multiple blood transfusions.

Our research team was not the first to think about using magnetic resonance imaging to measure iron in tissues. What made us different, however, was that we hypothesised that it was impossible to make such measurements reliably because of the variability of the magnetic properties of iron in different patients. So we set about trying to show why MRI techniques would *not* work as a method for non-invasive measurement of tissue iron. To do this we had to be very careful that we had not missed any trick for ensuring we gave MRI its best shot at success. In the process, we unexpectedly showed that there *was* a way of using MRI scanners to measure liver iron concentration.

In my presentation I will cover the hurdles we had to overcome to turn the discovery into a commercially viable business including the filing of provisional patents and subsequent PCT and full patent filings, setting up a company, finding investors, dealing with regulatory authorities, setting up ISO 9001 quality control management systems, dealing with audits from regulators, building relationships with customers, marketing, and lobbying for government reimbursements to patients for use of our medical technology. It seems to be a never ending process because investors always expect you to have new offerings in the pipeline. I will also cover our newer products including non-invasive heart iron measurements and non-invasive liver fat measurements.

I will conclude by looking at alternative and possibly better pathways than ours to commercialising new ideas.

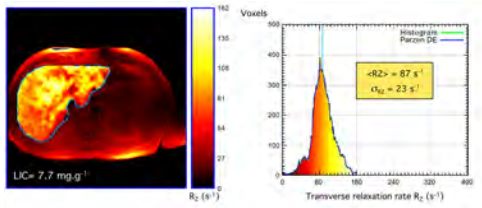

Fig. 1. Proton transverse relaxation rate (R_2) image of human liver with corresponding R_2 distribution. A calibration curve relates the mean R_2 to the patient's liver iron concentration (LIC).

Rheology of biocompatible ferrofluids

J. Nowak, S. Odenbach

Chair of Magnetofluiddynamics, TU Dresden, 01062 Dresden, Germany

Ferrofluids used for medical applications as e.g. magnetic drug targeting will usually contain comparably large magnetic particles with diameters about 50nm - 200nm to enable strong forces necessary to ensure significant targeting. For fluids containing large particles it is well known that magnetic field influence will cause an increase in the viscosity of the fluids. Up to now this change of the fluid properties and the resulting consequences for medical applications has not been addressed at all. Thus it will be the scope of this contribution to determine the magnetoviscous properties of ferrofluids for biomedical applications and to discuss possible impact for such applications.

Fast and Reliable *in situ* Analysis of Dispersions of Magnetic Nanoparticles

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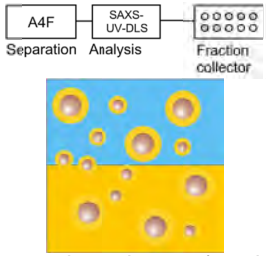
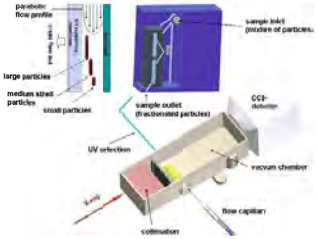
Typical analytical methods for the size analysis of magnetic nanoparticles like dynamic light scattering (DLS) and transmission electron microscopy (TEM) are either very time-consuming, are disturbed by the stabilizers covering the particles or cannot deliver conclusive results for mixtures of nanoparticles of varying mass (polydispersity). The latter being the original arch enemy of reliable particle analysis.

A new analytical method, which solves both the problem of polydispersity and also quickly yields reliable, representative data about the shape and quantity of nanoparticles in liquids (suspensions), was developed.

This process is based on coupling asymmetric flow field-flow fractionation (A4F) with small-angle X-ray scattering (SAXS). With the help of A4F, the particles are separated from each other according to size, without running the risk of altering the particle or its shell. After separation, the fractionated particle stream can be measured using small-angle X-ray scattering, determining the size and shape of the particles. In this case a special advantage is that with SAXS the particle nuclei themselves can be measured; the almost always present shell of the particles does not interfere. Besides, a large number of particles are detected simultaneously, which saves time and provides quality statistical data. Since this method is non-destructive, it can be followed by additional analytic processes such as the above mentioned DLS, in order to determine particle dimensions including their shell – which can even provide information about shell thickness. The applicability of this method will be demonstrated with commercial nanoparticles (Resovist) and rod-like particles from synthesis in a micro mixing procedure. The use of synchrotron radiation is discussed in comparison to the use of commercial X-ray tubes as sources of X-rays for SAXS. Finally the method is helpful to prepare well-defined samples for toxicity studies as will be demonstrated for commercial iron oxide powders (J. Chromatography A, 2011, 1218, 4160-4166)



Upper Figure. Scheme sample preparation for toxicological studies. Lower Figure. The different contrast of core and shell in DLS (blue) and SAXS (yellow) gives detailed information on polymer stabilized magnetic nanoparticles



Separation of rod-like magnetic nanoparticle and online analysis with small-angle X-ray scattering

Talk 15

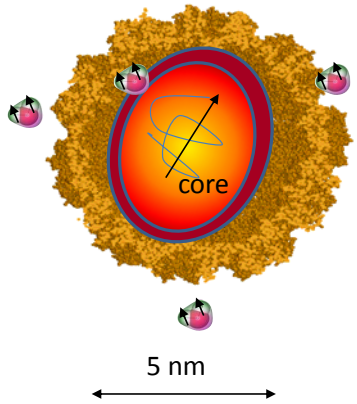
Modeling Nanoparticle Properties by Electron Magnetic Resonance

Robert J. Usselman[1] and Stephen E. Russek[1]

[1]National Institute of Standards and Technology, Boulder, CO 80305

Abstract:

Electron magnetic resonance (EMR) is a powerful spectroscopic method that can be used to determine magnetic moment distributions and anisotropic energies of superparamagnetic metal-oxide nanoparticles. We investigate the EMR spectral evolution of maghemite (γ-Fe_2O_3) nanoparticles formed within size-constraining *Listeria innocua* (LDps) protein cages, which have an inner diameter of 5 nm. Ultra small nanoparticles (d < 5 nm) are systems that interface the classical and quantum regimes, where the opportunity exists to observe the possible coexistence of classical and quantum phenomena. A static model was used to simulate the lineshape trends as a function of temperature and frequency. The lineshape simulations were used to infer the magnetic properties of size-constrained iron-oxide nanoparticles. The work presented here indicates that the magnetic properties of these size-constrained nanoparticles, and more generally metal oxide nanoparticles with diameters d < 5 nm, are complex and that currently existing models are not sufficient for determining their magnetic resonance signatures.



Talk 16

Magnetic Luminescent Core-Shell Particles for Drug Delivery and Imaging

Hongyu Chen[1], Thomas Moore[2], Frank Alexis[2], Jeffrey N. Anker*

Department of Chemistry and Center for Optical Materials Science and Engineering Technologies (COMSET), Clemson, SC 29634, USA
Department of Bioengineering, Clemson, SC 29634, USA
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The effectiveness of chemotherapy treatment greatly depends upon the local concentration of drug released in tumors. In order to deliver drugs to tumors and study release, we have developed magnetic nanoparticles that can be loaded with drugs, and display release-dependent fluorescent spectra. We synthesized a monodispersed core-shell structure with spindle-shaped γ-Fe_2O_3 cores and europium-doped gadolinium oxide shells. The size and shape can be controlled by changing the synthesis conditions of the iron oxide cores. In order to provide space for drug loading, hollow nanoparticles were formed by etching the Fe_2O_3 in oxalic acid. Nanoparticles with intermediate core-size were synthesized controlling the etching time (see Figure 1). We studied the effect of the core upon the particles' fluorescence and radioluminescence spectra, magnetic hysteresis curves, and T_2/T_2* MRI contrast. The hollow structures displayed bright radioluminescence signals, could be loaded with dyes, were paramagnetic and had T_2 relaxivities of r_2=322 ml mg^{-1} s^{-1}. Compared to the hollow structures, addition of the iron oxide cores added a ferromagnetic component, and increased the relaxivities to r_2=514 ml mg^{-1} s^{-1}, but reduced the luminescence intensity. Nanoparticles with partially dissolved cores had luminescent and magnetic properties intermediate between the solid core and hollow particles. These particles offer multimodal MRI/fluorescence/X-ray luminescence contrast agents which are sensitive to optical absorption of the core. Our synthesis method offers a productive synthesis route that enables a wide range of biological applications of magnetic/luminescent probes.

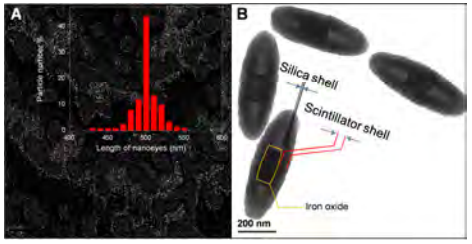


Figure 1. Monodispersed ellipsoidal particles with a partially filled γ-Fe_2O_3 core and SiO_2@Gd_2O_3:Eu shell (A) SEM and (B) and TEM image of these "nanoeyes."

Talk 17

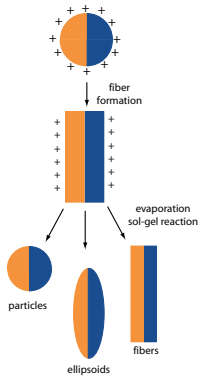
Thursday, May 24, 2012

8:00	**Registration desk opens / Posters with even numbers can be put up** today during the lunch break (pins will be provided)			
8:30	**Hawkins, Peter**	**Tutorial I - Magnetic Particles in Immunoassays**	Bristol, UK	Tutorial 1
	Session 4: Magnetic Nanoparticle Synthesis			
9:00	Andrew, Jennifer	Bi-phasic Magnetic Materials: Towards Multifunctional Contrast Agents	Gainesville, USA	Talk 18
9:15	Becker, Sören	Using nanotechnology for the formation of multimodal marker for in vivo ß-cell labeling	Hamburg, Germany	Talk 19
9:30	Jain, Nirmesh	Penetration of Solid Tumors by Sterically Stabilized Magnetic Nanoparticles	Sydney, Australia	Talk 20
9:45	Huber, Dale	Synthesis of Iron Oxide Nanoparticles by the in situ Generation of Iron Oleate	Albuquerque, USA	Talk 21
10:00	Lak, Aidin	Large single-core iron oxide nanoparticles: a tracer for magnetorelaxometry immunoassays	Braunschweig, Germany	Talk 22
10:15	**Coffee break**			
10:45	Marquina, Clara	MgO-coated magnetite nanoparticles for detection and treatment of Fusarium spp. fungi	Zaragoza, Spain	Talk 23
11:00	Niehaus, Jan	Adjusting the MRI contrast by formation of defined SPIO clusters	Hamburg, Germany	Talk 24
11:15	Peng, Mingli	A novel approach of transferring oleic acid capped iron oxide nanoparticles to water phase	Xi'an, China	Talk 25
11:30	Roig, Anna	Magnetically Labeled Endothelial Progenitor Cells for Cellular Therapy in Brain Ischemia Treatment	Bellaterra, Spain	Talk 26
11:45	Thanh, Nguyen T.	One-pot synthesis of hybrid magnetic and fluorescent core-shell structure of FePt@CdSe Nanoparticles	London, UK	Talk 27
12:00	**Guo, Haibo**	**Morphological Mapping of Iron Oxide Nanoparticles Using Thermodynamic Modeling**	Melbourne, Australia	Invited talk 3
12:45	**Lunch in the TCF Bank football stadium, DQ Clubroom / Exhibitors**			
	Session 5: Magnetic Hyperthermia			
14:30	**Rinaldi, Carlos / Ivkov, Robert**	**Debate: Do We Really Know How Magnetic Hyperthermia Works?** ***Moderated by Kannan Krishnan***	Baltimore/Mayaguez, USA	Invited talk 4
15:15	Borca-Tasciuc, Diana-Andra	Potential sources of errors in measuring and evaluating the specific absorption rate of magnetic nanoparticles in alternating magnetic field	Troy, USA	Talk 28
15:30	Creixell, Mar	EGFR-Targeted Magnetic Nanoparticles Kill Cancer Cells Under the Application of a Magnetic Field Without a Perceptible Rise in Temperature	Mayagüez, USA	Talk 29
15:45	Dennis, Cindi	Effect of Internal Magnetic Structure of Iron Oxide Magnetic Nanoparticles on the Field Dependence of the SLP for Hyperthermia	Gaithersburg, USA	Talk 30
16:00	Dutz, Silvio	Magnetic hyperthermia - Immobilisation state of magnetic multicore nanoparticles injected into living tumors	Vancouver, Canada	Talk 31
16:15	Goya, Gerardo	Is the magnetic hyperthermia mechanism a universal one? The case of dendritic cells.	Zaragoza, Spain	Talk 32
16:30	Hadjipanayis, Constantinos	Thermotherapy of Experimental Glioblastoma with Laponite-Embedded Magnetic Iron-Oxide Nanoparticles	Atlanta, USA	Talk 33
16:45	Bernau, Vianney	Injectable Nanocomposite for local Hyperthermia	Lausanne, Switzerland	Talk 34
17:00	Southern, Paul	Inter-Particle Interactions in the Static and Dynamic Magnetic Properties of Ferucarbotran Colloids	London, UK	Talk 35
17:15	**Group photograph**			
17:30	**Poster session (even numbers) with beer and pretzels - PLEASE RATE POSTERS** - *sponsored by* ***StemCell Technologies*** */ Exhibitors*			
20:00	**Free evening - use it to meet old friends, discuss new collaborations, and enjoy Minneapolis on your own!** - **please remove posters by end of the evening!**			

Bi-phasic Magnetic Materials: Towards Multifunctional Contrast Agents

Natalie Ganio, Justin D. Starr, Jennifer S. Andrew*
Department of Materials Science & Engineering, University of Florida, Gainesville, FL 32611-6400
*jandrew@mse.ufl.edu

Contrast agents are frequently used to increase clarity in images that results from magnetic resonance imaging (MRI). By utilizing contrast agents, clinicians can increase the sensitivity of an MRI, enhancing their diagnostic abilities. Currently, the most commonly used contrast agents are gadolinium based, where the paramagnetic Gd (III) is capable of shortening the T1 relaxation time of nearby protons. However, gadolinium-based agents have been shown to have some toxicity, and can lead to nephrogenic systemic fibrosis (NSF) in patients with renal disease. To address these challenges, particle based contrast agents have been developed, including manganese oxide (MnO) and iron oxide (e.g., Fe_3O_4, γ-Fe_2O_3), which are T1 and T2 and provide lightening or darkening contrast, respectively. T1 and T2 contrast agents are each better at illuminating different anatomical features. In this presentation we will present a multi-modal contrast agent capable of providing both T1 and T2 contrast on a single particle. By synthesizing Janus-type biphasic nanoparticles, where one hemisphere is MnO and the other is Fe3O4, we have realized a new type of MRI contrast agent capable of providing both T1 and T2 contrast. These Janus-type biphasic nanoparticles are fabricated via electrospraying. This presentation will focus on the synthesis, processing, and property relationships of these novel biphasic magnetic materials



Schematic formation mechanism of the biphasic type particles utilizing an electrospray based method.

Using nanotechnology for the formation of multimodal marker for *in vivo* β-cell labeling

S. Becker(1)*, J. Niehaus(2), C. Schmidtke(1), K. Werner(2) and H. Weller(1,2)

(1) University of Hamburg, Institute of Physical Chemistry, Grindelallee 117, 20146 Hamburg, Germany
(2) Center for Applied Nanotechnology (CAN GmbH), Grindelallee 117, 20146 Hamburg, Germany
*Corresponding author (becker@chemie.uni-hamburg.de)

In August 2011 the World Health Organization published their diabetes facts sheet showing that 346 million people worldwide suffer from diabetes.[1] The occurrence of diabetes type I as well as type II is related to a decline of β-cell functionality and/or mass. In the VIBRANT project we are currently developing a marker for the *in vivo* determination of the β-cell mass. This marker needs an intensive and stable read-out signal as well as a high specificity towards β-cells, both with negligible toxic effect.

Here we present our concept of this marker, a so called nanocontainer (see fig. 1). This container itself is a polymer micelle which can be loaded with hydrophobic tracers like superparamagnetic iron oxide (SPIOs) or fluorescent cadmium-based semiconductor nanoparticles (QDs) offering us the possibility of optical (QDs) and MRI (SPIOs) read-out. Beside the mediation of water solubility the polymer micelle is also a carrier of functional groups at their outer surface for further modification.

We will give a general overview of the nanocontainer's formation process, focusing on the synthesis of the used nanoparticle in high boiling organic solvents and the phase transfer into aqueous media. We will show physiochemical properties as well as toxicological data of our nanocontainers.

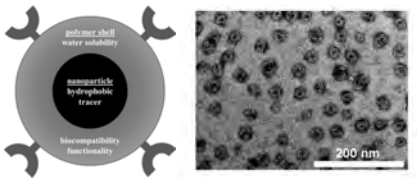


Fig 1: schematic concept of nanocontainer (left); TEM image of stained nanocontainers (right)

The research leading to these results has received finding from the European Community's Sevenths Framework Programme (FP7/2007-2013) under *grand agreement* n° 228933

[1] WHO fact sheet N°312, http://www.who.int/mediacentre/factsheets/fs312/en/index.html, **August 2011.**

Penetration of Solid Tumors by Sterically Stabilized Magnetic Nanoparticles

Nirmesh Jain[1], Binh T. T. Pham[1], Nicole S. Bryce[1], Nicole Fong[1], Trevor W. Hambley[1], Brian S. Hawkett[1], and Stephen K. Jones[2]

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Nanometer-scale magnetic nanoparticles (NPs) have attracted widespread attention due to the rapidly increasing number and variety of their applications in the biomedical sciences, including imaging, drug delivery and therapy.[1] Indeed, they possess unique nanoscale size dependent physical and chemical properties that can be controlled in a manner that is not possible in the corresponding bulk materials. When these tiny materials are introduced into biological systems, their small size and physicochemical properties enable them to operate as probes and delivery vectors suitable as candidates for the next generation of diagnostic and therapeutic techniques.

Moreover, in more than 90% of cancer patients undergoing chemotherapy with solid tumors, there is only a partial penetration of the drug into the tumor. Thus, treatments that achieve better targeting and deeper penetration of tumor cells are urgently required. Functionalized magnetic nanoparticles have been recognized as a promising vehicle for the targeted delivery of therapeutic moieties[2].

Synthetic chemistry has now developed to a stage where it is possible to produce magnetic NPs for in vivo biomedical applications such as medical imaging, drug delivery and magnetic thermotherapy achieving the so-called “find, fight, and follow” concept of early diagnosis and therapy control. Nevertheless, the difficulty in designing such nanoprobes consists in the fact that they should exhibit remarkable stability at physiological conditions and have adequate biocompatibility and targeting properties. Thus, the use of magnetic NPs for theranostic applications requires better control of the NP core, size, shape, chemical composition, degree of aggregation, and surface state.

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 developed 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. Our *in vitro* results (Figure 1) show the deeper penetration and enhancement in the uptake of Doxorubicin in spheroid from DLD-1 colon cancer cells in the presence of magnetic nanoparticles.

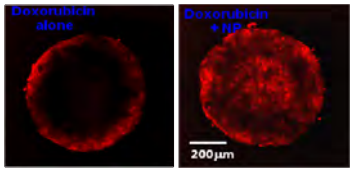

Figure 1: Confocal images of doxorubicin in the spheroid of DLD-1 colon cancer cells with and without magnetic nanoparticles after 24hr incubation.

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Synthesis of Iron Oxide Nanoparticles by the in situ Generation of Iron Oleate

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Thermal decomposition of iron oleate is a popular approach for the synthesis of iron oxide nanoparticles due to the high quality of the nanoparticles produced. The nanoparticle product is characterized by a narrow polydispersity, high crystallinity, and spherical morphology. Despite these advantages, it can be difficult to precisely reproduce nanoparticle sizes using this synthetic approach. One reason for this lack of reproducibility has been shown to be the non-stoichiometric nature of the iron oleate reagent, which has a tendency to form oligomers.[1] The result is that significant batch-to-batch variations in the iron content and the reactivity of iron oleate occur. We report here an approach to nanoparticle synthesis that retains the primary advantages of the iron oleate synthesis, while avoiding the significant drawbacks with the custom synthesis of the non-stoichiometric precursor. We generate iron oleate in situ by heating iron acetylacetonate in the presence of an excess of oleic acid, thereby forming iron oleate in situ. Further heating leads to decomposition of the iron oleate to generate iron oxide nanoparticles. The reaction has the advantage of requiring no custom synthesis of precursor, and using only inexpensive, commercially available compounds. Additionally, the iron precursor, iron acetylacetonate, is a crystalline, stoichiometric compound that can be purchased at very high purities. We will discuss details of the synthesis, temperature profile, spectroscopic evidence of the in situ formation of iron oleate, the size reproducibility, and magnetic properties of the resultant nanoparticles.

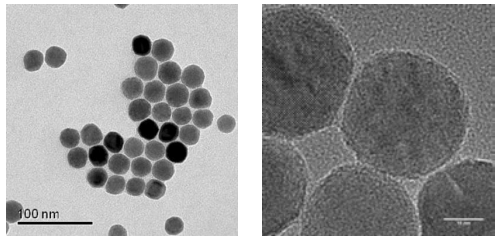

Figure 1. TEM (left) and high resolution TEM (right) of 27 nm diameter nanoparticles synthesized with this method. Scale bars are 100 nm and 10 nm respectively

Acknowledgements

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Large single-core iron oxide nanoparticles: a tracer for magnetorelaxometry immunoassays

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Magnetorelaxometry immunoassays (MARIA) have gained a lot of attention due to its capability of realizing homogenous immunoassays. MARIA is based on the change in relaxation behavior of magnetic particles by binding to analyte. Consequently, the synthesis of particles with $\tau_B < \tau_N \approx (d_{core} > 20\,nm)$ is a prerequisite for observing a substantial difference between bound and unbound particles, and eventually performing a successful immunoassay. Despite this fact, solely a few studies have been dedicated to optimizing a potential tracer for quantitative immunoassays using fluxgate magnetorelaxometry (MRX).

This study addresses the feasibility of using mono-disperse large single-core iron oxide nanoparticles (LSCNPs) for quantitative magnetorelaxometry immunoassays. We have established a robust synthesis protocol for the fabrication of LSCNPs with 25 nm core size and relatively narrow size distribution. The structural and morphological features and size distribution of the synthesized particles were explored by applying a variety of analysis methods, including transmission electron microscopy (TEM) (Fig. 1(a)), fluxgate magnetorelaxometry (MRX), photon cross correlation spectroscopy (PCCS) and alternating field susceptibility (ACS). The particle's size uniformity was additionally characterized using our magnetic particle spectrometer (MPS).

Preliminary magnetorelaxometry studies show a significant difference in Néel (immobile sample) and Brownian (mobile sample) relaxation time constants (Fig. 1 (b)), indicating an inherent capability of the self-synthesized particles for performing a successful relaxometry binding assay. Additionally, the synthesized particles depict a fast linear decay in the harmonics spectrum, revealing the mono-dispersity of the synthesized particles which ultimately allows one to conduct quantitative immunoassays. Furthermore, the particle functionalization, water stability and binding to analyte will be explored and discussed.

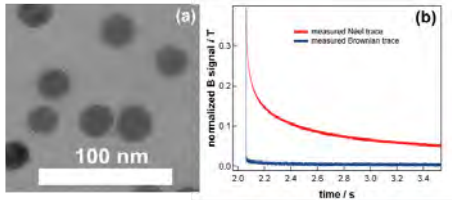

Fig. 1: (a) a typical TEM micrograph of LSCNPs and (b) Néel and Brownian relaxation traces of LSCNPs.

Acknowledgment

This work was financially supported by the DFG via SFB 578. Financial support by the International Graduate School of Metrology at Braunschweig (igsm) for PhD thesis (A.L.) is appreciated.

MgO-coated magnetite nanoparticles for detection and treatment of *Fusarium* spp. fungi

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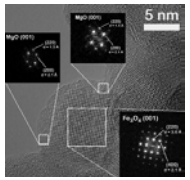
Nanometric magnetic iron oxide nanoparticles are very useful for biotechnological applications. We are interested in their use for the detection and control of the pathogenic fungus *Fusarium oxysporum.* Many *F. oxysporum formae especiales* are a major concern for agriculture and food industry, as they can infect a number of major crops (cereals, potato, etc.) and produce mycotoxins that may give rise to allergic symptoms or be carcinogenic in long-term consumption. These soil borne pathogens can survive as thick-walled chlamydospores, which remain viable in the soil for many years and hence control is problematic. In addition, several *ff. spp*. are non-pathogenic and even beneficial for agriculture exerting a bio-control effect on other pathogenic strains. Therefore it is crucial to provide control methods effective and directed to the pathogenic *ff. spp.*

We present the synthesis and characterization of superparamagnetic magnetite nanoparticles as well as a functionalization strategy, necessary for the study of the nanoparticles internalization by the *Fusarium* spp. Superparamagnetic Fe_3O_4 nanoparticles **coated with an ultra-thin (~1 nm)** MgO layer (total size ≈ 25 nm and hydrodynamic diameter ≈ 30 nm) have been synthesized combining co-precipitation and sol-gel methods. A thorough chemical and structural characterization has been carried out by HRTEM, XRD, EDS, DLS and TGA. Aberration corrected **HRTEM experiments with sub-angstrom spatial resolution** have allowed us to distinguish the ultra-thin MgO shell that grows epitaxially on the magnetic cores. The MgO shell protects the magnetic nuclei from oxidation, preserving the superparamagnetism even after calcination at high temperatures (≈ 600ºC[1]. To improve their water stability and to obtain a suitable surface for biochemical functionalization, our synthesized particles have been encapsulated in an aminated silica coating (also through a sol-gel process). After that the particles were **bio-functionalized with G protein/anti-HRP antibody,** as a preliminary study before the functionalization with an antibody which specifically recognizes the desired *Fusarium* species. All the functionalization steps have been verified by SDS-PAGE electrophoretical analysis, and show us that the antibody orientation is the required for an effective further immunological recognition.



Aberration-corrected HRTEM image of a MgO-coated Fe_3O_4 nanoparticle. Insets: Fast Fourier Transform from the selected areas (see L. De Matteis et al., Chem. Mater. **24** (2012) 451

Nanoparticles were incubated with a culture of *F. oxysporum* f. sp. *lycopersici* and tested for nanoparticles internalization and toxicity. Confocal microscopy images showed that, after 24 hours, the nanoparticles are internalized through the fungus hyphae. In addition several in vitro assays showed no differences in germination, growth, aggregation, biological activity and hyphal death between control and nanoparticles incubated samples, at different concentrations. This is important since they can be then functionalized for targeting specific pathogenic strains.

a) Overlay of light and fluorescence microscopy images of a *Fusarium* hypha after 24 hours incubation (x63); Confocal microscope images of a NBT (b) and DAB (c) stained *Fusarium* hypha after 24 hours incubation (x100).

Adjusting the MRI contrast by formation of defined SPIO clusters

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Today superparamagnetic iron oxide nanoparticles (SPIO) can be used to form contrast agents with improved sensitivity and specificity. Their magnetic read-out in MRI or MPI measurements avoids risks which occur from ionizing (CT) or radioactive (PET) radiation. To yield a high quality contrast agent the used SPIOs have to be monodispers with well defined and reproducible properties. These particles are typically produced by thermal decomposition with diameters only between 5 and 20 nm and only dispersible in non polar solvents. Nanoparticles with larger diameters for improved signal to noise ratio (SNR) lose their superparamagnetic behavior while those produced in water show a lack in crystallinity and monodispersity.

Here we present our continuous flow phase transfer approach for the encapsulation of hydrophobic nanoparticles in a hydrophilic container. As this procedure is fully automated and takes place in a microfluidic chip, the properties of the water soluble nanoparticles are highly reproducible and up-scaling can be done easily. As the used amphiphilic polymers can be made with different functional groups at the hydrophilic end, a coupling of affinity molecules to these containers is possible. In addition this approach can be used to produce equivalent capsules filled with different particles like magnetic iron oxide and fluorescent quantum dots so that a cross validation of the results is possible.

To yield a contrast agent with an increased MRI contrast we have developed a strategy to fill these capsules with a defined number of iron oxide particles. We will show the r_2 relaxivity of clusters with single particle sizes ranging from 5 to 15 nm and an overall hydrodynamic diameter between 50 and 200 nm to determine the optimal cluster size for a maximum MRI contrast.

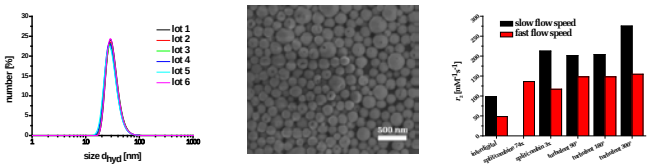

Fig 1 – DLS measurement of 6 equivalent batches of single encapsulated particles (left), SEM image of 170 nm sized capsules filled with iron oxide particles (middle) and relaxivity r_2 in dependence to flow speed and mixing chamber used during the phase transfer (right)

A novel approach of transferring oleic acid capped iron oxide nanoparticles to water phase

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Currently, high quality iron oxide nanoparticles (NPs) regarding monodispersity, size distribution, and crystallinity are normally produced in organic solvents and stabilized with oleic acid (OA). Therefore, phase transfer to achieve NP water solubility and functionality for further conjugation is essential and remains an outstanding challenge. Herein, a novel approach is presented to transfer mono-disperse iron oxide NPs to water based on the oxidation of OA stabilized on the surface of magnetic particles. Firstly, the oleic acid-capped NPs were synthesized using a thermolysis process of $Fe(OA)_3$ in high boiling-point solvent and redispersed in hexane[1], then oxidation of OA was carried out by adding the iron oxide NPs into the mixture of ethyl acetate/acetonitrile in the presence of sodium periodate at room temperature[2]. Two hours later, the magnetic NPs was separated and washed with ethanol and distilled water for several times, and dispersed in water. The characterization of NPs indicated that the phase transfer was successful performed without change in the size and shape of the iron oxide NPs. The hydrophilic groups on the iron oxide surface stabilized the NPs in aqueous solution and the oxidized NPs can be applied to bimolecular immobilization such as BSA.

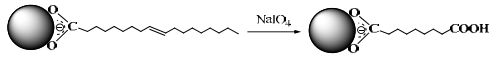

Fig 1. Scheme of water phase transger of magnetic particles

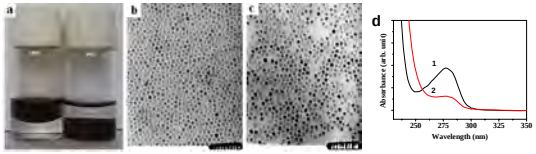

Fig 2: (a) Photographs of 12 nm iron oxide nanoparticles before (left) and after phase transfer (right); TEM images of nanoparticles (b) before (c) after phase transfer. The black bar was 100 nm; (d) UV-Vis spectra of BSA before (1) and (2) after immobilization.

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Magnetically Labeled Endothelial Progenitor Cells for Cellular Therapy in Brain Ischemia Treatment

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Endothelial progenitor cells (EPCs) show stemness characteristics with the ability of differentiating into endothelial cells (1). These cells constitute a new model for angiogenesis, endothelial regeneration and vessels repair (2). In recent years stem cell labeling with superparamagnetic iron oxide nanoparticles (SPIONPs) has been used as strategy for cellular therapy and tissue repair, as for instance in central nervous system diseases (3).

Our final objective is to enhance angiogenesis and tissue repair in peri-infarcted brain areas by engrafting functional EPCs using an external magnetic field. For that, citrate coated SPIONPs were synthesized through thermal decomposition route (4) yielding a γ-Fe_2O_3 core of 6 ±1 nm in diameter and were subsequently transferred to water by using anionic surfactants. Stable aqueous dispersion at pH= 7.5 imaged by Cryo-TEM showed nanoparticles aggregates with hydrodynamic sizes of 50 nm and 30% polydispersity. Magnetic measurement at room temperature confirmed the absence of remnant magnetization and a high saturation magnetization value (54 emu/g Fe_2O_3). Early EPCs from mouse were successfully labeled with the SPIONPs after 24h of co-incubation with a non-toxic iron concentration of 50 µg/ml. Labeled cells show an uptake of 24 pg Fe/cell. TEM images established cellular uptake and the storing of SPIONPs into endosomal compartments.

Furthermore, we observed that magnetized outgrowth EPCs were fully functional since they shaped vessel-like structures as non-magnetized cells and we have found that magnetized human and mouse EPCs secrete more VEGF and FGF than control cells. Finally, a preliminary *in vivo* cell tracking experiment demonstrates that magnetized EPCs can be guided to cortical areas of the brain by an external magnetic field as confirmed by MRI images.

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One-pot synthesis of hybrid magnetic and fluorescent core-shell structure of FePt@CdSe Nanoparticles

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Magnetic fluorescent FePt@CdSe core–shell nanoparticles were directly synthesized by sequential addition of precursors and using tetraethylene glycol as a solvent and a reducing agent. The core–shell NPs were successfully formed over a wide range of temperature (240–300 °C). The size and composition of the FePt core were tuned by changing the ratio of surfactant (oleic acid and oleylamine) to metal precursors [$Fe_3(CO)_{12}$ and $Pt(acac)_2$] and the feeding ratio of the precursors, respectively. The CdSe shell thickness also could be varied from 1 to 8.5 nm by rational control of the total amount of Cd and Se precursors. FePt@CdSe core–shell NPs with a core size of about 4.3 nm and shell thickness of about 2.5 nm displayed a fluorescence emission around 600 nm. They exhibited superparamagnetic behaviour at room temperature and the blocking temperature was about 55 K, which was almost the same as uncoated FePt NPs, while the coercivity decreased from 400 Oe for the FePt NPs to 200 Oe. Detailed characterization of intermediates and synthesized FePt@CdSe NPs revealed the fine structure and formation mechanism.

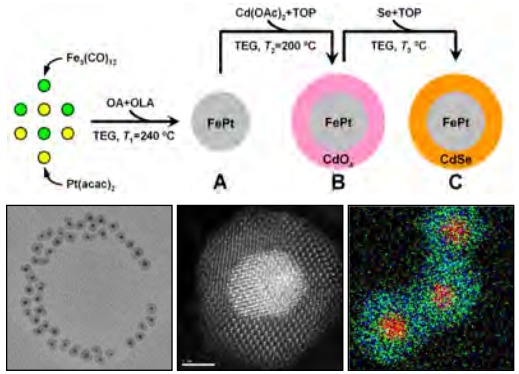

Fig 1. Core@Shell Structure of FePt@CdSe Nanoparticles

Morphological Mapping of Iron Oxide Nanoparticles Using Thermodynamic Modeling

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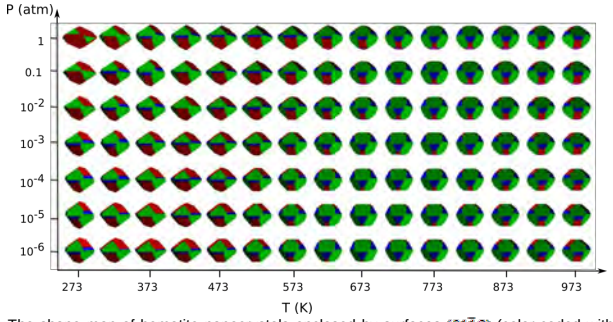
The morphology of iron oxide nanostructures is closely related to the magnetic properties and chemical stability. In synthesizing the nanostructures of specific shapes and sizes, a morphology model is particularly useful to understand the relationship between morphology, surface chemistry, magnetic properties, and environmental conditions.

We have used an advanced thermodynamical model capable of describing a range of nanomorphologies, with reliable input parameters from first principles calculations, to construct a map relating the size, shape, and environmental conditions. We also modeled the effect of passivation by water molecules that are common in aquatic and hydrous environments, such as water and humid air, and discussed the surface chemistry of exposed facets. The model covers diverse environmental conditions of supersaturation of water and oxygen, over which we build the mapping of the thermodynamic equilibrium shapes of iron oxide nanocrystals. By analysing the electronic structures of the equilibrium shapes we can connect the morphology with magnetic properties.

For hematite nanocrystals, we have built a collection of shapes enclosed by several low-index surfaces. We found that under thermodynamic equilibrium conditions, hematite nanocrystals are of the truncated pseudocubic or truncated rhombohedral morphology, exposing mainly the surfaces $(01\bar{1}2)$ and $(10\bar{1}4)$. The fractions of surfaces are dependent on the environmental conditions, and are insensitive to the particle size.

The thermodynamic morphology model in combination with electronic-structure calculations enables us to predict the sizes and shapes of iron oxide nanocrystals spanning a wide range of chemical and thermal conditions. The information by the model should provide guidance for not only synthesis, but also storage and application of iron oxide nanostructures.



The shape map of hematite nanocrystals enclosed by surfaces $(01\bar{1}2)$ (color-coded with green), $(10\bar{1}4)$ (red), $(10\bar{1}1)$ (blue, and (0001) (magenta). The pressure is of water vapor.

Invited Talk 3

Our First Debate Ever:

Do We Really Know How Magnetic Hyperthermia Works?

Carlos Rinaldi[a], Robert Ivkov[b]

Moderated by Kannan Krishnan[c]

[a] Professor, Chemical Engineering, University of Puerto Rico, Mayagüez, PR, USA, [b] Assistant Professor, Radiation Oncology & Molecular Radiation Sciences, Johns Hopkins University School of Medicine, Baltimore, MD, USA, [c] Professor of Materials Science and Adjunct Professor of Physics, University of Washington, Seattle, WA, USA

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This is a new format for our meeting with two seasoned experts, Prof. Rinaldi and Prof. Ivkov in magnetic hyperthermia giving us a short introduction into the area, followed by an exchange of opinions over some of the still controversial areas of this exciting field. To not let the debate get too much out of hand, we will have Prof. Krishnan moderate the debate.

Invited Talk 4

Potential sources of errors in measuring and evaluating the specific absorption rate of magnetic nanoparticles in alternating magnetic field

S. Huang[*1], S-Y. Wang[*1], A.Gupta[2], D.-A. Borca-Tasciuc[†1] and S. J. Salon[3]

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Rensselaer Polytechnic Institute 110 Eighth Street, Troy, NY, 12180, USA

Measuring accurately the specific absorption rate (SAR) of magnetic nanoparticle suspensions in alternating magnetic field is the key to a better understanding of suspension's attributes that affect the heat generation rate. However, much of the literature reporting on thermal power of magnetic nanoparticles pays little attention to the SAR measurement technique. In general, SAR measurement method is considered as being very simple and the setup consists of a coil surrounding a container holding the fluid sample. The temperature rise of the magnetic fluid is monitored once the field is turned on and SAR is determined from the initial slope of the temperature as a function of time. Due to the time scale of the measurement, heat losses are assumed to be negligible and not to affect the reported results. However, when the volume to surface area ratio of the sample is lower, volumetric heat storage rate within the solution becomes comparable to surface heat losses to the container holding the sample. Our theoretical and experimental investigations show this may lead to non-negligible errors in reported SAR. In addition, an accurate measurement also requires determining the position of highest temperature rise within the sample, which depends on the natural convection effects. In practice, this can be achieved by measuring the temperature at multiple locations along the axis of the sample.

To enable a meaningful comparison between the thermal power of different nanoparticle systems, SAR is often normalized to the product between the squared magnetic field and its frequency. For this purpose, the frequency and the magnetic field intensity, *H*, are reported along with SAR. Typically *H* is calculated from simple solenoid formula, based on the applied current and the number of turns of the coil. However, here we show that *H* also depends on the geometry of the magnetic suspension sample. The variation in the magnetic flux with fluid sample geometry arises because the magnetic flux lines must close and go around the coil loops. Most of the magnetic reluctance is due to the long path of the flux in the air. For identical volumes of the magnetic suspensions, long samples (i.e. of small radius) effectively inserts more magnetic material in the direction of the flux path than short ones (i.e. of large radius). This effectively reduces the reluctance, increasing the flux density. Since the field H is proportional to the magnetic flux, this is also higher, producing a higher SAR. Numerical calculations were carried out to determine the average squared magnetic field experienced by the magnetic suspensions in containers of different geometries and they were found to agree well with experimental trends in SAR.

[*] Authors have contributed equally to this work.
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Talk 28

EGFR-Targeted Magnetic Nanoparticles Kill Cancer Cells Under the Application of a Magnetic Field Without a Perceptible Rise in Temperature

Mar Creixell, Ana C. Bohórquez, Madeline Torres-Lugo, Carlos Rinaldi

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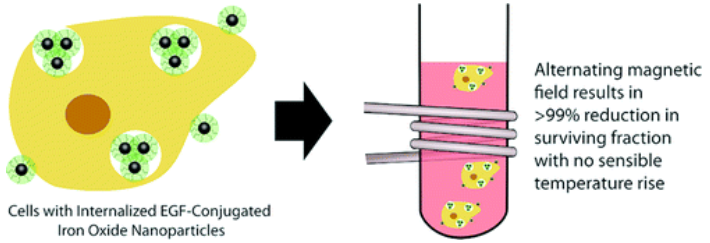
Magnetic nanoparticles have the ability to dissipate energy under an oscillating magnetic field, causing a rise in temperature that translates into a decrease in cell viability. This phenomenon is being explored as an alternative treatment to eradicate localized cancer tumors by what is referred to as magnetic fluid hyperthermia (MFH). Magnetic nanoparticles have been used successfully for MFH; however, these nanoparticles typically possess non-specific binding affinity that correlates with lack of specificity and low internalization rates. Because it's been theoretically reported that the heat generated by a single cell loaded with nanoparticles is negligible, MFH would not be useful for small tumors or metastatic cancers in which single cells need to be treated. In order to increase the specificity and efficiency of internalization to specific cell-types, epidermal growth factor (EGF) was conjugated iron oxide (IO) magnetic nanoparticles to obtain magnetic nanoparticles that target the epidermal growth factor receptor (EGFR), which is overexpressed in many types of cancer. Both targeted and non-targeted nanoparticles were incubated with cancer cells that either overexpress or do not 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. Nanoparticle localization within the cell was visualized by confocal laser scanning microscopy, showing a different distribution of accumulated nanoparticles in EGFR overexpressing cells compared with non overexpressing cells. Single cells were treated by MFH after incubation with targeted and nontargeted nanoparticles at different magnetic fields. 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 nontargeted nanoparticles. Cell viability was significantly reduced in a specific absorption rate (SAR) and Total Heat Dose (THD) dependent manner, although there was no measurable increase in temperature. The affect appears to be cell type specific, but indicates that MFH may be successfully applied under conditions previously considered as not possible.



Talk 29

Effect of Internal Magnetic Structure of Iron Oxide Magnetic Nanoparticles on the Field Dependence of the SLP for Hyperthermia

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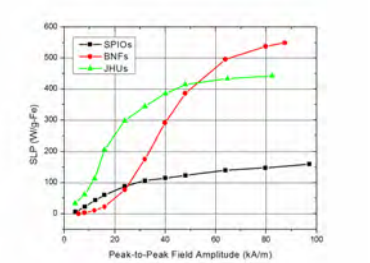
Magnetic nanoparticles are being developed for a wide range of biomedical applications. In particular, for hyperthermia, the magnetic nanoparticles are subjected to an alternating magnetic field in order to deposit energy in the form of heat into the surrounding cancerous tissue. However, the amount of energy deposited, as quantified by the specific loss power (SLP), is determined, in part, by the magnetic field amplitude. The magnetic field amplitude, in turn, is limited by the power generation requirements to produce a uniform field over for whole body vs. localized regions. Therefore, it is critical to understand the parameters important in determining how a given magnetic nanoparticle system responds to the magnetic field amplitude at fixed frequency.



Here, we considered three nanoparticle systems: the first (BNF) is comprised of Fe_3O_4 with a dextran shell; the second (JHU) is comprised of an Fe_3O_4/γ-Fe_2O_3 mixture with a dextran shell; the third (SPIO) is comprised of Fe_3O_4/γ-Fe_2O_3 nanocrystallites in a dextran matrix. The BNFs fail to generate significant heat until ~20 kA/m, and the SLP is still increasing past 500 W/g-Fe at 65 kA/m. The JHUs start to generate significant heat above ~5 kA/m, and then plateau at about 440 W/g-Fe at ~50 kA/m. The SPIOs plateau at about 150 W/g-Fe at ~30 kA/m. All three nanoparticles have a mean hydrodynamic diameter of 100 nm (as measured by dynamic light scattering). The 15% variation in the saturation magnetization, as determined by magnetometry, is insufficient to explain the differences in SLP as a function of magnetic field amplitude. Therefore, we used polarization analysed small angle neutron scattering (PASANS) to probe the internal magnetic domain structure of the nanoparticles. PASANS demonstrates that when multiple domains are present within a single nanoparticle, these domains must be aligned with a larger field before significant SLP will be generated. In addition, the stronger the coupling between the domains, the larger the field required before the SLP plateaus. Therefore, the internal structure of the magnetic nanoparticles plays a significant role in determining the SLP.

Magnetic hyperthermia – Immobilisation state of magnetic multicore nanoparticles injected into living tumors

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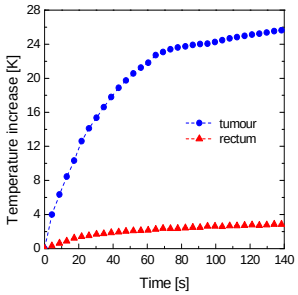
One of the main applications of magnetic nanoparticles (MNP) is magnetic heating therapy represented by magnetic particle hyperthermia and thermoablation for the treatment of malignant tumors. Due to different relaxation mechanisms and the related losses during reversal of magnetization the MNP are heated up when exposed to an alternating magnetic field of sufficient strength.

The proportion of each single reversal mechanism on the heating effect depends on the structural and magnetic properties of the particles and the surrounding medium. For the heat generation inside a living tumor it is of particular interest whether the particles are mobile in the extra- and intracellular liquid or if they are fixed to the tumor tissue because these conditions may determine the dominating relaxation mechanism. Up to now the behavior regarding the immobilization state of the MNP in tumor tissue is more or less unknown.

Aim of this contribution was the investigation of this magnetic behavior of MNP injected into tumors in vivo. To this end magnetic multicore particles were injected into MDA-MB-231 tumors subcutaneously grown between the shoulder blades of female immunodeficient mice. Mice were sacrificed before or after magnetic heating treatment in an alternating magnetic field (H = 24 kA/m, f = 400 kHz) and tumors were removed for further investigations. The spatial distribution of the particles inside the tumor was investigated by X-ray images and histological examination. Magnetic properties of the MNP in the tissue were determined by vibrating sample magnetometry and were compared to the properties of the same particles dispersed in water or fixed to a gelatine matrix.



Temperature rise within the tumor (circles) and the rectum (triangles) during magnetic heating treatment.

It was found that the injected MNP are relatively inhomogeneously distributed as spots in the tumor tissue. In each spot the spatial distribution of the MNP is predominantly homogeneous without formation of clusters. They show a magnetic behavior like the MNP fixed to a matrix which means that the MNP are bound relatively tight to the tumor tissue. This is very important for the choice of suitable MNP for magnetic heating therapy. In this case MNP with high hysteresis losses or high Neel-losses (depends on frequency and amplitude of the field) are promising for a successful magnetic heating. In this contribution we show the experimental methods and the results for the investigation of this behavior, describe the performance of magnetic heating treatment on mice, and discuss the importance of our results for further development of MNP for magnetic particle hyperthermia and thermoablation.

Is the magnetic hyperthermia mechanism a universal one? The case of dendritic cells.

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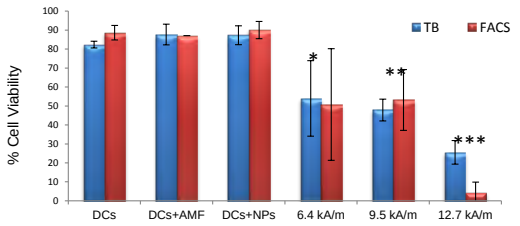
We present a series of *in vitro* experiments using *human-monocyte-derived* dendritic cells (DCs) previously loaded with magnetic nanoparticles (MNPs) as heating agents, as a proof of concept of a 'Trojan horse' strategy for immune-related therapies. Direct observation using TEM and dual-beam SEM/FIB techniques demonstrated that the final distribution of MNPs was in the cytosolic area inside phagocytic/endosomal vesicles and a negligible amount attached to the cell membrane. In a series of experiments, these magnetically-loaded DCs were submitted to alternating magnetic fields (AMF) of f = 255 kHz and different amplitudes (up to 12.7 kA/m) for different times from 5 to 30 min. These experiments showed that it is possible to induce cell death with no detectable temperature increase in the culture medium. Up to 98% of induced cell death occurred for quantities of uptaken MNPs as low as 1.5 pg Fe_3O_4/cell. Moreover, we found that it is possible to control the magnitude of induced cell death by an adequate tuning of the physical AMF parameters and exposure time as well as through the control of the amount of loaded MNPs. Fluorescence-activated cell sorting and trypan blue analysis performed concurrently allowed us to identify this cell death route as a necrotic-like process. This process also resulted in cell membrane disruption and the loss of the cell structure, which was most likely related to cytoskeletal damage.

We propose that the cell death mechanism could be due to MNPs confined within the lysosomes that cause the disruption of lysosomal membranes when submitted to AMF, thus releasing the lysosomal material into the cytoplasmatic space and triggering cell death. These results represent a new and challenging concept of cell death caused by the action of an intracellular agent and, at the same time, pose the question of whether there is a universal mechanism by which magnetic hyperthermia will work on different cell types.



Effect of the different AMF amplitudes (f = 260 kHz, t = 15 min) on the cell viability of loaded DCs (1.5±0.8 pgFe_3O_4/cell), as measured by Trypan Blue and Fluorescence-activated cell sorting (FACS). For all experiments n=6. The AMF amplitude for the control (DCs+AMF) was 12.7kA/m.

Thermotherapy of Experimental Glioblastoma with Laponite-Embedded Magnetic Iron-Oxide Nanoparticles

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Local hyperthermia generated by magnetic nanoparticles (MNPs) has recently been described in human patients for the thermotherapy of the most lethal form of brain cancer known as glioblastoma (GBM) after intratumoral implantation in high concentration.[R] In this work, we describe the use of magnetic iron-oxide nanoparticles (IONPs) embedded on a synthetic clay matrix (laponite) for thermotherapy of experimental GBM. We have recently reported these biocompatible laponite-embedded magnetic nanoparticles exhibit high saturation magnetization at low applied magnetic fields which permits generation of high temperature elevations [R].

A comparison of temperature elevation was made with amphiphilic triblock copolymer-coated IONPs (6 mg/ml; mean diameter of 15 nm) and laponite-embedded IONPs (3 mg/ml; mean diameter of 13 nm) after application of an alternating magnetic field (288 kHz) for 10 min. Therapy-resistant human glioblastoma cells (U87MG, U87wtEGFR, U87ΔEGFRvIII) that overexpress the wild-type (wt) epidermal growth factor receptor (EGFR) or the deletion mutant EGFRvIII, were treated with laponite-embedded IONPs (3 mg/ml) or control (medium). After 24 h of incubation with the laponite-embedded IONPs, GBM cells were treated with an alternating magnetic field (288 kHz) for 10 min. Cell survival and proliferation were assessed by a crystal violet assay. Toxicity studies were performed with human GBM cells (U87ΔEGFRvIII) and human foreskin fibroblasts (HFF) after treatment with laponite-embedded IONPs (12, 24, and 48 h) or control (medium) and no application of applied magnetic fields.

A greater than thirteen-fold elevation in temperature was achieved after application of an alternating magnetic field (288 kHz) with laponite-embedded IONPs (68 °C) in comparison to polymer-coated IONPs (5 °C) double in concentration. A large drop in GBM cell survival and proliferation was found in all therapy-resistant cell lines after application of alternating magnetic fields and thermotherapy. Minimal to no toxicity was found in GBM or HFF cells at 12, 24, and 48 h of treatment with laponite-embedded IONPs in comparison to control treatment of cells.

The results of our study suggest that Laponite-embedded IONPs represent an ideal magnetic nanoparticle composite material for the thermotherapy of experimental GBM at low concentration when exposed to safe alternating magnetic fields.

Injectable Nanocomposite for local Hyperthermia

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Cancer death results from metastasis in 30 to 70% of the patients. Breast, prostate and thyroid cancers develop metastasis in more than 60% of the cases, among which 70% occur in the spine. To prevent and heal bone cancer related to weakening and fracturing of the spine, a established therapeutic method is the injection of bone cement at the location of the cancer, so called vertebroplasty.

In the present work, we developed a new system for spine cancer treatment by superparamagnetic iron oxide nanoparticle (SPION)-mediated hyperthermia. A maximal volume fraction of SPION was added to a bone cement formulation for combined vertebroplasty and hyperthermia therapies. Additionally a human size magnetic field applicator for delivering homogeneous high frequency magnetic field in the spine region with minimal parasite body heating was developed.

Firstly, a highly concentrated SPION (Fe_3O_4) suspension was prepared by alkaline co-precipitation of ferric and ferrous chlorides in aqueous solution and then washed and concentrated by magnetic sedimentation up to a concentration of > 100mg/ml. Secondly, a continuous silica matrix containing SPIONs was formed by adding tetramethyl orthosilicate (TMOS) directly to the SPION suspensions and drying at 200°C Celsius for 24 hours. The formed Silica-SPION nanocomposite with ~70%(w/w) Fe_3O_4 was then ground down in a ball mill to make beads with diameters ranging from 20 to 50 µm. The obtained Silica-SPION beads (SSB) show a strong heating behavior (2.8 W/g) in a commercial high frequency magnetic field generator operated at 144kHz and 12mT. Commercial acrylic cement loaded with SSB was injected in nude mice. The temperature profile indicates a high in vivo temperature increase (> 10° C) which induced a 5 mm large necrosis area in the tumor.

At the same time, a lightweight, human sized (coil diameter = 400mm) magnetic field applicator has been developed with excellent field uniformity, that can operate at high frequencies (300 kHz). Much analysis and optimisation was performed using numerical models of humans in full anatomical detail to maximize homogeneity while minimizing unwanted heating of normal tissue.

Inter-Particle Interactions in the Static and Dynamic Magnetic Properties of Ferucarbotran Colloids

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Ferucarbotran colloids and similar products consisting of maghemite/magnetite nanoparticles (Fig. 1) coated with biocompatible molecules are set as the industry standard for MRI in medicine, and more recently, for experimentation in magnetic hyperthermia. In the pursuit of assessing their effectiveness, the benefits of dextran and some of its derivatives as biocompatible coatings for these colloids have been extensively studied, but much less attention has been paid to the effects on the magnetic properties with respect to the stability of the coating. Any departure away from normal storage or handling conditions may bring a significant change in the magnetic performance of Ferucarbotran, modifying its characteristic relaxation and specific absorption rate, both critical in medical applications.

Standard magnetic characterisation techniques often rely on MH curves as the only source of practical information about magnetic nanoparticles for bio-applications. We show how a suitable combination of both static and dynamic measurements provides a more realistic picture of the magnetic structure. For example, Henkel plots (and ∂M plots) are an effective technique to determine the presence of inter-particle interactions in a magnetic system. It is shown that in the event of a partial coating destabilisation there is an increase in inter-particle interactions resulting in demagnetising dipolar interactions (Fig. 2).

A complementary set of measurements have been taken using transmission electron microscopy, X-ray diffractometry, inductively coupled plasma, Mössbauer spectroscopy and high frequency AC susceptometry in order to fully characterise the physical-chemical characteristics of Ferucarbotran colloids under different conditions, with special emphasis on the inter-particle interactions.

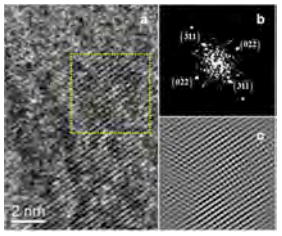

Fig. 1 HRTEM image of ferucarbotran nanocrystals (a); indexed diffraction pattern (b) and Fourier-filtered image (c) of the selected area viewed under the [233] zone axis.

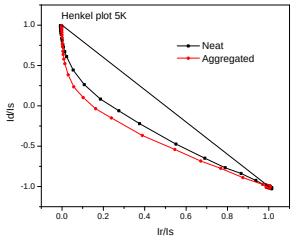

Fig. 2 Henkel plot showing an increase in demagnetising interactions as a result of aggregation.

Friday, May 25, 2012

8:00	**Registration desk opens** / No poster sessions today!			
8:30	**Hawkins, Peter**	**Tutorial II - Magnetic Particles in Immunoassays**	Bristol, UK	Tutorial 2
	Session 6: Magnetic Imaging / MPI			
9:00	Bales, Brian	GEH121333: A New Iron Oxide Nanoparticle for Magnetic Resonance Imaging in Inflammation and Oncology	Niskayuna, USA	Talk 36
9:15	Begin-Colin, Sylvie	Why a dendritic approach to biocompatible iron oxide nanoparticles for bioimaging ?	Strasbourg, France	Talk 37
9:30	Kim, Jongsik	High Speed Magnetomotive Optical Coherence Tomography	Urbana, USA	Talk 38
9:45	Liebl, Maik	In-Vivo Quantification of Magnetic Nanoparticle Distributions after Magnetic Drug Targeting in a Rabbit Carcinoma Model using Magnetorelaxometry	Berlin, Germany	Talk 39
10:00	**Coffee break**			
10:30	Saville, Steven	The Influence of Linear Magnetic Chain Formation on Transverse Relaxation Rates in Magnetic Resonance Imaging	Clemson, USA	Talk 40
10:45	Rahn, Helene	Quantitative Tomographic Examination of Magnetic Nanoparticles	Dresden, Germany	Talk 41
11:00	Riffle, Judy	Magnetic Block Ionomer Clusters (MBIClusters) with Ultrahigh Transverse NMR Relaxivities for Dual Imaging and Therapeutic Agents	Blacksburg, USA	Talk 42
11:15	Trekker, Jesse	Optimizing Superparamagnetic Nanoparticles towards Contrast Enhanced MRI for sensitive in vivo cell detection	Leuven, Belgium	Talk 43
11:30	**Krishnan, Kannan**	**Developing Tracers for Enhanced Resolution and Sensitivity in MPI**	Seattle, USA	Invited talk 5
12:15	**Lunch at McNamara Center / Exhibitors**			
12:30	**POSTER PRIZE** - *Presented by Cordula Gruettner during the lunch*			
	Session 7: Magnetic Separation			
13:30	Herrmann, Inge	Cleaning Blood: Applications of Ultra-strong Metal Nanomagnets in Nanomedicine	Zurich, Switzerland	Talk 44
13:45	Benelmekki, Maria	Magnetophoretic transport of non-magnetic latex colloidal particles across a magnetic fluid volume under a uniform magnetic field gradient	Braga, Portugal	Talk 45
14:00	Dapprich, Johannes	Identification of New Sequence Variants by Targeted Enrichment and Next-Generation Sequencing	Lawrenceville, USA	Talk 46
14:15	Dutz, Silvio	A Microfluidic Chip for Size Dependent Fractionation of Magnetic Microspheres	Vancouver, Canada	Talk 47
14:30	Ijiri, Yumi	Novel method for magnetic field and size based purification of magnetic nanoparticles	Oberlin, USA	Talk 48
14:45	Ooi, Chin Chun	Effect of Magnetic Field and Magnetic Field Gradient on Effectiveness of the Magnetic Sifter for Cell Purification	Stanford, USA	Talk 49
15:00	Plouffe, Brian	Magnetic Particle-Based Microfluidic Circulating Tumor Cell Separation for Oncological Therapeutic Monitoring	Boston, USA	Talk 50
15:15	Samia, Anna Cristina	pH-Controlled Adsorption of Cd ions on Carboxyl-Terminated Superparamagnetic Iron Oxide Nanoparticles	Cleveland, USA	Talk 51
15:30	Sharma, Anirudh	Magnetic barcode nanowires for osteosarcoma cell control, detection and separation	Minneapolis, USA	Talk 52
15:45	Yang, Liangrong	A novel separation technique: gas-assisted superparamagnetic extraction for scale-up of protein isolation (bovine serum albumin BSA)	Beijing, China	Talk 53
16:00	**Coffee break**			
	Session 8: Magnetic Gene Deliver / Interesting Magnet Systems			
16:30	Schuerle, Simone	An Electromagnetic Manipulation System for Pre-clinical Testing of Targeted Drug Delivery	Zurich, Switzerland	Talk 54
16:45	Mykhaylyk, Olga	Silica-Iron Oxide Magnetic Nanoparticles for Viral Gene Delivery	Munich Germany	Talk 55
17:00	Zimmermann, Katrin	Optimization of magnetic nanoparticles assisted lentiviral gene transfer and cell positioning	Bonn, Germany	Talk 56
17:15	Shapiro, Benjamin	Treating Tinnitus by Magnetic Pushing of Therapy: Rat Experiments	College Park, USA	Talk 57
17:30	Dumas-Bouchiat, Frederic	Magnetic tool for in-vivo magnetic capture: Applications in blood filtering and proteomic diagnostics	Grenoble, France	Talk 58
17:45	Nacev, Alek	Improving the Treatment of Hypoxic Breast Cancer Liver Metastases by using Dynamic Magnetic Shift	College Park, USA	Talk 59
18:00	**Traditional boat cruise on the Mississippi - Buses leave in front of the University Hotel ;** dress appropriately, it gets windy on the boat!			

GEH121333: A New Iron Oxide Nanoparticle for Magnetic Resonance Imaging in Inflammation and Oncology

B. C. Bales[1*], D. Meyer[1], B. Hay[1], E. Pressman[1], T. Luttrell[1], P. Buckley[1], P. Bui[1], B. Lee[1], C. Morton[1], N. LaPlante[1], S. Bhaumik[1], L. Lowery[1], J. Lee[1], D. deMoulpied[1], K. Langseth[2], P. Sontum[2], S. Fairway[2], I. Henricksen[2], D. Grant[2], P. Gordon[2]

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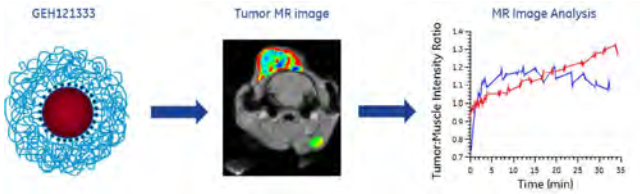
There is currently significant interest in the use of superparamagnetic iron oxide (SPIO) nanoparticles as contrast agents for magnetic resonance (MR) imaging. Despite this fact, there are currently no clinical SPIO nanoparticles marketed in the U.S. for use as MR contrast agents.

We have developed a SPIO nanoparticle, GEH121333, for use as a MR contrast agent in inflammation and oncology imaging. GEH121333 is synthesized using a two-step, scalable approach, wherein the SPIO nanoparticles are first synthesized in an organic solvent and then a subsequent ligand exchange provides an aqueous suspension of SPIO nanoparticles following rigorous purification. GEH121333 has a size of 22 nm as measured by dynamic light scattering, is very monodisperse as measured by asymmetric field flow fractionation, and has relaxivity properties which allow both T2 and T1 weighting MR imaging in the same exam.

GEH121333 has been shown to image macrophage cells present in inflammation and to allow quantitative measurement of the blood volume and vessel permeability in tumors by following the change in the SPIO signal over time. In addition to imaging efficacy, GEH121333 was designed with patient safety as a very high priority. GEH121333 does not show indications of immune activation in vitro, is highly stable and resists protein interactions in biologic media, and is well tolerated in rats at least to 250x above the anticipated clinical dose. In addition, a rapid processing of the nanoparticles in the liver has been observed, providing the ability to re-image patients sooner. The unique combination of physical and biological properties of GEH121333 make it well suited for inflammation and oncology imaging.



Why a dendritic approach to biocompatible iron oxide nanoparticles for bioimaging ?

Delphine Felder-Flesch,[a*] Claire Billotey,[b] Sylvie Begin-Colin, [a*]

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[b] Laboratoire de Physico-chimie des Matériaux Luminescents LPCML-UCBL UMR 5620 5 place d'Arsonval F-69437 Lyon, France.

Superparamagnetic iron oxide nanoparticles (NPs) with appropriate surface coating are widely used for numerous in vivo applications and in particular for MRI contrast enhancement. To improve the contrast enhancement and targeting properties as well as the biodistribution of functionalized iron oxide NPs, challenges have to be overcome such as: (i) the design of an organic coating favouring ideal biodistribution, ensuring multifunctionalization (targeting, optical imaging…) and preserving a small size distribution of coated NPs in physiological media (<50 nm), (ii) the synthesis of iron oxide NPs with optimal magnetic properties and (iii) the development of strong anchors at the NPs surface avoiding desorption of molecules in blood. Indeed, the coating designs as well as the interaction nature between the organic shell and the nanocrystal surface are key points to address. We thus propose a concept combining a dendritic coating with phosphonate anchors. Indeed, phosphonates ensure a strong anchoring at the NPs surface while preserving their magnetic properties, and dendritic shells, in addition to their small and easily controllable size (as a function of their generation), are promising building blocks simultaneously solving the problems of biocompatibility, large in vivo stability and specificity. Iron oxide NPs synthesized by co-precipitation and thermal decomposition were coated with functional oligoethyleneglycol or poly(amido)amine (PAMAM) dendrons to improve colloidal stability, graft fluorophores and investigate cell interactions. Different grafting strategies were optimized as a function of the NPs synthesis and dendron nature. The size distribution, colloidal stability in isoosmolar media, nature of surface complex, biodistribution and contrast enhancement properties evaluated through in vitro and in vivo MRI experiments were compared as a function of the nature of both dendrons and nanoparticles. All functionalized nanoparticles (whatever the synthesis method) display good colloidal stability in water but, in isoosmolar media, best results were observed with functional dendronized NPs bearing carboxylates at their periphery. The *in vitro* contrast enhancement properties of all dendronized nanoparticles were found higher than those of commercial products (polymer-decorated) and the best values were recorded for the nanoparticles synthesized by coprecipitation due to their higher saturation magnetization. However the NPs synthesized by thermal decomposition were more efficient *in vivo* due to their lower particle size distribution. Moreover, no evident adverse effect was observed in rat after injection, even at high concentrations and a long time after injection. The biodistribution of such nanohybrids was also studied by optical imaging thanks to Alexa labelling at the dendron periphery. In this case, a fast hepatobiliary, together with a low urinary, elimination was observed. Luckily, no RES uptake could be highlighted. Such study confirmed the interest of the dendritic approach to develop new, smart and multimodal contrast agents.

Chem. Comm. 2010, *46*, 985-987; *Contrast Med. and Mol. Imaging*, 2011, *6*, 132-138; *Biomaterials*, 2011, 32, 8562-8573; *New J. Chem. invited Perspective review in DENDRIMER 2,* 2012, DOI: 10.1039/C1NJ20416E**;** *Eur. J. Inorg. Chem., invited microreview on manganese-based Contrast Agents,* 2012, DOI: 10.1002/ejic.201101163

High Speed Magnetomotive Optical Coherence Tomography

Jongsik Kim,[a*] Adeel Ahmad,[a,b] Stephen A. Boppart[a,b,c,d]

[a]Beckman Institute for Advance Science and Technology, [b]Departments of Electrical and Computer Engineering, [c]Bioengineering, and [d]Internal Medicine,
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*E-Mail : kim37@illinois.edu

Optical coherence tomography (OCT) [1] has been used to perform high-resolution cross-sectional imaging in clinical and research applications. OCT is analogous to ultrasound imaging, but uses near-infrared light rather than sound to achieve resolutions that are at least an order of magnitude higher. Magnetomotive OCT (MM-OCT) is a functional extension of OCT which utilizes magnetic nanoparticles (MNPs) that are modulated by an external magnetic field for contrast enhancement and for elastography to assess the structural and viscoelastic properties of the surrounding tissues.[2,3] The contrast mechanism of magnetomotive (MM) imaging relies on the presence of a restoring force acting on the particles after MNP displacement induced by a relatively weak external magnetic field (≥ 100 Gauss). When the restoring force from a sample with MNPs is weak or non-existent, the MM-OCT signal-to-noise ratio (SNR) can degrade significantly. These factors make MM-OCT imaging almost impossible with a sample that does not have an elastic restoring force, such as magnetic particles within a low-viscosity liquid medium.

We have developed a novel solenoid configuration to enable MM-OCT imaging in samples that do not have an elastic restoring force. Moreover, this coil is air-cooled with no significant heating, potentially enabling real-time MM-OCT imaging for extended durations. The modulation signal is provided by a two coil configuration setup based on Lenz's law from electromagnetic theory,

$$\text{Voltage (emf)} = - N \Delta\phi / \Delta t$$

where N is the number of turns in the coil, Δt is the time interval, and $\Delta\phi$ is the magnetic flux equal to BA. B is the external magnetic field and A is the cross-sectional area of coil. The alternating magnetic field (B; ~200 Gauss) in the primary coil (45 turns; Fig. 1A) induces the B-field gradient change on the MNPs. This alternating B field gradient between the two coils induces MNP translation in the direction of the B field.

Figure 1 shows the experimental results with a polydimethylsiloxane (PDMS) gel phantom embedded with MNPs (1000 ppm). The MM-OCT system (Fig. 1A) is based on a spectral domain OCT (SD-OCT) system with a Ti:Al_2O_3 femtosecond laser (KMLabs, Inc.) producing 800 nm light with a bandwidth of 120 nm. The axial and transverse resolutions were about 3 µm and 16 µm, respectively. The SD-OCT B-mode image is superimposed with the MM signal (Fig. 1C). The modulation frequency of 200 Hz is clearly visible (about 0.65 dB higher than background noise) in the frequency spectrum (Fig. 1D).

These results demonstrate the potential for a restoring-force-free MM-OCT imaging. Currently, we are investigating the maximum scan speed in liquids. Ongoing studies are also being conducted to explore the possibility of extending these techniques to an *in-vivo* animal model.

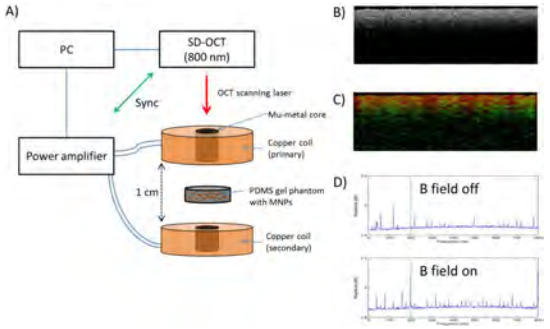

Figure 1. Schematic representation of the MM-OCT system: A) MM-OCT solenoid configuration, B) SD-OCT B-mode image, C) superimposed image of MM signal (green) and OCT B-mode image (red), and D) frequency spectrum (B field on /off) of MM signal.

References

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In-Vivo Quantification of Magnetic Nanoparticle Distributions after Magnetic Drug Targeting in a Rabbit Carcinoma Model using Magnetorelaxometry

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In Magnetic Drug Targeting (MDT) magnetic nanoparticles (MNP) carrying chemotherapeutic agents are administered into a tumor supplying artery and accumulated at the tumor region by an external magnetic field gradient. A quantitative and spatially resolved knowledge about the MNP distribution inside an animal model is provided by magnetorelaxometry (MRX). This technique detects the relaxation of the magnetic moment of MNP by a magnetic field sensor after the sample has been magnetized.

We present an adapted MRX procedure capable for *in-vivo* MRX on a rabbit tumor model (VX2 squamous cell carcinoma) after Magnetic Drug Targeting using a 304-channel SQUID system. The magnetization field was generated by a large Helmholtz coil. To account for the large animal extension (about 50 cm) compared to the sensor array diameter (about 20 cm) two consecutive MRX measurements were performed. First, the sensor array was focused at the tumor position near the rabbit's hind leg and then the liver region. The magnetic field pattern of two small copper coils allowed the localization of the two regions and the combination of both measurements.

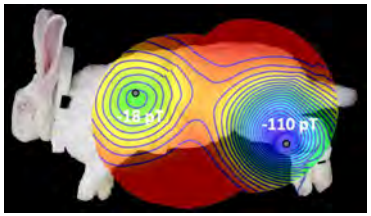

Figure 1: Combined magnetic field map of the nanoparticle distribution inside the rabbit with the estimated centers of gravity.

The MNP amount and centre of gravity of the MNP distribution in liver and tumor was determined by an inverse procedure using a magnetic point dipole model (tumor) or an extended homogenous 3D magnetization distribution (liver). We demonstrate the capability of MRX to quantify the MNP accumulation in an *in-vivo* setting providing valuable information for MDT. With reference measurements the minimum magnetic relaxation moment that is sufficient for MNP quantification was determined and used as guidance for suppression of artifacts in the MRX signals caused by breathing and cardiac activity of the animals. Additionally, the magnetic signals from the breathing and heart activity can be used for monitoring the vital function of the rabbit.

The Influence of Linear Magnetic Chain Formation on Transverse Relaxation Rates in Magnetic Resonance Imaging

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There have been significant advances recently in the use of magnetic nanoparticles for various biomedical applications, with one potential use as contrast enhancement agents in magnetic resonance imaging (MRI).[1] In MRI, the size of the nanoparticle and the stabilizing layer play a tremendous role in not only particle stability, but in how they interact with their surroundings.[2] It is well known that magnetic nanoparticles will chain under in the influence of an external magnetic field,[3] but it is not well understood how the formation of these linear magnetic structures affect the transverse relaxation rate. In order to study the effect chain formation on MRI contrast enhancement and its relationship with the stabilizing brush length, a matrix of 22nm particles with varied ligand lengths was synthesized using iron oxide particles with poly(ethylene glycol) ligands. These systems were characterized with dynamic light scattering, transmission electron microscopy, dark-field scattering, and proton transverse relaxation measurements. The dark field scattering experiments and transverse relaxation measurements were done in a similar magnetic field under the same time scale to correlate the reduction of the transverse relativity with the formation of linear magnetic chains. Our results suggest that varying the ligand length has a direct effect on the transverse relaxation mechanism due to the contribution of ligand length to the colloidal stability of the system, including differences in chain formation rate and size. With increasing ligand length, interparticle interactions are limited, which results in slower chain formation and shorter widespread chains. This data indicates that understanding the colloidal arrangement of these systems is paramount, and that both particle stability and time dependence both play a key role in determining the effect of iron oxide nanoparticles on surrounding water protons.

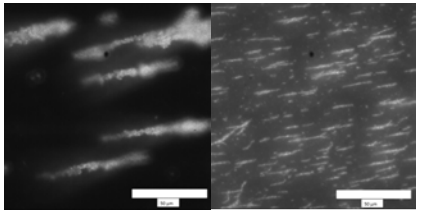

Figure 1: Dark Field Scattering for A) 550MW and B) 5000MW PEG coated 22nm nanoparticles under .1T external magnetic field

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Quantitative Tomographic Examination of Magnetic Nanoparticles used as Drug Carriers

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The success of the minimal invasive cancer therapies magnetic drug targeting (MDT) and magnetic heating treatment (MHT) depends strongly on the correct distribution of the magnetic nanoparticles as well as on the fact that a sufficient amount of magnetic nanoparticles carrying drugs is accumulated in the target region. A study whether the mentioned requirements are fulfilled has been done using a non-destructive 3-dimensional imaging method X-ray-micro computer tomography (XµCT).

A calibration procedure for tomographic equipment with adequate phantoms has been developed. This opens now the possibility to analyze tomographic data in a quantitative way with respect to the nanoparticle content. Thereby, the nanoparticle concentration is assigned voxel-wise to the grey values of the three-dimensional tomographic data. Thus, the tomographic data of a biological tissue samples as e.g. tumors after MDT can be analyzed with regard to 3-dimensional nanoparticle distribution as well as the nanoparticle content. The tomographically acquired nanoparticle contents have been compared with results obtained with the quantitative measurement method magnetorelaxometry (MRX). A good agreement between the quantitative tomographic data and quantitative MRX data has been figured out.

a) b) c)

a) Optical photography of a tumor treated with MDT and then embeded in paraffin; b) 3-dimensional representation of the tumor shown in a). The nanoparticle accumulation can be qualitatively seen. This is displayed in red to black labeled grey values correlating with magnetic nanoparticles. c) Quantitatively evlauted data set of the tumor, the different colors are linked to different nanoparticle content. The total nanoparticle content figured out with the help of the calibration procedure amounts to 24.41 mg while the reference MRX value is 25±2 mg. The calculated deviation between the quantitative tomographic result and quantitative MRX-result is thus 2.36 % only.

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Magnetic Block Ionomer Clusters *(MBIClusters)* with Ultrahigh Transverse NMR Relaxivities for Dual Imaging and Therapeutic Agents

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Magnetite nanoparticles are powerful contrast enhancement agents for T_2-weighted MRI because of their high magnetization, low toxicities and particulate nature. Multifunctional magnetite nanocarriers that also contain therapeutic agents are of great interest for delivering drugs and tracking their biodistribution via MRI. This is challenging since the contrast agents disperse broadly *in vivo* and sufficient concentrations of the agents must accumulate in close proximity to provide good contrast. Another challenge in terms of method sensitivity is the potential use of magnetite labels in cellular therapies, for example in stem cells, where cell tracking *in vivo* with MRI as the cells differentiate and migrate is desirable.

This paper introduces magnetic block ionomer clusters *(MBIClusters)* that combine the attributes of core-shell block ionomer complexes containing electrostatically-bound drugs with magnetite nanoparticles designed for MRI. Our strategy has been to co-encapsulate cationic antibiotics and magnetite with anionic, doubly-hydrophilic poly(ethylene oxide-*b*-acrylate) copolymers to form discrete polymer-magnetite nanoparticles, then to crosslink the tips of the shells to provide controlled size clusters (figure 1). These differ from previous clusters in that the space between particles within the clusters is hydrophilic. The average cluster size is controlled by adjusting the reactant concentrations in the crosslinking step. Transverse relaxivities of these materials scale with the average size and transverse relaxivity measured at 1.4T and 37°C can be reproducibly controlled over almost an order of magnitude (figure 2). *In vitro* studies have shown that these MBIClusters are efficiently taken up by macrophage-like cells that have been infected with pathogenic bacteria.

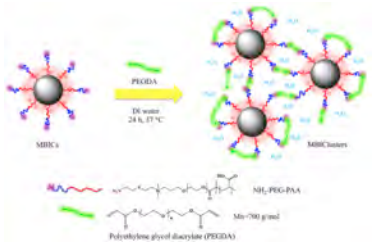

Figure 1. Crosslinking of preformed, discrete magnetite-polymer nanoparticles through the tips of the coronas enables formation of doubly-hydrophilic clusters.

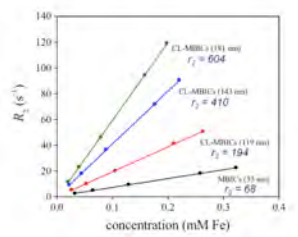

Figure 2. *MBIClusters* with hydrophilic cores and coronas have r_2's that scale over an order of magnitude with increased size.

Optimizing Superparamagnetic Nanoparticles towards Contrast Enhanced MRI for sensitive in vivo cell detection

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Commercially available magnetic nanoparticles (MNPs) used as contrast agents in Magnetic Resonance Imaging (MRI) show significant heterogeneities in shape and size and are in their composition not optimized for in vitro cell labeling (1). To overcome these drawbacks, we have synthesized MNPs through the thermal decomposition method (2), and obtained small, spherical MNPs with a narrow size distribution. By using a seed mediated growth approach, we obtained sizes from 6.9 to 12.9 nm with size dispersions ≤ 10%. We conducted a successful ligand exchange of the original hydrophobic coating with a hydrophilic covalently attached poly-ethylene-glycolated silane.

In this report, we compared the labeling properties of the small cored MNPs (6.9 nm) versus the large cored MNPs (12.9 nm). Mesenchymale stem cells (MSCs) were labeled under different conditions with the MNPs. Limited to no toxicity was observed on the labeled cells in a Prestoblue™ cell viability assay and in cell proliferation studies. Fluorescence confocal microscopy revealed a perinuclear localization of the MNPs in the cells (Figure A). This was further confirmed with transmission electron microscopy showing no effect of the cell uptake on the size and shape of the MNPs.

Next, we evaluated the contrast generating properties of the labeled cells. Firstly, the MSCs were labeled with different concentrations of MNPs and suspended in an agar phantom. The relaxation rate (R^*_2) was measured at 9.4 Tesla. The large cored MNPs generated higher R_2* per intracellular iron versus cells labeled with small cored MNPs (Figure B). This result was confirmed in the following in vivo tests. In this set of experiments, 100.000 or 300.000 cells labeled with different concentrations of small or large cored MNPs, were stereotactically injected in the brain of mice. Because of the local high concentration of MNPs a signal void was detected in the MRI images. The volume of this signal void was proportional to the total amount of iron injected in the brain, and to the size of the iron core of the MNPs used as cell label. Again, the cells labeled with the large cored MNPs produced larger void signals compared to cell labeled with the smaller cored MNPs (Figure C). In addition both synthesized MNPs created larger signal voids compared to the commercial contrast agent Endorem©.

The proposed route of MRI contrast agent development demonstrated the strength of the applied synthesis and functionalization methods to produce a range of high performing MRI contrast agents.

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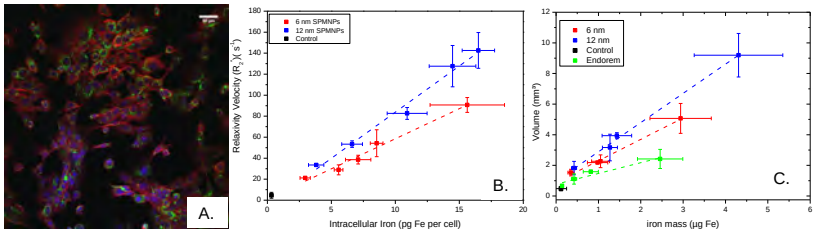

Figure. (A) Confocal fluorescence microscope image of MSCs labeled with fluorescent MNPs. The MNPs were coated with a FITC-silane and the actine of the MSCs was stained with a red alexa fluor actine stain. The nuclei of the MSCs were stained with Hoechst 33342.Scale bar is 50 µm. (B) Graph of R_2 versus intracellular iron of labeled MSCs with MNPs and measured in vitro in an agar phantom. (C) Graph of hypointense MR volume vs total iron mass of in vivo injected labeled MSCs.

Optimized tracers for Magnetic Particle Imaging

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The performance -- sensitivity and spatial resolution -- of Magnetic Particle Imaging (MPI), a new medical imaging technology for whole-body imaging of magnetic tracers, depends largely on the structural, chemical & magnetic characteristics of the tracers, in addition to instrumentation parameters. However, tracer magnetization varies strongly with particle size at the nanoscale, making MPI the first clinically relevant imaging technology that depends on nanotechnology. Hence, for a given imaging system (field gradient, applied frequency etc.), best performance can be achieved by optimizing tracer magnetic (static and dynamic relaxation) response, crystallographic (phase purity) structural (size and size distribution) and morphological (agglomeration and aqueous stability) properties. In addition, tracer performance critically depends on their biocompatibility as well as surface functionalization for maximum circulation and/or specific targeting.

With simulations and experiments we have determined the ideal size for MPI at multiple driving frequencies, from 5 kHz to 250 khz. Using wet-chemical methods, we synthesize optimized and highly uniform tracers, over a wide size range, that are matched to the requirements at a specific frequency and show improved signal intensity (by 30x), spatial resolution (by 2x), determined from the point-spread function: the full-width-half-maximum of $M'(H(t))$, and uniformly greater intensity in harmonic spectra (for all of the 40 harmonics measured by our device), compared with commercially available tracers (such as Feridex I.V™ or Resovist™). Finally, for the first clinical application of angiography, tracer availability in the vasculature is of utmost importance and hence pharmacokinetics and dose-dependent biodistribution characteristics of tracers in an animal model (CD-1 mice) were determined. On the other hand for molecular imaging of cancer a binding-model that arrest the Brownian relaxation is relevant. Incorporating the details of these experiments and measurements, our comprehensive approach to the development of optimal MPI tracers for these two translational applications will be presented.

Cleaning Blood: Applications of Ultra-strong Metal Nanomagnets in Nanomedicine

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Nanomagnets with metal core have recently been shown to be promising candidates for magnetic drug delivery and hyperthermia due to superior magnetic properties compared to commonly used beads. This presentation will discuss the direct removal of harmful substances from human whole blood by the use of functionalized magnetic nanoparticles. As a successful application strongly relies on a safe implementation, a particular focus is put on possible interactions of nanomagnets with the vascular compartment. The presentation will also discuss the implementation of the technology into an extracorporeal blood purification device and further steps in the direction of a clinical application of the concept.

Carbon coated metallic nanoparticles were equipped with various functional groups, including heavy metal scavengers, fab-fragments and antibodies. Carbon coated metal nanomagnets were added to fresh human whole blood where they scanned the liquid volume and captured the target compounds. After removal of the toxin-loaded nanomagnets by magnetic separation, the blood was analyzed for remaining toxin or inflammatory mediators, iron metabolism and blood integrity.
The applicability of the concept is demonstrated utilizing three examples: The removal of a heavy metal (lead), a steroid drug (digoxin) and whole proteins (Interleukin-6 and Interleukin-1b) was achieved by spiking human whole blood with the contaminant and applying appropriately functionalized magnetic beads for the detoxification. The contaminant concentration in intoxicated whole blood could be significantly decreased in a dose-dependent manner using a magnetic separation-based blood purification technology. The integrity of the blood was not affected by the process as depicted by monitoring a series of clinically important parameters.

Noxious compounds differing in chemical nature (ions, small molecule drugs, and proteins) can be efficiently and selectively removed from whole blood without being limited by filter cut-offs or slow pore diffusion. 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 or auto-immune diseases.

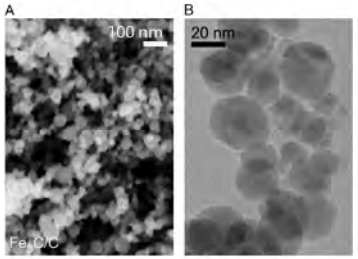

Figure 1. Scanning Electron Micrograph (A) and Transmission Electron Micrograph (B) of carbon encapsulated iron carbide nanomagnets.

Magnetophoretic transport of non-magnetic latex colloidal particles across a magnetic fluid volume under a uniform magnetic field gradient

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ABSTRACT

In this work, we present a novel method for the separation and recovering of the latex particles across an aqueous volume of magnetic fluid under a uniform magnetic field gradient. We show experimentally that non-magnetic latex particles (Estapor® microspheres, K100 Red) in a dispersion of superparamagnetic NPs experience a nontrivial, two-step "go and come-back" motion when brought under a uniform magnetic field gradient. Our theoretical analysis indicates that the observed motion is due to the combined effect of the behavior of latex particles as magnetic holes and the adsorption of NPs onto the latex surface. The NPs adsorption on latex particles has been confirmed in our experiments by three independent experimental techniques (EDS, SEM and electrophoresis).

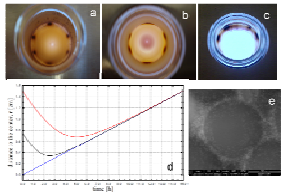

Figure1. Magnetophoretic behavior of a suspension of latex particles and SDS-NPs in water. (a) Initial mixture; (b) latex particles are concentrated in the center of the vessel forming a ring. (c) Both SDS-NPs and latex particles are trapped on the walls of the vessel. (d) Trajectories of the latex particles in the mixture at 60T/m. (e) SEM images in the backscattered detection mode of a sample extracted from the ring.

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Identification of New Sequence Variants by Targeted Enrichment and Next-Generation Sequencing

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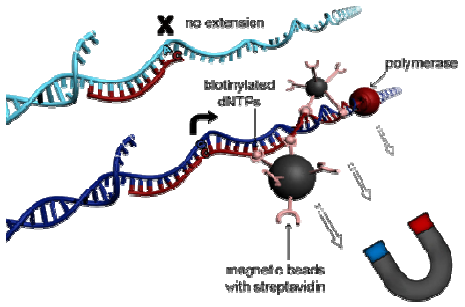
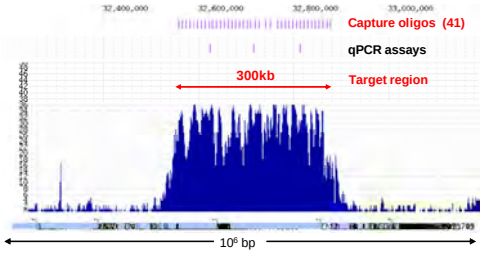
Next-generation sequencing (NGS) platforms are generating sequencing data at unprecedented rates, and the accurate determination of individual genomes at low cost would significantly advance the development of personalized medicine. However, the cost for the bioinformatic assembly after sequencing is significant and can exceed the cost of sequencing itself. The reason is that the precipitous drop in sequencing cost is not being matched by an equally improved efficiency of computing power and storage to keep up with the data overload.

One approach is to free up the sequence assembly pipeline by feeding it only with 'meaningful' data, i.e. by eliminating any reads up front that are likely irrelevant, or by not generating them in the first place. The risk, of course, is that unexpected but potentially valuable data inadvertently gets thrown out as well in the process. Problems typically occur when the genome of interest is highly polymorphic, polyploid, or when a reference sequence is incomplete, contains gaps of unknown sequence or structural variations, or does not exist yet.

It appears that targeted enrichment methods for the selection of genomic regions of interest can address the need for accurate sequence assembly, provided they offer sufficient flexibility: Target capture has to be adjustable - depending on the circumstance - between streamlining the data as desired versus preserving sufficient genomic context as necessary to understand more complex or yet unknown genomic regions.





Region- and haplotype-specific extraction (RSE/HSE) is an automated, magnetic particle-based target enrichment method that captures large (10-40kb) segments of genomic DNA with high efficiency in a sequence- or SNP-specific way.

RSE/HSE provides the ability to accurately determine extended molecular sequence and haplotypes from each target point. A target point can be any specific sequence or known polymorphic position.

RSE/HSE has been used for the resolution of complex genomic regions and ambiguous allele combinations, the identification of previously undetected structural variants, QTL and transposon / T-DNA insertion point mapping, and to determine breakpoints and unknown sequence surrounding a specific associated marker, such as a SNP.

RSE also promises to be a useful tool to avoid any amplification and go directly from sample preparation to sequencing. This will not only streamline operations and allow for the analysis of minute amounts of DNA from individual biopsies, but it can also preserve epigenetic signatures such as methylation, DNA-bound regulatory factors, which are valuable targets for screening in oncology.

A Microfluidic Chip for Size Dependent Fractionation of Magnetic Microspheres

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Magnetic microspheres (MMS) typically exhibit broad size distributions. For clinical magnetic drug targeting, uniform MMS of identical magnetic material content are preferred, so that each microsphere responds in the same way to externally-applied guiding magnetic field gradients. Furthermore, the MMS must be smaller than red blood cells, but large enough to react to applied magnetic field gradients. Since the preparation of monodisperse MMS can be challenging, there is considerable motivation to develop tools for size-dependent fractionation of polydispere MMS to obtain narrow size distributions.

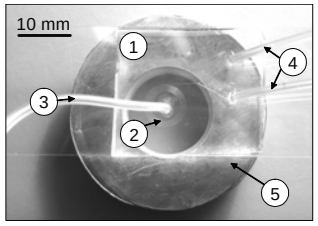

Fig. 1: Photograph of the microfluidic chip (1) showing the spiral structure (2), inlet (3), and two outlets (4). The octupole magnet (5) is visible beneath the chip

In this contribution we present a microfluidic chip for continuous MMS fractionation based on shear-induced inertial lift forces that act on particles flowing in a curvilinear channel (i.e., the Dean effect). As shown previsouly, fractionation achieved using the Dean effect alone does not distinguish between magnetic and non-magnetic particles of the same size. An additional level of control over the fractionation process can be obtained by imposing a magnetic force that acts toward the outer wall of the channel. In our experiment this is accomplished using a radially-directed magnetic field gradient realized by a magnetic octupole. The spiral microfluidic structure was made using polydimethylsiloxane moulding. The chip consists of an inlet (3) at the centre of the spiral, the spiral structure itself (2), and a 1:1 flow splitter at the spiral exit, separating the flow into inner and outer streams that can be collected at the inner and outer outlets (4) and the magnetic octupole (5) mounted below the chip (**Fig. 1**).

The suitability of the chip for separating MMSs into distinct size distributions was confirmed through several experiments. The forces acting on the MMS could be adjusted such that, when 10 µm diameter particles used, nearly all were found in either the inner stream or the outer stream. In a different test, a mixture of 2 µm- and 12 µm-diameter particles was separated into its constituent components. A typical MMS batch with a broad size distribution and a mean diameter of 3.5 µm was also fractionated into samples with mean diameters of 2.8 and 4.3 µm.

In conclusion, the chip described here is appropriate for applications where one wants to exclude particle sizes above or below a given size threshold or magnetic concentration from an MMS batch in continuous operating mode. Furthermore the chip can be used to concentrate particles of from highly dilute suspensions.

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

Novel method for magnetic field and size based purification of magnetic nanoparticles

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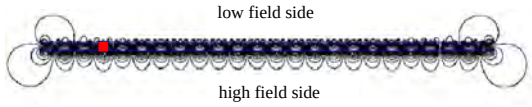
Magnetic nanoparticles are of much interest for a wide range of biomedical applications including hyperthermia, MRI contrast agents, magnetic particle imaging, and magnetic tagging and cell separation. In these applications, features such as efficiency and resolution depend critically on uniformity in not only the size but explicitly the magnetic properties of the nanoparticles. Unfortunately, comparatively few purification methods exist to sort nanoscale materials on the basis of differences in magnetic behavior.

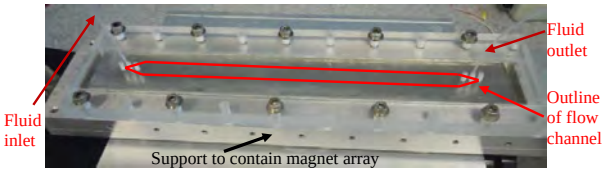
Here, we describe an unusual approach to this problem, based on a so-called Halbach array of permanent NdFeB magnets. Specifically, we have constructed a linear arrangement of ~ 50 magnets with 90 degree shifts in the relative magnetization orientation for successive blocks. While typically this configuration is used for its concentration of magnetic flux on one side of the array, we have been investigating the opposite side, which has the unusual combination of low magnetic field, yet high field gradient. We have then combined the array with a flow channel for magnetic nanoparticle suspensions, such that for an appropriate distance away from the magnets, nanoparticles of higher magnetization will accumulate against the channel wall, with lower magnetization nanoparticles flowing unaffected. We present preliminary results investigating suspensions of 5 nm, 10 nm, and 20 nm magnetite nanoparticles.

This method offers a novel way to purify and analyze fluids containing magnetic nanoparticles. In comparison to other approaches, it more readily allows for scaling up to preparatory quantities of particles.

a) Finite element analysis of field lines for the magnet array, showing the concentration of field lines on one side vs. the other. Red square indicates one magnet block, ¼" x ¼".



b) Photo of the constructed magnet array with nearly transparent flow channel on top.



Effect of Magnetic Field and Magnetic Field Gradient on Effectiveness of the Magnetic Sifter for Cell Purification

C. Ooi[1*], C.M. Earhart[2], R.J. Wilson[2], S.X. Wang[2,3]

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Magnetic separation of biomolecules and cells has become increasingly common as a means of preparing biological samples as researchers utilize magnetic particles conjugated with specific antibodies to selectively isolate cells and proteins. Purification and isolation of these cells and biomolecules can facilitate further biological analysis such as flow cytometry or protein assays. However, in applications such as rare cell separation, magnetic separation devices must demonstrate high elution efficiencies in addition to high capture efficiencies for viable downstream analysis.

We have previously developed a microfabricated magnetic sifter for cell and biomolecular magnetic separation, and demonstrated its high capture efficiency with commercial magnetic nanoparticles such as the Miltenyi MACS microbeads. The sifter is a 7 x 7 mm^2 silicon die, with an array of slots in a soft magnetic material (permalloy) supported on a silicon nitride membrane. When an external magnetic field is applied, the permalloy is magnetized, and high magnetic field gradients in the slots will capture magnetically labeled biological entities in any biological sample being pumped through the sifter.

In our initial experiments with H1650 lung cancer cell lines labeled with magnetic nanoparticles via the Epithelial Cell Adhesion Molecule (EpCAM) antigen, we demonstrate capture efficiencies above 90% even at a sample flow rate of 5ml/hr, but elution efficiencies hover between 50% and 60%. A significant cause of the low elution efficiency is the lateral drift of labeled cells in the device due to magnetic field gradients from the permanent magnets. We obtain improved elution efficiencies close to 90% via optimization of the permanent magnet size and position, and explain the effect via the use of finite element software (Ansoft Maxwell 3D) for magnetic field and field gradient distributions, and a particle tracing algorithm for analyzing the final positions of the particles.

This improvement in elution efficiency, allied to previous optimization of sifter geometry to improve capture efficiency, is critical in enabling the sifter to be used for magnetic separation of biologically relevant but rare moieties such as cancer stem cells. Effective capture and elution is especially significant for sufficient numbers of these rare cells to be obtained for subsequent analysis.

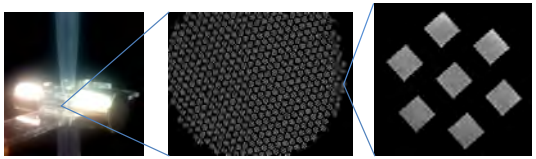

Figure 1. Magnetic Sifter Assembly

Magnetic Particle-Based Microfluidic Circulating Tumor Cell Separation for Oncological Therapeutic Monitoring

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With oncologists increasingly using circulating tumor cells (CTCs) to gather prognostic information on their patients, it is more and more apparent that CTCs can offer clinicians a non-invasive means of sampling for genetic markers, monitoring treatment responsiveness, aiding in treatment decision-making and indicating novel drug targets. With over 10 million cancer patients in the US along, a CTC diagnostic platform would have a tremendous impact on healthcare. Unfortunately, detecting rare CTCs in complex blood samples has been a major challenge, requiring an exceptionally specific and sensitive assay for discerning and capturing CTCs with high efficiency within a reasonable time frame.

This talk aims to describe a new reliable, reproducible, and rapid platform for the separation of CTCs from unprocessed whole blood. A mathematically based rational model will be presented for the design of a magnet-activated microfluidic cell isolation device. Briefly, the computational model is based on a first-principles force calculation for spherical, uniform cells labeled with superparamagnetic microbeads. Microbead and cell parameters, such as diameter, magnetic susceptibility, and particle binding characteristics, were experimentally determined and the resultant values directly inserted into the design equations to ensure realistic cell displacement estimates. The rationally optimized geometry is shown in Figure 1. Two current-carrying wires, with currents running antiparallel, allow for displacement in both the negative and the positive lateral directions into a central buffer stream. Cell were collected from the central stream and counted off-chip by flow cytometry and quantitative RT-PCR. The resulting device achieved greater than 95% efficiency down to single cell level while maintaining a 90% purity directly from whole blood samples. Based on the computation optimization and subsequent validation experiments separation takes less than 6 minutes, which represents the highest throughput currently available for CTC separation. Furthermore, the platform was designed to allow for the easy disposable of all components that contacted the blood, whilst preserving all the expensive components that drive the separation process, greatly reducing the cost in the clinic – and in turn the patient. Finally, the collected cells were shown to maintain viability and biological function after separation allowing for subsequent assay.

Overall, the presented device illustrates a viable separation platform for high purity, efficient, and rapid collection of rare CTC populations.

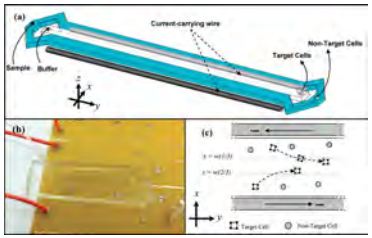

Figure 1. (a) Schematic illustration of the cell separation design. (b) Photograph of the microfluidic chip aligned on the electromagnet wire array. (c) Principle of magnetophoretic cell separation

pH-Controlled Adsorption of Cd ions on Carboxyl-Terminated Superparamagnetic Iron Oxide Nanoparticles

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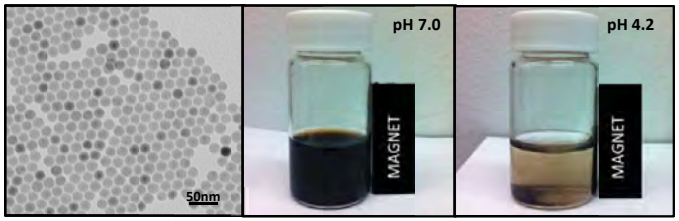
The adsorption of Cd^{2+} on carboxyl group terminated superparamagnetic iron oxide nanoparticles, was investigated *in situ* using rotating disk electrode techniques. By utilizing negatively and positively charge redox active species in buffered aqueous media (pH = 7) devoid of Cd^{2+}, we could demonstrate that the presence of dispersed carboxyl-terminated iron oxide nanoparticles does not affect the measured diffusion limiting currents. This finding made it possible to determine the concentration of unbound Cd^{2+} in solutions directly from the Levich equation. The results obtained yielded Cd^{2+} adsorption efficiencies of about 20 μg of Cd/mg of carboxyl-terminated iron oxide nanoparticles, which are among the highest reported in the literature employing *ex situ* methods. Desorption of Cd^{2+} from the functionalized iron oxide nanoparticles could be accomplished by lowering the pH of the solution, which leads to agglomeration making it possible to easily capture the nanoparticles by an external magnet. By raising the pH of the solution, the carboxyl-terminated iron oxide nanoparticles can be redispersed and regain their ability to adsorb Cd^{2+} in solution.



Water dispersible carboxyl-terminated superparamagnetic iron oxide nanoparticles exhibiting pH-controlled Cd ion adsorption response.

Magnetic barcode nanowires for osteosarcoma cell control, detection and separation

Anirudh Sharma[1], ShengShee Thor, Yuechen Zhu, Bethanie Stadler, Allison Hubel

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We will present the control and separation of osteosarcoma cells (OSCA) using gold/nickel multi-component barcode nanowires of various lengths and functionalization of the gold segments. Identifying and separating cell populations has both clinical and commercial significance. Existing technologies such as immunomagnetic isolation and flow cytometric methods have significant limitations. Multi-segment nanowires provide a unique combination of optical and magnetic readout signals for each type of cell based on the multilayers of the nanowires, much like how a unique barcode is associated with a product at a store. Optical microscopy images confirm uptake of these nanowires by osteosarcoma (OSCA) cells and TEM images suggest that the nanowires are enclosed by membranes inside the cells. Barcode nanowires have been shown to have higher separation efficiency for a fixed applied magnetic field than commercially available superparamagnetic beads owing to greater binding of nanowires to cells due to higher surface area and also due to higher magnetic moment. The effects of incubating nanowires with osteosarcoma cells under different conditions were studied. Specifically, the effects of varying concentration of nanowires, incubation time, nanowire dimensions and kinetics such as sedimentation and diffusion were observed to have values that compared favorably with literature. Also, different lengths of gold segments were fabricated to act as contrast agents for imaging and as thermal therapeutic agents to terminate OSCA cells using heat generation through localized surface plasmon resonance (LSPR). Fabrication of the nanowires was done using electrodeposition in anodic aluminum oxide (AAO) pores followed by etching the oxide to release the nanowires. LSPR for different Au length segments was tested using UV/VIS spectrometer. OSCA cells were used since they have low tolerance to heat changes.

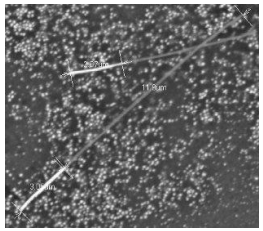

Fig 1: SEM image showing multilayers in two Gold/Nickel nanowires.

A novel separation technique: gas-assisted superparamagnetic extraction for scale-up of protein isolation (bovine serum albumin BSA)

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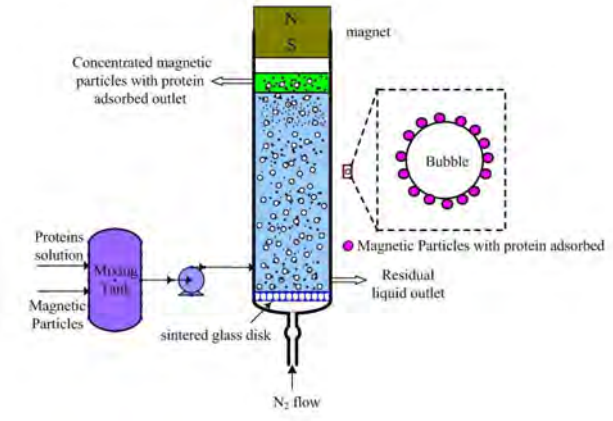
A novel separation technique, gas-assisted superparamagnetic extraction (GASE) was proposed for the first time to improve magnetic separation process efficiency for protein and magnetic sorbent separation from plenty of liquids. BSA was chosen as a model protein, citrate modified superparamagnetic particles was chosen as the sorbent. By combining superparamagnetic extraction for separation of targeted substances with bubble adsorption separation technology for concentration of this sorbent, BSA could be quickly and efficiently separated and concentrated from plenty of water. Firstly, the adsorption properties of the magnetic sorbent were tested. Subsequently the effects of the mass of sorbent, nitrogen flow rate, initial PH and liquid loading volume on the separation efficiency of BSA were investigated. The results indicated that initial pH was a crucial factor. In pH range of 3.8-5.8, magnetic sorbents with BSA adsorbed were quickly separated and concentrated, while in pH range of 6.0-8.0, no separation efficiency was obtained. Particularly, separation and concentration could be well carried out under the presence of unsteady foam. Under the optimal conditions, the enrichment ratio and recovery percentage of superparamagnetic particles with BSA adsorbed reached more than 20 and 95% within 3 min, respectively. The proposed method has potential application for separation of large scale of stock solution in less scale of magnetic separation apparatus.



Gas-assisted superparamagnetic extraction for protein separation

An Electromagnetic Manipulation System for Pre-clinical Testing of Targeted Drug Delivery

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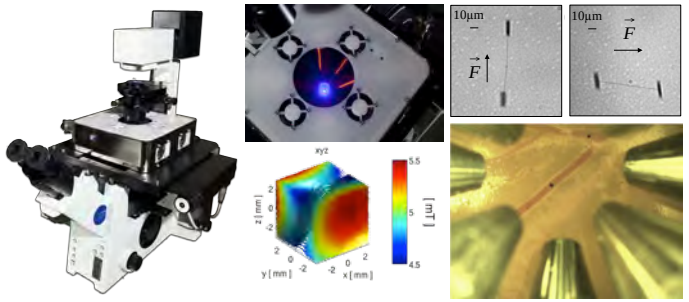
Site-specific drug delivery is a goal of many pharmaceutical research efforts. Side effects can be reduced, and the required drug dose can be minimized while achieving an optimal efficacy. One approach for delivery methods is based on wireless magnetic control. Magnetic vehicles with incorporated or attached drugs can be navigated through bodily fluids by the application of magnetic fields and field gradients. Many magnetic approaches show promise but are limited in their control.

We present a hemispherical magnetic manipulation system capable of 5 degree-of-freedom (DOF) control of an untethered micro or nanocarrier (3-DOF position, 2-DOF pointing orientation). The system, termed the MiniMag, consists of 8 stationary electromagnets with soft-magnetic cores. Arbitrary fields and field gradients up to 80 mT and 20 T/m at frequencies as high as 6 kHz can be achieved. This is accomplished through the superposition of the magnetic fields of the individual coils and capitalizes on a linear representation of the coupled field contributions. The tabletop system has been integrated with an optical microscope and incorporated into an inverted fluorescence microscope.

Various control strategies can be explored with the system. Examples include pulling with magnetic field gradients, cork-screw like motion of helical structures by rotating magnetic fields, stick-slip actuation on surfaces by resonating structures, and stick-slip actuation on surfaces induced by oscillating magnetic fields. Gradient based magnetic pulling and motion induced by rotating magnetic fields are particularly interesting for in-vivo applications and have been investigated for structures of various shapes and materials. Magnetic manipulation experiments have been carried out with micro/nanospheres made of superparamagnetic and soft and hard magnetic materials. Cylindrical structures of nickel and iron nanowires encased in carbon shells and liposomal tubular and helical structures with a CoNiReP coating have also been investigated. Dispersion media including water and a blood analog fluid have been used, and fluid flow has been applied to simulate the dynamic conditions of the blood circulatory system. In addition, microspheres have been navigated through the blood vessels of a chicken embryo. These experiments provide valuable insight into in-vivo control of magnetic micro/nanocarriers.



Left: The MiniMag system incorporated in an inverted microscope; middle: close-up of coils (top), simulation of magnetic field distribution for a field of 5mT along x (bottom); right: magnetic gradient based control of Ni nanowires (top) and NdFeB microspheres in blood vessels of a chicken embryo (bottom).

Silica-Iron Oxide Magnetic Nanoparticles for Viral Gene Delivery

Olga Mykhaylyk[1*], Titus Sobisch[2], Isabella Almstätter[3], Markus Döblinger[4], Dietmar Eberbeck[5], Rickmer Braren[3], Dietmar Lerche[2] and Christian Plank[1]

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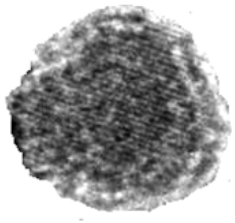
The purpose of the work was to find optimal formulation of silica-iron oxide magnetic nanoparticles with surface phosphonate groups decorated with 25-kD branched polyethylenimine (PEI) for gene delivery.

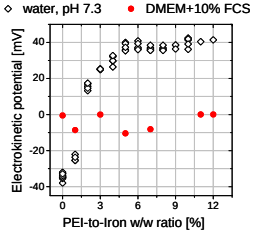
Surface composition (XPS), charge, associations with adenovirus, magneto-transduction efficiencies, cell internalizations, *in vitro* toxicities and MRI relaxivities were tested for the particles decorated with varying amounts of PEI. The dispersion stability was quantified by multisample analytical centrifugation with photometric detection of space and time resolved extinction profiles (STEP-technology) over the entire suspension sample.

Moderate PEI-decoration of MNPs results in charge reversal and destabilization. Analysis of space and time resolved concentration changes during centrifugation clearly revealed that at > 5% PEI loading flocculation gradually decreases and sufficient stabilization is achieved at > 10%. The association with adenovirus occurred efficiently at levels over 5% PEI, resulting in the complexes stable in 50% FCS at a PEI-to-iron w/w ratio of ≥ 7%; the maximum magneto-transduction efficiency was achieved at 9-12% PEI. Primary silica iron oxide nanoparticles and those with 11.5% PEI demonstrated excellent r_2* relaxivity values (> 600 $s^{-1}(mM\ Fe)^{-1}$) for the free and cell-internalized particles. The results of this study show that the attachment of PEI to the designed silica-iron oxide nanoparticles with PEI-to-iron w/w ratios of 10-12% allows for high colloidal stability and the efficient association with viral vectors into optimized magnetic viral complexes being stable in the presence of high protein concentrations and enabling highly efficient magnetotransduction *in vitro* without causing cell toxicity. The high r_2* relaxivities of the materials are suggestive of the MRI imaging modality. The lentiviral complexes of the PEI-modified silica-iron oxide magnetic nanoparticles allowed for the excellent transduction of cultured and primary cells as hematopoietic stem cells and human mesenchymal cells in a procedure that we have termed Magselectofection. The productivity of an oncolytic adenovirus can be dramatically increased *in vitro* and *in vivo* when combined with PEI-decorated silica-iron oxide nanoparticles.



M_s= 94 Am²/kg(Fe)
d_{mv} = (7.3±0.6) nm
d_{TEM}=(6.7±1.5) nm

~8.4 phosphonate groups per nm²



Optimization of magnetic nanoparticles assisted lentiviral gene transfer and cell positioning

Katrin Zimmermann[✉1,2], Christina Trueck[1,2], Olga Mykhaylyk[3], Martina Anton[3], Sarah Vosen[4], Daniela Wenzel[4], Ulrich M. Becher[5], Bernd K. Fleischmann[2,4,6], Alexander Pfeifer[✉1,2,6]

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Abstract

Targeting of specific cells and tissues is of great importance for successful gene- and cell-based therapies. However, local and high efficient cell and tissue targeting is still a major hurdle for successful application of viral gene therapy vectors. Lentiviral vectors (LVs) are versatile tools, because they are able to integrate into the host genome, which is essential for stable long term expression. Here, we combined targeting of LVs and positioning of transduced cells by applying magnetic nanoparticles (MNPs) and magnetic gradient fields. Importantly, we also studied LV targeting under non-permissive conditions which is of clinical relevance e. g. for transplantation medicine.

The MNPs of the core-shell type have a ferrimagnetic core (Fe_3O_4) surrounded by different coatings. The aim of this study was the optimization of MNP assisted lentiviral gene transfer with focus on different endothelial cell lines ranging from human umbilical vein endothelial cells (HUVECs) and bovine pulmonary arterial endothelial cells (bPAECs) to endothelial precursor cells (meEPCs, murine embryonal endothelial progenitor cells (EPCs), and hlEPCs, human late EPCs). Analysis of diverse MNPs resulted in the identification of nanoparticles with improved LV association and enhanced transduction properties of the complexes in the different endothelial cell types. These MNPs were either coated with polyethylenimine (PEI-Mag2, PEI-Mag3), palmitoyldextran (PALD2-Mag1) or with silicon oxide and surface phosphonate groups (SO-Mag5). An optimal binding of MNPs to LVs was achieved in the range of about 300fg Fe per viral particle. None of the MNPs tested showed cytotoxicity as analyzed by different assays that are either based on cell metabolic activity, cell proliferation or cell lysis. Importantly, in three different endothelial cell types (i.e. bPAECs, meEPCs, hlEPCs), 1.8 to 2.4 fold higher transduction efficiencies as compared to classical overnight transduction were achieved even under non-permissive conditions (4°C, 30min). The magnetic moments of the LV/MNP complexes were even high enough to achieve magnetically-guided local gene targeting of perfused endothelial cells with high transduction efficiencies. In first *in vivo* experiments using an *Arteria carotis* model, we successfully achieved local gene targeting in the native as well as in the injured vessel upon magnetic gradient field application.

Transduction with LV-MNP complexes renders the target cells magnetic. Thereby, the MNP-loaded cells can be positioned by magnetic field application. *Ex vivo* perfusion of a mouse aorta with LV/MNP transduced cells under clinically relevant flow conditions led to local cell accumulation at the intima of the vessel. Additionally, we tested positioning of endothelial cells transduced with magnetic lentiviral complexes *in vivo* in the carotid artery and observed re-endothelialization of parts of the injured commune carotid artery.

Taken together, MNP guided lentiviral transduction of endothelial cells can be significantly enhanced and localized by using optimized MNPs and magnetic gradient field application. Furthermore, the LV/MNP complexes can be applied *in vivo* leading to local gene targeting and cell positioning of LV/MNP transduced cells showed local cell attachment *ex vivo* as well as *in vivo*.

Treating Tinnitus by Magnetic Pushing of Therapy: Rat Experiments

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Tinnitus affects ~10% of the adults in the US. It can result from trauma, long-term exposure to noise, be associated with or be the result of noise-induced hearing loss, ear and sinus infections, diseases of the heart or blood vessels, Ménière's disease, and sometimes brain tumors. Its effect can be debilitating, leading to increased suicidal tendencies in some patients. Treating tinnitus, and other inner ear diseases, is made difficult by the blood-labyrinthine barrier (similar to the blood-brain barrier) that prevents blood-borne therapy from reaching the inner ear.

Previously, we showed experimental results for safely bypassing the blood-labyrinthine barrier by magnetically injecting ferromagnetic particles from rat middle into inner ears. To do so, we used a novel magnetic injection device that creates a magnetic push node. For human face-to-ear distances, magnetic push outperforms pulling from the other side of the head by a factor of ×20 and enables magnets that meet FDA safety requirements to delivery therapy to the inner ear [1].

Figure 1: A) The magnetic push system for rat experiments. Two pairs of magnets are inside the white polymer holder– their position is marked by the blue outline, and a steel ball can be seen levitated by the push force (enlarged on right). B) Experimental setup. The device is placed upside down above rat's head in order to align its ear with the push node which is about 2 cm away from corner of the "V" geometry. C) No fluorescent particles are visible in an inner ear tissue scrape for a rat where push was not applied versus D) many particles for a rat where a magnetic push was used.

Now we demonstrate that this magnetic push treatment can successfully treat an inner ear disease. A rat with normal hearing has its startle response to a brief loud noise attenuated by the administration of a short silent-gap warning in an otherwise steady background tone ("pre-pulse inhibition of startle response"). Rats with tonal tinnitus do not hear this silent gap and their startle response is not attenuated when tinnitus is present [2]. Quantitative behavioral monitoring was achieved by placing the rat on a platform that measures acceleration induced by the startle response. We compared gap/no-gap response for magnetically treated vs untreated rats. Rats treated with magnetically pushed steroid-laden 300 nm diameter particles showed lack of tinnitus immediately after and three weeks after trauma compared to untreated rats.

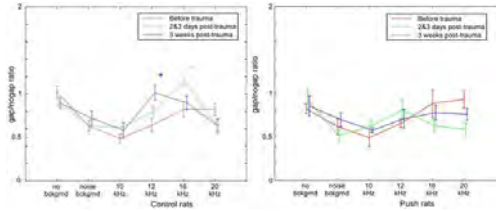

Figure 2: Gap/no-gap ratio for untreated vs magnetically treated rats. For the treated rats, there is no evidence of tinnitus at any frequency.

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Magnetic tool for in-vivo magnetic capture: Applications in blood filtering and proteomic diagnostics

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We have recently reported on the development of arrays of high performance micro-magnets (NdFeB, SmCo) on the one hand using a laser-based technique (Thermo-Magnetic Patterning) [1] and on the other hand using a lithography-based process (Topographic Patterning) [2]. The extrinsic magnetic properties (remanence, coercivity) of the µ-magnets are comparable to high quality commercial bulk magnets. A combination of Scanning Hall Probe Microscopy and simulations indicate that these µ-magnets produce stray magnetic field gradients as high as 10^6T/m [3].

Based on the high quality and on the autonomous nature of these µ-magnets, they are very well adapted to use in point-of-care devices to trap magnetic carriers and very small nanoparticles (down to the sub-10 nm size).

To study the trapping efficiency of µ-magnet-based systems, different magnetic structures were investigated ex-vivo in microfluidic devices [4] and fluid circulation with different micro/nano magnetic particles.

Based on these results, we have developed a specific magnetic tool, the "MAGPIE" (MAGnetic Probe for In-vivo Experiments) to trap magnetic carriers circulating in the human body (blood, cerebrospinal fluid).

In this communication, we will show results on the ex-vivo and in-vivo trapping of magnetic particles using such a tool. The efficiency of the device was tested using commercial MRI magnetic particles used today in humans.

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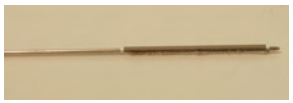

Fig.1 : Optical image of a MAGPIE implant (600µm×2cm) stuck on a cylindrical holder.

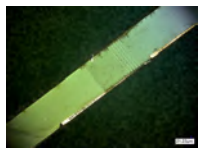

Fig.2 : Optical image of a MAGPIE surface capture of MRI magnetic nanoparticles (30 nm) from circulating blood. The dark stripes constitute stacks of nanoparticles.

Improving the Treatment of Hypoxic Breast Cancer Liver Metastases by using Dynamic Magnetic Shift

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Magnetic drug targeting has historically been viewed as a way to focus therapy to one specific location, to a solid tumor, blood clot, or to a desired location in the brain, eyes, or ears. However, cancer patients die from metastatic disease – from the spread of thousands of tumors throughout their body.

Based on human autopsy studies carried out at the National Cancer Institute, we have been evaluating whether magnetic forces can potentially improve the simultaneous treatment of thousands of metastases. For terminal breast cancer patients, we focused on liver metastases since there is evidence suggesting that treatment of metastatic hepatic lesions can lead to improvement in patient outcomes [1-4]. Consistent with other studies [5-7], it was found that advanced liver metastases are typically poorly vascularized compared to surrounding normal liver tissue (Figure 1a). Since chemotherapy must diffuse from blood to tissue, a longer distance from vessels to cancer cells (compared to vessels to normal liver cells) is anticipated to lead to higher dosing of normal versus cancerous tissue – the inverse of the desired outcome, and this too is consistent with studies that have shown tumor drug concentration can remain unchanged even if cellular uptake is increased [8-10].

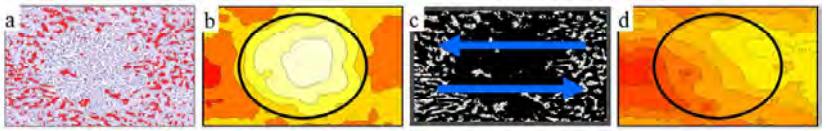

Figure 1: a) Measured autopsy blood vessel distribution for a typical liver metastatic tumor in a breast-cancer patient. Compared to surrounding normal liver, the blood vessels (red) are misshapen and fewer in number. b) Simulation of chemotherapy spatial distribution after a 2 hour treatment – a dense collection of vessels create a high dose in normal tissue but a therapy cold spot remains at the tumor (marked by the black circle). c) Potential solution: external strong magnets (not shown) can be used to shift magnetized therapy left and right across all tumors, thus normalizing the distribution of therapy and eliminating cold spots at many metastatic tumors at once. d) Simulation of chemotherapy distribution at the same tumor with applied magnetic shifting – the therapy cold spot at the tumor has been eliminated.

However, magnetic forces grant the ability to shift therapy. The anticipated issue with poorly vascularized (hypoxic) metastatic tumors is that chemotherapy will go everywhere in the body *except* to these tumors (Fig 1a,b). Thus the anticipated pattern of chemotherapy is high throughout the whole body but with small gaps at poorly vascularized metastatic tumors, which are exactly those tumors that have been linked with cancer recurrence [6, 11, 12]. By pulling on the whole chemotherapy pattern from outside the body using large external magnets, the entire therapy pattern could be shifted left and right to "paint over" many hypoxic metastatic tumors that would otherwise receive insufficient treatment (as shown for one sample tumor in Fig 1c,d).

Figure 2 shows an optimization result based on our breast-cancer autopsy data. The timing of magnetic shift was chosen to optimize the distribution of therapy in a first set of cases (group A). This same timing was then applied to a second independent group (B) and showed a statistical improvement in tumors versus normal tissue dose response across group B patients.

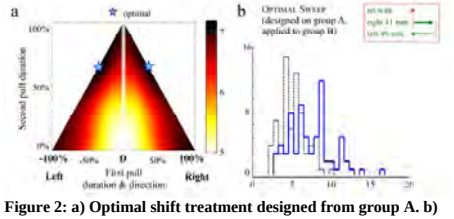

Figure 2: a) Optimal shift treatment designed from group A. b) Optimal treatment improves group B's cancer treatment.

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Saturday, May 26, 2012				
8:00	**Registration desk opens**			
8:30	Hawkins, Peter	Tutorial III - Magnetic Particles in Immunoassays	Bristol, UK	Tutorial 3
	Session 9: Magnetic Drug Delivery			
9:00	Bahadur, Dhirendra	Hyperthermia and other stimulants for drug release: in vitro and in vivo investigations through nano particulates and hybrids	Mumbai, India	Talk 60
9:15	Brazel, Christopher	Magnetite Nanoparticle-Loaded Poly(caprolactone-b-ethylene glycol) Micelles as Magnetic-Heating Activated Carriers for Anticancer Drugs	Tuscaloosa, USA	Talk 61
9:30	Gabbasov, Raul	Biodegradation of Magnetic Nanoparticles in Mouse Liver from Combined Analysis of Mössbauer and Magnetization Data	Moscow, Russia	Talk 62
9:45	Kempe, Maria	Magnetic Nanoparticles for Implant-Assisted Magnetic Drug Targeting in Cardiovascular Medicine	Lund, Sweden	Talk 63
10:00	Klostergaard, Jim	Anti-Tumor Activity of Drug-Loaded Magnetically Responsive Nanoparticles	Houston, USA	Talk 64
10:15	**Coffee break**			
11:00	Prina-Mello, Adriele	Multiparametric toxicity evaluation of SPIONs as identification for multifunctional nanoparticles for theranostic applications	Dublin, UK	Talk 65
11:15	Tietze, Rainer	Mitoxantrone delivery via Magnetic Drug Targeting for tumor therapy-Characterisations and biological outcome	Erlangen, Germany	Talk 66
11:30	Gregory-Evans, Kevin	Focused Magnetic Stem Cell Targeting to the Retina Using Magnetic Nanoparticles	Vancouver, Canada	Invited talk 6
12:15	**Lunch boxes at Van Vleck Auditorium**			
	Session 10 : Biological Applications			
13:15	Riegler, Johannes	Magnetic delivery of mesenchymal stem cells after arterial injury	London, UK	Talk 67
13:30	Tefft, Brandon	Magnetizable Duplex Steel Stents Enable Endothelial Cell Capture	Rochester, USA	Talk 68
13:45	Bonnaud, Cecile	Magnetic Janus Liposomes: Design of magnetic thermosensitive biomembranes for MRI and drug delivery	Marly, Switzerland	Talk 69
14:00	Marten, Gernot	Modular Construction of Tailor-Made Bioactive Hybrid Nanoparticles	Vancouver, Canada	Talk 70
14:15	Gazova, Zuzana	BSA-modified magnetic fluids as therapeutic agents targeting insulin-associating amyloidosis	Kosice, Slovakia	Talk 71
14:30	Guan, Yueping	Peroxidase-like activity of magnetic nanoparticles and their applications in immunoassay	Beijing, China	Talk 72
14:45	Gutierrez, Lucia	Renal iron load in sickle cell disease determined by magnetic resonance imaging measurements	Perth, Australia	Talk 73
15:00	Hight Walker, Angela	Bismuth-doped Cobalt Ferrite Nanoparticles for MRI and CT Contrast Enhancement	Gaithersburg, USA	Talk 74
15:15	Horak, Daniel	Anti-EpCAM-Immobilized Albumin-Coated Monodisperse Magnetic Poly(Glycidyl Methacrylate) Microspheres for Detection of Circulating Tumor Cells	Prague, Czech Rep.	Talk 75
15:30	Lee, Hakho	Novel core-shell magnetic nanoparticles as highly efficient contrasting agents for magnetic resonance detection	Boston, USA	Talk 76
15:45	**Closing Comments and Announcement of the NEXT MEETING: Urs Hafeli / Stefan Odenbach**			
16:00	**Meeting ends**			

Hyperthermia and other stimulants for drug release: *in vitro* and *in vivo* investigations through nano particulates and hybrids

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[2]Bhabha Atomic Research Centre, Trombay, Mumbai-400085
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Magnetic nano particulates (MNPs) with different shapes, composites, porous assembly, hybrids, biphasic system and core shell structure forming magnetic fluids have been developed by various soft chemical methods. Several magnetic nanohybrids based on lipids, hydrogel, dendrimers and other biodegradable polymers have been investigated for a combination therapy which includes hyperthermia and chemotherapy, and some of these hybrids have been made temperature and pH sensitive which have been exploited as stimulant for drug delivery in combination with hyperthermia. We have further studied the role of AC magnetic field and ultrasound as the stimulants for drug release as well as a source of hyperthermia. We have observed that synergistic effects of chemotherapy and hyperthermia are significant particularly with lipid and hydrogel based systems. We report a detailed *in vitro* and *in vivo* experiment with some of these systems. *In vivo* experiments with magnetic hydrogels and magnetic liposomes are of particular significance. Biodistribution in different organs have been investigated after sacrificing the animals using magnetic measurements (VSM) and ICP analysis which match very well. A detailed biocompatibility study comprising of haemotological and histopathological tests of vital organs indicate that these formulations are nontoxic. There is a significant suppression of tumor growth with both kinds of systems under an applied AC field. With higher field strength, a stronger inhibition of tumor growth is seen. Double dose treatment exhibits even better effect. We discuss here some of these aspects based on the work carried out in our laboratory. In addition, we discuss development of multifunctional magnetic hybrid nanostructures, which may be used for theragnostic applications. These nanohybrids/encapsulates of chemotherapeutic drug, MNPs and targeting moiety (like folic acid) have been investigated for both therapeutic and diagnostic applications. Finally hyperthermia is found more effective in animal model in combination with chemotherapy for inhibition of tumor growth.

Refernce:

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Magnetite Nanoparticle-Loaded Poly(caprolactone-b-ethylene glycol) Micelles as Magnetic-Heating Activated Carriers for Anticancer Drugs

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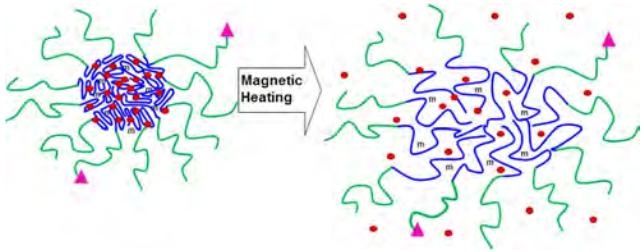
The great potential to delivery hyperthermia therapy locally in cancerous tumors via magnetic nanoparticles has progressed as far as human clinical trials and it has been shown to be clinically effective at minimizing or reversing tumor growth. At the same time, a number of studies indicate that a multifaceted approach to cancer therapy can result in synergistic effects that contain cancer more effectively than any single therapeutic approach can achieve. Thus, combinations of surgery, chemotherapy, radiation and hyperthermia should be strongly considered. This paper investigates the design of materials for combining hyperthermia with chemotherapy; specifically, the polymeric micelles containing magnetic nanoparticles are being developed to achieve a magnetically-triggered drug delivery system that can be combined with magnetic hyperthermia for localized cancer therapy.

Block copolymers of poly(caprolactone-b-ethylene glycol), P(CL-b-EG), have been synthesized with varying chain lengths of both CL and EG segments. The block copolymers self-assemble in aqueous solution to form micelles with number average diameters under 100 nm. The CL blocks form the core, which crystallizes and has melting points that are in an effective therapeutic window to be melted by hyperthermia temperatures (42 - 45 ^{o}C). The micelles are stable above the critical micelle concentrations, and custom-synthesized magnetite nanoparticles (approximately 8 nm in diameter) can be loaded into the core along with doxorubicin, an anti-cancer drug. Localized heating in the micelles has been achieved using an electromagnetic induction coil with frequencies in the hundreds of kilohertz range. Depending on the concentration of nanoparticles, field strength and magnetic field frequency, heating of micelle solutions above 45 ^{o}C can be achieved in less than 10 minutes. Drug release experiments using isothermal baths have been conducted using Floatalyzers® with MWCO of 50 kDa to separate the nano-sized micelles from released drug. Initial experiments indicate that the release rate of doxorubicin from micelles is significantly higher when the micelles are heated above the PCL melting temperature.

In addition to developing MNPs and block copolymers that can carry drugs and be magnetically-activated to release, the micelles can be targeted to cancer cells through receptor-ligand interactions. We have developed micelles that contain a cyclic RGD peptide in the PEG corona, and have studied the localization of such micelles to human embryonic kidney cells (which express integrin receptors through which the RGD peptides attach). Preliminary results indicate that the RGD-targeted micelles have a significantly higher targeting effectiveness compared to untargeted micelles.



Schematic of drug release from targeted (triangle) poly(caprolactone (core)-b-ethylene glycol (corona)) micelles containing magnetite nanoparticles (m) and doxorubicin (circles).

Biodegradation of Magnetic Nanoparticles in Mouse Liver from Combined Analysis of Mössbauer and Magnetization Data

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[b]Institute of Physics and Technology, Russian Academy of Sciences, Moscow, Russia
[c]Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry, Russian Academy of Sciences, Moscow, Russia
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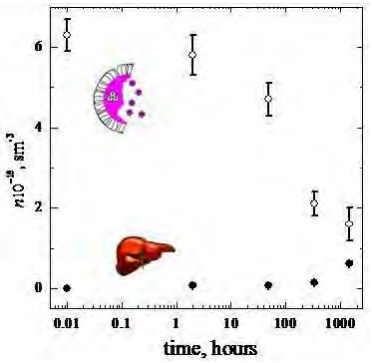
Two years ago at the previous Conference in Rostock we presented the preliminary results of the Mössbauer study of biodegradation *in vivo* of magnetic particles. Superparamagnetic particles in ferrofluid were injected intravenously into the tail vein of mice. At various time intervals after the injection the mice were sacrificed and their organs were investigated. The Mössbauer study showed that magnetic particles were well-detectable in the spectra of the liver. It was found that the shape of the Mössbauer spectra of liver changes with time after the injection, which allowed us to talk about the biodegradation process. Together with a six-line spectrum of the initial magnetic particles, we found a strong additional doublet of lines. This doublet may indicate both appearance in the liver of superparamagnetic nanoparticles, which size is smaller than size of intrinsic nanoparticles, and generation of non magnetic ferritin–like proteins.



The concentration of iron in the nanoparticles (white circles) and in the ferritin-like protein (black circles) in mouse liver depending on the time after injection.

One year ago at the Conference "Frontiers in BioMagnetic Particles 2" in Charleston we suggested the experimental method for determination of the origin of the doublet by measuring the Mössbauer spectra of each sample of mouse liver at two different temperatures and in an external magnetic field. We suggested a calculation procedure for simultaneous fit of these three Mössbauer spectra, corresponding to the same stage of the nanoparticles biodegradation, within self-consistent set of parameters. As a result we separated the partial superparamagnetic component of the spectra, which characterizes changes of exogenous magnetic nanoparticles in the process of their biodegradation, from the partial paramagnetic component of the spectra, which characterizes changes of ferritin-like proteins. In addition we have evaluated the particle's magnetic anisotropy energy, the Debye temperature, the critical field for their magnetization reversal and their volume concentration at each stage of biodegradation.

However Mössbauer spectroscopy can not principally distinguish the average particles size, which requires for additional measurements. In this report we demonstrate that the efficiency of the proposed technique can be enhanced by adding to the set of three Mössbauer spectra a magnetization curve measured on the same sample. The whole set of data can be simultaneously processed within the same model of magnetic dynamics with the same set of parameters so that the additional measurements of magnetization curves allow one to determine the change in the nanoparticle size during the biodegradation process.

Magnetic Nanoparticles for Implant-Assisted Magnetic Drug Targeting in Cardiovascular Medicine

Henrik Kempe and Maria Kempe*

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Targeted drug administration is attractive for drugs that are costly, toxic, or prone to provoke side effects. A high local dose is obtained while the systemic exposure is minimized and the overall dose reduced. Drug-loaded (super)paramagnetic nanoparticles can, after intravascular injection and transportation with the blood, be captured at the desired site in the body by the aid of extracorporeal magnets. The magnetic force exerted on the particles must overcome the drag force produced by the blood flow. The magnetic force on a particle of diameter d is proportional to d^2 while the drag force is proportional to d; small particles are therefore more difficult to retain than large ones. Furthermore, the drag force is proportional to the velocity of the blood; large blood vessels are therefore more difficult to target than small ones. Finally, the magnetic field diminishes with the cube of the distance; deep locations are therefore considerably more difficult to target than superficial ones. It is therefore challenging to reach large vessels at deep locations in the body with nano-sized magnetic particles.

In this work, we have developed magnetic nanoparticles in the size-range 10–30 nm for implant-assisted magnetic drug targeting for treatment of in-stent thrombosis. The magnetic nanoparticles were synthesized in a one-pot procedure by precipitation of ferrous hydroxide followed by oxidation to magnetite. The nanoparticles were subsequently silanized and conjugated with the thrombolytic drug tissue plasminogen activator (tPA). The nanoparticles were characterized regarding magnetic properties, composition, size, and shape. Hemolysis assays showed that the hemolytic activity of the tPA-nanoparticle conjugates was negligible. The nanoparticles were captured efficiently to the surface of a ferromagnetic coiled wire at the fluid velocities typical for human arteries in an *in vitro* flow-through model. A preliminary test of the tPA-nanoparticle conjugates in a pig model demonstrated that the conjugates were useful for treatment of in-stent thrombosis in coronary arteries (Figure 1).

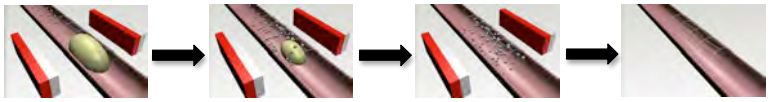

Figure 1. Schematic representation of lysis of an in-stent thrombus by injection and capture of tPA-nanoparticle conjugates at the stent surface under the influence of an external magnetic field (a-c). The nanoparticles are released after removing the magnets (d).

Anti-Tumor Activity of Drug-Loaded Magnetically Responsive Nanoparticles

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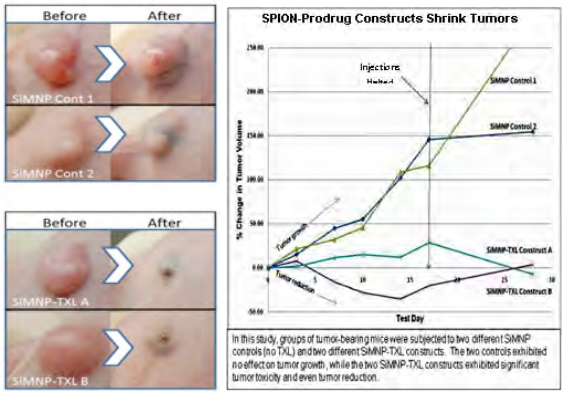
Superparamagnetic iron oxide nanoparticles (SPION), carrying a covalently-linked chemotherapeutic agent, can be magnetically directed to concentrate at a tumor under the influence of external shaped/focused magnetic field gradients. Following extravasation, the pro-drug will be released following cleavage of the bio-reversible linker within the tumor microenvironment, thus both selectively enhancing drug delivery to tumor tissue and minimizing harm to normal tissue: raising the therapeutic index compared with that of free drugs, offering potentially improved treatment.

A possible limitation with chemadsorptive approaches for binding of drugs to magnetically-responsive carriers has been the minimal control over subsequent desorption, which may either occur prematurely, before the platform arrives at the tumor site--causing free drug toxicity--or incompletely at the tumor site--reducing anti-tumor efficacy. These deficiencies can be addressed using a pro-drug construct approach in which the drug is covalently linked via bio-reversible ester or hydrazone bonds to magnetite-based, magnetically-responsive nanoparticles (MNP)--offering site-specific release.

In the present study, paclitaxel (TXL) was covalently linked to silica-coated MNPs (SiMNP) using the above pro-drug strategy and the activity of each conjugate was then evaluated in orthotopic human breast adenocarcinoma xenograft nude mouse models. In initial *in vitro* evaluations, MDA-MB-231 and MDA-MB-468 triple-negative human breast adenocarcinoma cells were treated with SiMNP-TXL prodrug and SiMNP control (silane linker only) formulations over a range of concentrations for up to five days before staining with MTT to determine tumor cell survival. There was a rank order of potency among three TXL-loaded MNP formulations: an ester-linked formulation being the least potent, and another ester-linked formulation, which also had PEG attached to the MNP, and a formulation with a pH-sensitive hydrazone bond both being 10-100-fold more potent. *In vivo*, tumors of mice given either of the control MNP-linker preparations alone (SiMNP Cont 1 and 2) formulations grew steadily; however, the more potent prodrugs from the *in vitro* analyses, SiMNP-TXL A and SiMNP-TXL B, demonstrated the ability to delay or reverse tumor growth following a multiple-dosing regimen. A year after treatment initiation, 40% of treated mice were still surviving with no palpable tumor evident. A pilot toxicology study revealed transient hepatocellular necrosis following i.v. administration of the parental, SiMNP; however, no lesions were observed in mice given the PEG-MNPs loaded with TXL.

We propose to further develop the lead MNP-TXL constructs for systemic administration followed by magnetic vectoring to tumors in preparation for planned clinical trials.



Multiparametric toxicity evaluation of SPIONs as identification for multifunctional nanoparticles for theranostic applications

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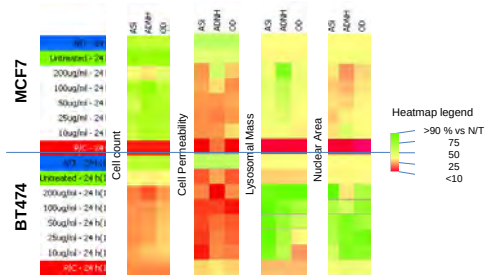
Superparamagnetic iron oxide nanoparticles (SPION) have shown potential as multifunctional nanoparticles for theranostic applications because they have been used as magnetic resonance imaging (MRI) constrast agents in clinic and their features could be easily tailored by coupling with targeting moieties, fluorescence dyes, or therapeutic agents. The assessment of toxicity and biocompatibility of the tailored product is therefore paramount to deliver commercially sound theranostic tools.

This work focuses on the toxicity and biocompatibility assessment of 3 differently coated SPIONs derived from two synthesis methods: water and thermal decompositon. The nature of the coating under assessment where Aminodextran (ADNH), Aminopropilsilane (ASi), dimercaptosuccininc acid (DMSA). SPIONs where charaterised by transmission electron microscope (TEM), vibrating sample magnetometer (VSM). dyanimic light scattering (DLS), electrophoretic zeta potential and Nanoparticle Tracking and Analysis (NTA) technique.

Toxicity and biocompatibility were assesd by automated high content screening of two breast cancer cell lines (MCF7, and BT474) and healthy (MCF10A) control exposed to incremental doses of the SPIONs up to 24 hours *in vitro*. Toxicity and biocompatibility was recorded by the variation of total cell count, lysosomal mass and cell permeability compared to their respective controls. Multiparametric analysis allowed for normalization and biocompatibility comparison between the coated SPIONs developed in this study.



The unique combination of physico-chemical properties and multiparametric toxicity analysis is therefore allowing for the optimal identification of the basic nanoparticle requirements prior to functionalisation wit the moieties, fluorescence dyes, or therapeutic agents for targeted cancer theranostic applications.

ACKNOWLEDGEMENTS
This work is partially funded by the European Commission Framework programme 7 under MULTIFUN project (NMP large ref 262943).

Mitoxantrone delivery via Magnetic Drug Targeting for tumor therapy- Characterisations and biological outcome

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Nano-sized magnetic drug carriers offer a splendid prospect for directed drug application. The increased drug concentration in cancer tissue reached for this kind of application enables therapeutic effects on tumors that cannot be achieved using conventional chemotherapeutic treatment. Characterizing the drug targeting system and its behavior *in-vitro* is necessary for understanding the biological outcome. In our studies IR-spectroscopy and thermogravimetry show covalent binding of the biocompatible layer, whereas this could not be shown for mitoxantrone (MTO), an anthracenedione derivative that is coordinated to nanoparticles´ surface. XPS reveals the binding of this agent and leads to some changes in its structure that cannot be detected for the pure unbound substance. Although binding of MTO to particles is hardly defined, considerable amounts of drug can be carried by ferrofluids (Fig. 1). The behavior of magnetic nanoparticles in biological fluids is difficult to predict. A useful approach to understand magnetic particle enrichment influenced by external magnetic fields is an *ex-vivo* flow-model, whereby magnetic nanoparticles are rinsed through a bovine artery. Variation of the magnetic field strength influences the attraction, which can be monitored by different quantifying and imaging techniques. Magnetorelaxometry reliably detects and quantifyies magnetic iron oxide (Fig. 2) and HPLC-UV could measure MTO, which was linked to the particles. µCT enlightens the spatial arrangement of particles inside vessels and histological investigations give a closer view on the cellular stage.
Furthermore, the nanosized drug carrier complex has to be investigated due to its biological outcome. Real time cell analysis is useful to observe the therapeutic particles concerning their outcome in cell culture time-resolved up to several days. First experiments in concordance to conventional assays show that effectivity of nanoparticles bound MTO in cell culture is higher than treatment with pure mitoxantrone.
The proven efficiency of Magnetic Drug Targeting in animal experiments shown by complete tumor remissions has to be studied more detailed to close existing gaps in knowledge for nanomedicine.

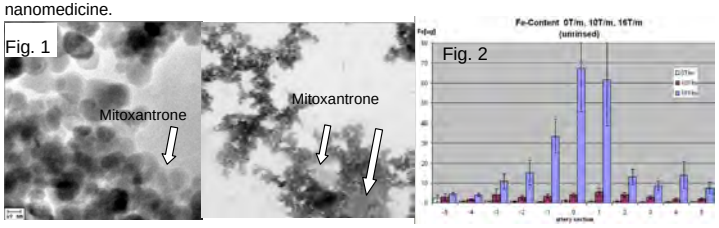

Fig. 1: TEM images of MTO loaded particle complex whereas MTO is both spherical surrounded and encapsulated inside agglomerates. Fig. 2: particle distribution in a bovine artery model after MDT

Focused Magnetic Stem Cell Targeting to the Retina Using Magnetic Nanoparticles

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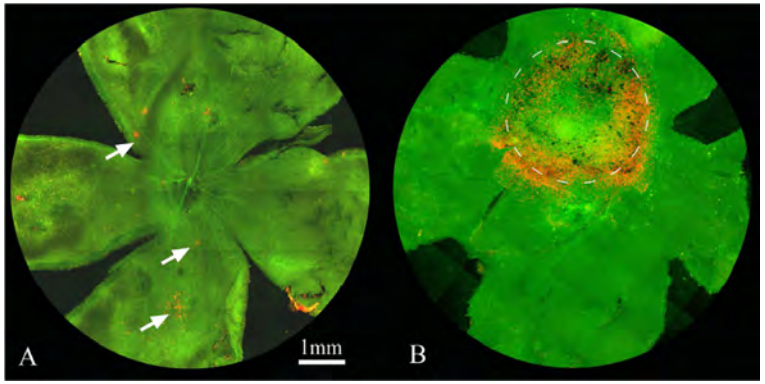
Developing new ways of delivering cells to diseased tissue will be a key factor in translating cell therapeutics research into clinical use. Magnetically targeting cells enables delivery of significant numbers of cells to key areas of specific organs. To demonstrate feasibility in neurological tissue, we targeted cells magnetically to the upper hemisphere of the rodent retina. Rat mesenchymal stem cells (MSCs) were magnetized using superparamagnetic iron oxide nanoparticles (SPIONs). FluidMAG-D labeled MSCs were injected intravitreally or via the tail vein of the S334ter-4 transgenic rat model of retinal degeneration with or without placing a gold-plated neodymium disc magnet within the orbit, but outside the eye. Retinal flatmount and cryosection imaging demonstrated that after intravitreal injection cells localized to the inner retina in a tightly confined area corresponding to the position of the orbital magnet. After intravenous injection, similar retinal localization was achieved and remarkably was associated with a tenfold increase in magnetic MSC delivery to the retina. Treatment also resulted in significantly higher retinal concentrations of anti-inflammatory molecules interleukin-10 and hepatocyte growth factor. Animals injected at post-natal day 21 were followed for 8 weeks. *In vivo* electroretinography, optokinetic tracking responses and optical coherence tomography results were consistent with subsequent retinal histology in showing an area of neuroprotection corresponding to the orbitally placed magnet after multiple intravenous magnetic MSC injections. These results establish a focal therapeutic benefit for magnetic MSC therapy in a rodent model of retinal degeneration.



Magnetic delivery of mesenchymal stem cells after arterial injury

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Introduction

Peripheral arterial disease is one of the major manifestations of atherosclerosis leading to critical limb ischemia. Chronic undersupply of oxygen and nutrients to the legs leads to rest pain, non-healing ulcers and can ultimately culminate in limb amputation [1]. Restoration of pulsatile blood flow is a primary treatment criterion. This can be achieved via angioplasty, stenting, endarterectomy or bypass grafting. However, long term vessel patency is affected by restenosis and neointimal hyperplasia. Preclinical experiments have shown that neointimal hyperplasia can be prevented via the restoration of a functional endothelial layer [2]. But suitable stem/progenitor cells for that are scarce and their retention following conventional delivery is poor.

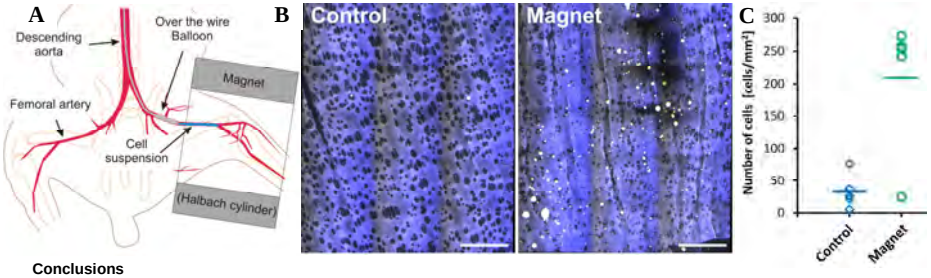
The aim of this project was to establish the feasibility of magnetic cell delivery to lower leg arteries after arterial injury in a clinically relevant setting. We have previously demonstrated the ability to design a scalable magnet for this application [3]. We have now investigated magnetic targeting mesenchymal stem cells in femoral arteries of rabbits following balloon angioplasty and demonstrated a 6 fold increase in cell retention.

Methods

Initial *in vitro* experiments were performed to test the effect of different iron oxide particles (fluidMAG-D, -lipid, -DEAE, -P, -Q; Endorem; Ferrocarbotran) and their concentration on cell viability (metabolic activity, MTS assay). The uptake of iron oxide particles was assessed using SQUID magnetometry. FluidMAG-D was selected for *in vivo* experiments. The differentiation potential of rabbit MSCs labelled with fluidMAG-D was assessed, as well as the secretion of angiogenic factors.
In vivo experiments: New Zealand White rabbits (2-2.5 kg, n=5) underwent arterial injury via the inflation of an over-the-wire balloon (2mm x 15mm, Sprinter, 8atm) in the left or right femoral artery under general anaesthesia and fluoroscopic guidance. Following this initial injury, the balloon was deflated retrieved by about 20mm and inflated to stop the blood flow while 300µl saline containing 1E5 labelled MSCs (fluidMag-D, PKH26) were infused over one minute. Four minutes later, the blood flow was restored and the balloon retrieved. The same procedure was performed on the other leg but a Halbach cylinder was placed around the leg before cell infusion started. This magnet was kept in place for an additional 40 minutes after blood flow restoration and balloon retrieval. Arteries were harvested 24 hours after cell delivery, sectioned longitudinally and the distribution of cells was assessed via enface confocal microscopy.

Results

Labelling rabbit MSCs for 24 hours with FluidMAG-D lead to an internalisation of 8E-9 emu/cell or 57 pg/cell. Labelling efficiency was approximately 80%. There were no negative effects on cell growth or differentiation to adipocytes or osteoblasts but chondrogenic differentiation was impaired. Cell labelling did not change the levels of angiogenic factors released into the culture medium. **Figure A** shows the experimental set up for cell delivery after angioplasty. In addition to iron oxide, cells were also labelled with a florescent dye for *in vivo* experiments and appear yellow on confocal microscopy images. **Figure B** shows increased cell retention on the arterial wall for the magnet group 24 hours after cell delivery. **Figure C** shows the number of cells retained per square millimetre of arterial lumen surface for the control and magnet group. The placements of the magnet around the leg during and after cell delivery lead a 6 folds increase in cell retention.



Conclusions

We have designed and produced a magnet that is scalable to clinical studies: this magnet will generate the same magnetic force per cell as the human scale. Magnetic labelling of rabbit MSCs did not negatively affect their growth and angiogenic potential. Delivery of these labelled cells via over-the-wire balloons was safe and lead to increased cell retention when a magnet was placed around the leg of the animal.

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Magnetizable Duplex Steel Stents Enable Endothelial Cell Capture

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Emerging medical nanotechnology applications often utilize magnetic forces to guide the movement of superparamagnetic particle linked cells and drugs in order to achieve a therapeutic effect. Superparamagnetic particle labeled endothelial cells (Figure 1) have previously been captured on the surface of prototype nickel-plated stents in proof of concept studies. Facilitated endothelialization may help improve the healing of stented arteries and reduce the risk for stent thrombosis and restenosis.

Extensive evaluation of candidate materials led to the development of a magnetizable 2205 duplex stainless steel stent. Magnetic field strengths of approximately 630 mG were induced within these stents by holding them in close proximity to a 0.7 T rare earth magnet. The magnetic field strength was reliably maintained over several days, but was partially reduced upon mild mechanical shock or plastic deformation. Mechanical testing demonstrated that stents could withstand crimping and expansion necessary for vascular implantation, however, magnetic field strength was significantly reduced. When placed in an endothelial cell suspension of 1×10^6 cells/mL, magnetized stents captured approximately 310 cells/mm^2 compared to approximately 35 cells/mm^2 for non-magnetized control stents (Figure 2).

These data provide quantitative support to the observation that low level magnetization of stents may be adequate to attract labeled, autologous, blood-derived endothelial outgrowth cells following stent placement. This, in turn, may lead to more rapid and complete healing of vascular stents with a concomitant improvement in stent performance.

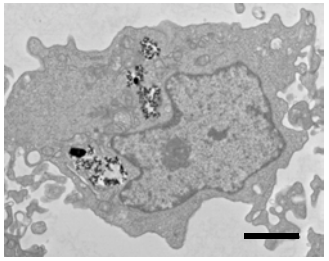

Fig 1. Transmission electron microscopy image of endothelial outgrowth cell labeled with superparamagnetic nanoparticles.
Scale bar = 2 µm.

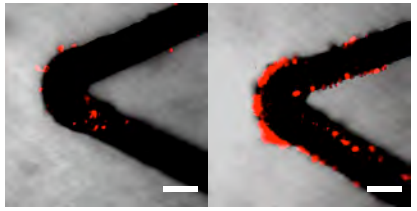

Fig 2. Confocal microscopy images of a non-magnetized stent (left) and a magnetized stent (right) showing capture of porcine blood-derived endothelial outgrowth cells that have been labeled with superparamagnetic nanoparticles and stained red with a fluorescent dye.
Scale bar = 100 µm.

Magnetic Janus Liposomes:

Design of magnetic thermosensitive biomembranes for MRI and drug delivery

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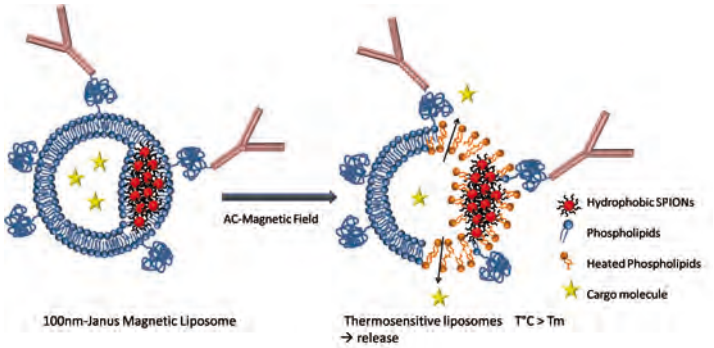
Liposomes have been increasingly developed over the last years as drug carriers to overcome poor pharmacokinetics and inappropriate biodistribution. Their serum-stability is enhanced thanks to a "stealth" outer layer, such as polyethylene glycol (PEG), which prevents their uptake by phagocytic blood cells. Functionalized with cancer recognition factors, liposomes can achieve passive and active targeting of tumoral tissues. The biggest challenge in their application is to trigger and control the release of the encapsulated drug. First and second generation nanovectors possess the ability to remote responsiveness to the tumor environment, such as pH-sensitive liposomes. Our work aims at the design of a third generation liposomal nanovectors capable of more complex functions. We produced magnetic Janus liposomes with a mean diameter size of 100nm, entrapping a cluster of hydrophobic iron oxide nanoparticles inside the thermosensitive bilayer. Widely used as MRI contrast agent, iron oxide nanoparticles have also shown significant heating in an AC magnetic field. Here, we have used alternating magnetic fields to release cargo from liposomes by locally heating the membrane and thus changing its permeability. Self-assembly of our particle-phospholipid- systems was thoroughly investigated by dynamic light scattering and cryo-transmission electron microscopy and the release was characterized using a self-quenched fluorophore encapsulated inside the liposomes.



Talk 69

Modular Construction of Tailor-Made Bioactive Hybrid Nanoparticles

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Nanomaterials offer new opportunities and applications in the biological and medical field due to the coincidence of dimensions as compared to proteins and viruses, offering novel pathways for to addressing new targets on the sub-cellular scale. The combination of inorganic and polymeric components is an elegant option to combine their unique functional properties to modular-designed nanoscopic carriers tailor-made for different biomedical application areas.

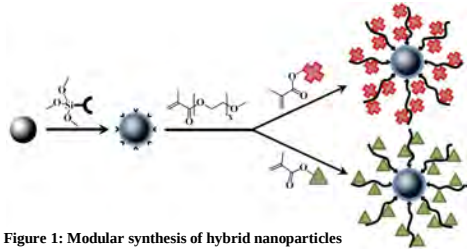

Figure 1: Modular synthesis of hybrid nanoparticles

In our work we present the synthesis (Figure 1) of such modular hybrid nanocarriers based on superparamagnetic iron oxide cores, a biocompatible polymer shell and various bioactive functional groups.

While the magnetic core provides the opportunity for magnetic separation in magnetic field gradients, and for local heat development in dynamic fields due to relaxation processes, the brush-like polymer shell prevents agglomeration and excellent dilution stability under biological operation conditions.[2] In addition, the modular synthesis allows introduction of (bio)functional groups and thermoresponse.

The shell is prepared by surface-initiated (co)polymerization (ATRP) of oligo(ethylene glycol) methylether methacrylate (OEGMA) monomers[1] and different comonomers as primers for functional groups like active esters (*N*-hydroxysuccinimide, SI) and nanocontainers (β-cyclodextrin, CD).

The hybrid nanoparticles can be tailor-made for several applications in biomedicine, e. g. protein separation,[3] biocatalysis[3,4], drug delivery and ROS-based cancer therapies. SI-functional nanoparticles can be shown to covalently bind amino-functional substances like proteins (membrane proteins or enzymes) for labeling and easy separation above the LCST of the polymer shell. Furthermore the catalytic activity of immobilized enzymes can be found to be strongly temperature dependent and can be manipulated by applying a magnetic AC-field to heat the particles. For active drug delivery purposes, core-shell nanoparticles functionalized with cyclodextrin units can be used in this work to reversibly bind and release small molecule drugs. In certain cases, the complex formation can be highly temperature sensitive due to entropic effects leading to release burst upon heating conventionally or in an alternating magnetic field. The use of iron oxide nanoparticles in redox active cancer therapy can reduce the presence of reactive oxygen species (ROS) in healthy cells and thus decrease the communication with cancer cells to prevent cancer cell propagation and tumor spreading.

Acknowledgement: We gratefully acknowledge Prof. H. Ritter, HHU Düsseldorf, for providing the CD-functional comonomer, Prof. J. Schrader, Universitätsklinikum Düsseldorf, for cell membrane labeling tests, Prof. P. Brenneisen, Universitätsklinikum Düsseldorf, for cell ROS studies, DFG and FCI for financial support.

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Talk 70

BSA-modified magnetic fluids as therapeutic agents targeting insulin-associating amyloidosis

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Protein self-assembly that leads to the formation of amyloid aggregates is a major cause of cell damage or death in amyloid-related diseases such as Alzheimer's disease, dialysis-related amyloidosis or various forms of systemic amyloidosis. Insulin amyloid deposits have been reported in patients with diabetes undergoing long-term treatment by injection of insulin. Insulin amyloid fibrillization also causes problems in the production and storage of this drug and in application of insulin pumps. Currently, there is no real cure available toward treating the amyloid-related diseases. However, experimental data indicate that reduction of amyloid aggregates is beneficial for cells and animals.

We have investigated anti-amyloid ability of albumin magnetic fluids (MFBSAs) consisting of Fe_3O_4 nanoparticles (NPs) stabilized by sodium oleate and modified by different amounts of bovine serum albumin (BSA) (w/w ratios of BSA/Fe_3O_4 from 0.005 to 15). It was observed that BSA content in MFBSAs affects the size of dispersed nanoparticles, isoelectric point and zeta potential of magnetic fluids.

Incubation of insulin amyloid fibrils with MFBSAs leads to significant destruction of aggregates as it was observed by atomic force microscopy (Fig. 1) and ThT fluorescence assay where lowering of the intensity indicates a reduction of insulin amyloid aggregates. The obtained results suggest a correlation between the amount of protein in the state of amyloid aggregates and the size of nanoparticles. The increasing of the NP size leads to a decline of destructive activity of the studied magnetic fluids. The most effective were MFBSAs with hydrodynamic diameters up to 60 nm [1].

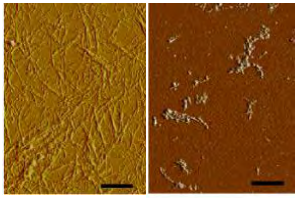

Fig.1. AFM image of insulin amyloid fibrils (a) and after overnight incubation with MFBSA0.1 (b). Bars represent 1 μm.

We suppose that interactions of MFBSAs with bonds creating and/or stabilizing the β-sheets forming amyloid fibrils lead to disruption of these bonds and thus to depolymerization and interruption of the interface between two neighboring β-sheets. For the most active magnetic fluids (BSA/Fe_3O_4 ratios 0.01 and 0.02) the DC50 values were determined in the range of micromolar Fe_3O_4 concentrations ($\mu g.ml^{-1}$) indicating their ability to interfere with insulin fibrils at stoichiometric concentrations [1].

It can be concluded that the most active MFBSAs have potential to depolymerizate insulin amyloid fibrils. We assume that the present findings represent a starting point for the application of the selected active MFBSAs as therapeutic agents targeting insulin-associating amyloidosis.

Acknowledgement: This work was supported within the projects 26110230061 and 2622012033 in the framework of the SF of EU, CEX of SAS Nanofluid, VEGA 0079, 0041, 0077, APVV-0171-10 and SK RO-0012-10, SAS NSC Taiwan and VVGS 38/12-13.

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Peroxidase-like activity of magnetic nanoparticles and their applications in immunoassay

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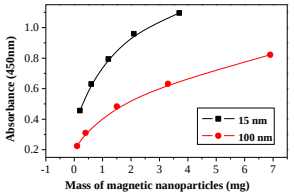
Functionalized Magnetic nanoparticles have found successful application in various fields of bioscience and biotechnology. Using functionalized magnetic nanoparticles for immunoassay instead of traditional enzyme-linked immunosorbent assay（ELISA）has been proved to be a simple，fast，and sensitive method. Especially, it was reported that bare magnetic nanoparticles possess peroxidase-like activity, which are widely used in ELISA. It makes the development of a more economical, accurate, and sensitive detection method possible. However, weakening or loss of peroxidase activity was shown after functionalization of bare magnetic nanoparticles.



The catalytic activity of magnetic nanoparticles.

Here, we report a kind of functionalized magnetic nanoparticles with large specific surface, small particle size, and remarkable amino groups on the surface possess an intrinsic enzyme mimetic activity similar to that found in natural peroxidases. The peroxidase-like activity was investigated by detecting the characteristic color of enzyme substrates produced by the catalytic oxidation of magnetic nanoparticles . The peroxidase-like activity of magnetic nanoparticles was PH, temperature, and quantity dependent. The optimal temperature was approximately 40℃. The catalytic activity enhanced with the increase of magnetic nanoparticles. Further study revealed that magnetic nanoparticles were reusable with high catalytic activity, which was superior compared with peroxidase. Application of the magnetic nanoparticles in a double-antibody sandwich enzyme-linked immunosorbent assay proved to be effective, in which magnetic nanoparticles were separation carrier and detection indicator. It holds promising potential for sensitive and robust bioassays in biomedical applications.

Renal iron load in sickle cell disease determined by magnetic resonance imaging measurements

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In a similar way that magnetic nanoparticles are used as contrast agents to alter the images obtained by magnetic resonance imaging (MRI) scanners, pathological iron accumulation in tissues can be tracked by MRI.

Sickle cell disease (SCD) is an inherited haemoglobin disorder characterized by chronic haemolytic anaemia and recurrent acute vaso-occlusive clinical events. Blood transfusion therapy has become a major therapeutic option, and with that comes secondary iron loading as the human body has no active mechanism for iron excretion. Although iron accumulation usually occurs in the liver and spleen, studies suggest that iron accumulation also occurs in the kidneys. In SCD patients, renal iron load has been shown to correlate with intravascular haemolysis rather than transfusion load.

In this study, mean kidney proton transverse relaxation rates (R-R2) were measured for 40 SCD patients and 17 controls as a surrogate measure of iron accumulation in the kidneys. Liver iron concentrations (LIC) were also measured to assess correlations with kidney R2 values. Axial images of the abdomen covering the liver and kidneys were obtained from clinical magnetic resonance imaging scanners operating at 1.5 T. Kidney and liver data were acquired simultaneously using a single spin-echo sequence (FerriScan®) with 5 echo times between 6 and 18 ms and a repetition time of 2500 ms.

Mean R-R2 was significantly higher ($p<0.0001$) in the SCD patients ($26.87 \pm$ SD $8.89\ s^{-1}$) compared with healthy controls ($17.77 \pm$ SD $2.94\ s^{-1}$). Furthermore, R-R2 values correlated with transfusion history ($r^2=0.23$, $p=0.004$) and markers of haemolysis such as bilirubin ($r^2=0.39$, $p<0.0001$) and lactate dehydrogenase levels ($r^2=0.37$, $p<0.0001$). These results suggest that iron accumulates in kidney tissues from SCD patients and is associated with haemolysis indicators.

Several iron metabolism disorders result in tissue iron accumulation, but the biodistribution of the iron within the body in each disease may have a different pattern. The non-invasive measurements of tissue iron loading in different organs by spin density projection assisted R2-MRI (FerriScan®) are crucial to understand the patterns of iron biodistribution in each disease and to adjust iron chelation therapy doses.

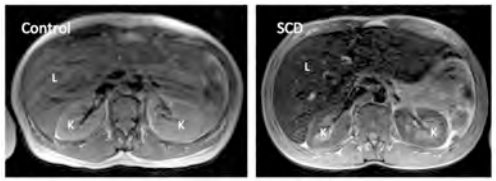

Figure. Hypointense MRI signal can be observed in the liver (L) and kidneys (K) of sickle cell disease patients in comparison with controls.

Bismuth-Doped Cobalt Ferrite Nanoparticles for MRI and CT Contrast Enhancement

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Nanomaterials play a defining role in the development of advanced contrast agents for biomedical imaging due to their unique, tunable physical properties. One of the advantages offered by nanoparticle-based contrast media over traditional contrast is the ability to integrate moieties of complimentary imaging techniques into one deliverable agent. One gains the advantages of both by combining two different imaging modalities.. Additionally, when used as a pharmacokinetic model for drug-loaded nanoparticles, multi-modal nanoparticles provide a fundamental understanding of their biodistribution.

Currently, iron oxide-based nanoparticles (e.g. Feridex ®) have found clinical applications as MRI contrast agents. Several studies have also examined the use of bismuth-containing lipid-based nanostructures and other bismuth compound-based nanoparticles as contrast agents for x-ray imaging techniques.[1,2] As of this writing, iron oxide-based contrast agents and bismuth-based contrast agents have not been combined into a single contrast moiety.

This study aims to develop bismuth-doped, cobalt ferrite nanoparticles for use as a combined magnetic resonance imaging (MRI) and x-ray computed tomography (CT) contrast agent using simple, aqueous-based synthesis. These nanoparticles rely on the x-ray attenuation of bismuth and the ferromagnetism of the cobalt ferrite domains for x-ray and MRI contrast enhancement, respectively. Amine-terminated nanoparticles were precipitated by mixing appropriate ratios of Fe^{3+}, Co^{2+} and Bi^{3+} ions in a high-pH solution containing cetrimonium bromide (CTAB), a tertiary amine-containing surfactant. Cobalt ferrite nanoparticles without bismuth were prepared similarly as a control. The nanoparticles were characterized using energy-dispersive x-ray spectroscopy (EDS), transmission electron microscopy (TEM), Raman spectroscopy and superconducting quantum interference (SQUID) magnetometry. X-ray attenuation was measured using a Cu K_α x-ray source and a photostimulable x-ray storage phosphor.

EDS measurements indicated the presence of iron, cobalt and bismuth in the bismuth-doped cobalt ferrite nanoparticles. Bismuth-doped nanoparticles also showed ferromagnetic behavior when analyzed using SQUID. Raman spectroscopy indicated the presence of magnetite domains within the nanoparticles. X-ray attenuation measurements showed contrast comparable to commercially available diatrizoic acid. MRI and CT phantom imaging studies are currently under development.

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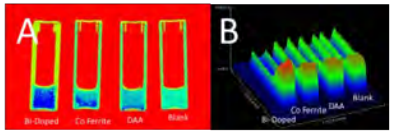

(A) X-ray attenuation false-color image and (B) pixel intensity image of nanoparticle samples suspended in 1% agarose. "DAA" refers to diatrizoic acid control, and "blank" refers to pure agarose gel.

Anti-EpCAM-Immobilized Albumin-Coated Monodisperse Magnetic Poly(Glycidyl Methacrylate) Microspheres for Detection of Circulating Tumor Cells

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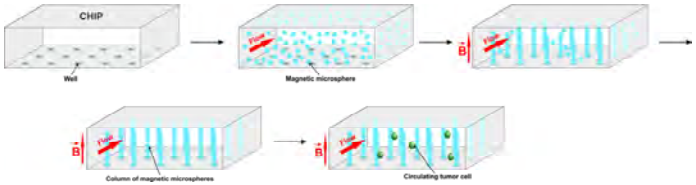
Highly magnetic and monodisperse microspheres were prepared for use in microfluidic immunomagnetic cell sorting, with a specific application to the capture of circulating tumor cells (CTCs). Multi-step swelling polymerization method in the presence of cyclohexyl acetate porogen was used for preparation of monodisperse (4 μm) macroporous poly(glycidyl methacrylate-*co*-2-[(methoxycarbonyl)methoxy]ethyl methacrylate-*co*-ethylene dimethacrylate) microspheres. After their hydrolysis and ammonolysis, carboxyl and amino groups were introduced in the microspheres. Iron oxide was then precipitated in the microspheres to render them magnetic. Repeated precipitation made possible to raise the iron oxide content to more than 30 wt.%. The microspheres were characterized by electron microscopy, atomic absorption and IR spectroscopy and superconducting quantum interference device (SQUID), To minimize non-specific adsorption of the microspheres in a microchannel, and of cells on the microspheres, they were coated with albumin crosslinked with glutaraldehyde. Antibodies of epithelial cell adhesion molecule (anti-EpCAM) were then immobilized on the albumin-coated magnetic microspheres using the carbodiimide method. Capture of MCF7 cells as a model of CTCs by the microspheres with immobilized anti-EpCAM IgG was performed in a batch experiment. Finally, MCF7 cells were captured by the anti-EpCAM-immobilized albumin-coated magnetic microspheres in an Ephesia chip. A very good rejection of lymphocytes was achieved. Thus, the feasibility of capturing of circulating tumor cells by albumin-coated monodisperse magnetic poly(glycidyl methacrylate) microspheres with immobilized anti-EpCAM in a microfluidic device was confirmed.



The Ephesia system. Alligment of magnetic microspheres into columns inside the chip under magnetic field and capture of epithelial cells.

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Novel core-shell magnetic nanoparticles as highly efficient contrasting agents for magnetic resonance detection

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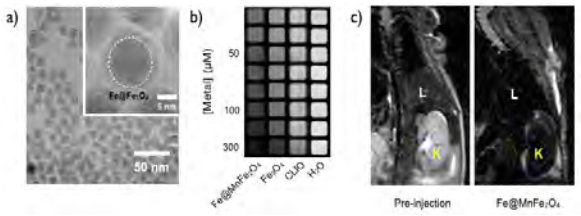
Magnetic nanoparticles (MNPs) with high magnetic moments and small size are under active development for biomedical applications. Ferromagnetic metals, rather than their oxides, have been suggested as an ideal constituent for MNPs because of their superior magnetization. Unfortunately, monometallic MNPs typically require protective layers to prevent progressive oxidation. To date, most core/shell approaches have yielded sub-optimal magnetization, as the shell was formed either by oxidizing the core or by coating it with non-magnetic materials. Here we present a new approach to preparing highly magnetic, *monometallic* MNPs. These hybrid core-shell particles consist of an iron (Fe) core and a ferrite shell (Fe@MFe_2O_4, M = Fe, Mn, Co). The Fe cores were first enlarged to increase the overall magnetization. Protective ferrite shells were then grown onto the cores and metal-doped to further improve magnetization. TEM analysis confirmed the encasing of the core with crystalline ferrite that provided robust protection on the core from oxidation (Fig. 1a). The resultant particles exhibited a novel magnetic feature: the presence of hysteresis but only negligible remanence. Comprehensive numerical analysis revealed that the ferromagnetic core and the superparamagnetic shell have a cooperative effect on magnetization. Thus, whilst the Fe cores have coercivity similar to that of bulk iron, the ferrite shells effectively reduce it by leading the magnetization at low external magnetic fields. This allows the particles to achieve high transverse relaxivity (r_2) of 365 s^{-1} mM^{-1} and yet remain superparamagnetic for biological applications. A comparative study of phantoms (Fig. 1b**)** confirmed the superiority of Fe@$MnFe_2O_4$ as a MR contrast agent; compared to the widely-used CLIO (cross-linked iron-oxide), Fe@$MnFe_2O_4$ was able to produce the same signal changes at ~10 times lower doses. The same trend was observed in preliminary *in vivo* imaging (Fig. 1c). With specific antibody conjugation, the particles also enabled a detection sensitivity of ~10 cancer cells in human whole blood *in vitro*.



a) TEM image of 16 nm Fe@Fe_3O_4. Fe core (dotted) was preserved during coating. b) Phantom MRI verified the superiority of Fe@$MnFe_2O_4$. c) *In vivo* mouse imaging with Fe@$MnFe_2O_4$. L, liver; K, kidney.

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Poster Presentations (in alphabetical order of the first author)

Poster List as of May 16, 2012

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1	Abubaker-Sharif	Budri	Development and application of a rapid and sensitive assay to quantify the cellular uptake of iron oxide nanoparticles	Ivkov	Robert	rivkov1@jhmi.edu
2	Ahmed	Naveed	Biodegradable magnetic nanoparticles for in vivo theranostics	Elaissari	Abdelhamid	elaissari@lagep.univ-lyon1.fr
3	Almstätter	Isabella	Design and evaluation of a multifunctional nano-carrier system for targeted drug delivery in gastro-intestinal cancer	Almstätter	Isabella	isabella.almstaetter@tum.de
4	Almstätter	Isabella	Characterization of magnetic viral vectors by MRI	Almstätter	Isabella	isabella.almstaetter@tum.de
5	Andreu	Jordi	A Coarse-Grain approach for the simulation of superparamagnetic dispersions under high magnetic fields	Andreu	Jordi	jandreu@icmab.es
6	Hajdu	Angela	Surface functionalization effect on protein corona; interactions for magnetite core nanoparticles	Hajdu	Angela	angela.hajdu@net.sote.hu
7	Arghir	Iulia	Nanostructuring Optical Surfaces towards FO-SPR Biosensors with Improved Performances	Arghir	Iulia	iulia.arghir@biw.kuleuven.be
8	Baehring	Franziska	Suitability of vitality assays for testing biological effects of coated superparamagnetic nanoparticles	Baehring	Franziska	franziska.baehring@med.uni-jena.de
9	Belousov	Andrey	Influence on cell regulation by magnetite nanoparticles (MCS-B)	Belousov	Andrey	an.belousov2012@yandex.ru
10	Belousov	Andrey	Effecti of magnetite nanoparticles MCS-B on microorganisms	Belousov	Andrey	an.belousov2012@yandex.ru
11	Blanco-Andujar	Cristina	Application of magnetic alternating current hyperthermia to induce cell death in melanoma	Blanco-Andujar	Cristina	ucap007@ucl.ac.uk
12	Blanco-Andujar	Cristina	Unraveling the formation pathway of iron oxide nanoparticles	Thanh	Nguyen TK	ntk.thanh@ucl.ac.uk
13	Bleul	Regina	Magnetic Polymersomes - Multifunctional Carriers for Biomedical Applications	Maskos	Michael	maskos@imm-mainz.de
14	Brandão	Delfina	Immunomagnetic Separation of Pathogenic Bacteria for Multiplex Electrochemical Magneto Biosensing	Pividori	Maria Isabel	isabel.pividori@uab.cat
15	Brusentsov	Nikolai	Early Contrast MRI and Thermochemotherapy of Oncological Diseases	Brusentsov	Nikolai	brusentsov2005@yandex.ru
16	Budi	Maeve	Comparative Analysis of Phosphate and Nitro-DOPA Anchoring Strength on Magnetite Nanoparticles	Mefford	Thompson	mefford@clemson.edu
17	Calabresi	Marcos	Study of gastric emptying and orocecal transit time in gastrectomized rats by AC biosusceptometry	Calabresi	Marcos	mfcalabresi@ibb.unesp.br
18	Carenza	Elisa	Magnetically Labeled Endothelial Progenitor Cells for Cellular Therapy in Brain Ischemia Treatment	Roig	Anna	roig@icmab.es
19	Carinelli	Soledad	Magneto immunosensor for the enumeration of CD4+ T lymphocytes in HIV diagnosis	Pividori	María	Isabel.Pividori@uab.cat
20	Chalmers	Jeff	Characterization and Quantification of the intrinsic magnetization	Chalmers	Jeff	Chalmers.1@osu.edu
21	Chen	Hongyu	Multifunctional magnetic nanoparticles for deep-tissue manipulation and imaging	Anker	Jeffrey	janker@clemson.edu
22	Visbal-Onufrak	Michelle	Protein Denaturation During Magnetic Fluid Hyperthermia at a Constant Thermal Dose: Does it Matter How the Magnetic Field is Applied?	Visbal-Onufrak	Michelle A	michelle.visbal@ece.uprm.edu
23	Chen	Chuanfang	The Dynamic Simulation and Applications of the Magnetotactic Bacteria	Song	Tao	songtao@mail.iee.ac.cn
24	Colle	Frederik	Novel optomagnetic platform for particle detection in DNA assays	Colle	Frederik	frederik.colle@imec.be
25	Cooper	J.	Core/Shell Magnetization in NiO Nanoparticles	Love	David	dml42@cam.ac.uk
26	Cornaglia	Matteo	Balancing Magnetic Dipolar and Viscous Forces on Superparamagnetic Beads for Improving the Specificity of Microfluidic Surface-based Immunoassays	Cornaglia	Matteo	matteo.cornaglia@epfl.ch
27	Corredor	E.	Magnetic nanoparticles penetration and transport in-planta	Marquina	Clara	clara@unizar.es
28	den Dulk	R.	Magneto-capillary valve for integrated biological sample preparation using magnetic microcarriers	Cappelli	Stefano	s.cappelli@tue.nl
29	Dutz	Silvio	The role of interactions in systems of single domain ferrimagnetic iron oxide nanoparticles	Dutz	Silvio	silvio.dutz@ipht-jena.de
30	Ebai	Tonge	Ultra-sensitive detection of proteins and infectious agents using magnetic particle-based Proximity Ligation Assay	Ebai	Tonge	tonge.ebai@igp.uu.se
31	Eberbeck	Dietmar	Magnetic Anisotropy and its Role in Magnetic Particle Imaging	Dennis	Cindi	cindi.dennis@nist.gov
32	Elbez	Remy	Nanoparticle induced Cell Magneto-Rotation: Monitoring Morphology, Stress and Drug Sensitivity of a Suspended Single Cancer Cell	Kopelman	Raoul	kopelman@umich.edu
33	Estevanato	Luciana	Selol-loaded magnetic nanocapsules for cancer treatment by hyperthermia	Estevanato	Luciana	lulislandim@yahoo.com.br
34	Garchov	T.	Efficient one-pot synthesis of polymer-coated USPIOs for image-guided nanoparticle-mediated cancer gene therapy	Bertin	Annabelle	annabelle.bertin@bam.de
35	Giles	Rory	Novel Magnetic Particles for Bioassays	Giles	Rory	rory.giles@espci.fr
36	Gitter	Kurt	Investigations on a branched tube model in magnetic drug targeting; systematic measurements and simulation	Gitter	Kurt	kurt.gitter@tu-dresden.de
37	Gomez Roca	Alejandro	Susceptibility and hyperthermia studies of fine magnetic particles	Gomez Roca	Alejandro	agr506@york.ac.uk
38	Gorschinski	Angelika	Magnetic Nanoparticles and Self-Assembled Magnetic Microspheres for Magnetic Separation: Preparation, Characterization, and Application	Gorschinski	Angelika	angelika.gorschinski@kit.edu
39	Gruettner	Cordula	An Easy Assay for measuring the Sheelding of Iron Oxide Cores by the Nanoparticle Shell	Gruettner	Cordula	gruettner@micromod.de
40	Gruettner	Cordula	Antibody Conjugation to Magnetic Nanoparticles by Strain-Promoted Alkyne-Azide Cycloaddition	Gruettner	Cordula	gruettner@micromod.de
41	Gutierrez	Lucia	Effect of the anesthesia on the magnetic nanoparticle biodistribution after intravenous injection	Gutierrez	Lucia	lucia.gutierrez@uwa.edu.au
42	Guttierez	Fabiola	The torsional properties of proteins studied by magnetic particle actuation	Guttierez	Fabiola	f.a.gutierrez.mejia@tue.nl
43	Hedayati	Mohammad	Cytotoxicity of intracellular nanoparticle-mediated hyperthermia depends on cell number	Hedayati	Mohammad	mhedayati@jhmi.edu
44	Hirsch	Vera	Surface charges influences the protein adsorption kinetics, colloidal stability and subsequent cell interaction of polymer coated SPIONs in vitro	Fink	Alke	alke.fink@unifr.ch
45	Horng	Herng-Er	An Easy and Simple method in manipulating temperatures for various sizes of magnetic nanoparticles on hyperthermia applications	Horng	Herng-Er	phyfv001@ntnu.edu.tw
46	Huang	Yu Yen (Eric)	Patterned Nano-magnet on-Chip for Screening Circulating Tumor Cells in Blood	Huang	Yu Yen (Eric)	n9692416@gmail.com
47	Ivkov	Robert	Magnetic nanoparticle hyperthermia: Magnetic nanoparticles for non-invasive cancer therapy	Ivkov	Robert	rivkov1@jhmi.edu
48	Ivkov	Robert	Devices for generating uniform alternating magnetic fields for non-invasive cancer therapy	Ivkov	Robert	rivkov1@jhmi.edu

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50	Johansson	Christer	Magnetic properties of magnetic multi-core particles	Johansson	Christer	christer.johansson@imego.com
51	Jurikova	Alena	Thermal properties of magnetic nanoparticles modified with polyethylene glycol	Jurikova	Alena	akasard@saske.sk
52	Kami	Daisuke	Efficient Transfection method using the Magnetic nanoparticles and Episomal vector	Kami	Daisuke	daisu777@gmail.com
53	Kekalo	Katsyarina	Magnetic Heating of Fe-Co Ferrites: Experiments and Modeling	Kekalo	Katsyarina	e.kekalo@gmail.com
54	Kennedy	David	AUTOMATED MEASUREMENT OF MAGNETOPHORETIC MOBILITY: METHOD AND APPLICATIONS	Kennedy	David	david.kennedy@ikotech.com
55	Khan	Aslam	Temperature Responsive Hydrogel-Coated Magnetic Nanoparticles	Khan	Aslam	aslamkhan@ksu.edu.sa
56	Khurshid	Hafsa	High Magnetic Moment Nanoparticles for MRI Contrast Enhancement	Khurshid	Hafsa	khurshid@usf.edu
57	Kinoshita	Takuya	Synthesis of $La_{0.75}Sr_{0.25}MnO_3$ Fine Particles for Magnetic Hyperthermia by Ultrasonic Spray Pyrolysis	Kinoshita	Takuya	t-kinoshita@chemeng.osakafu-u.ac.jp
58	Knopke	Christian	Quantification of Magnetic Nanoparticle Uptake in Cells by Temperature Dependent Magnetorelaxometry	Knopke	Christian	christian.knopke@ptb.de
59	Kolesnichenko	Vladimir	SYNTHESIS AND RELAXIVITY PROPERTIES OF SUPERPARAMAGNETIC IRON OXIDES WITH VARIABLE SIZE AND OXIDATION STATE	Kolesnichenko	Vladimir	vkolesni@xula.edu
60	Kopelman	Raoul	Asynchronous magnetorotation based Biomedical Platforms: From Biomarker Analysis to Rapid Testing for Microbial and Cancer Drug Sensitivity	Kopelman	Raoul	kopelman@umich.edu
61	Korinkova	Tereza	Monodisperse cobalt-zinc ferrite nanoparticles prepared by thermal decomposition and coated with silica in reverse microemulsion	Veverka	Pavel	veverkap@fzu.cz
62	Kozissnik	Bettina	Measuring the Behaviour of Antibody-Functionalised Magnetic Nanoparticles using Quartz Crystal Microbalance	Kozissnik	Bettina	b.kozissnik@me.com
63	Kralj	Slavko	Magnetic nanoparticles targeting into specific cells using epidermal growth factor as affinity ligand	Makovec	Darko	darko.makovec@ijs.si
64	Kuang	Min	Inorganic nanoparticles for biomedical applications.	Wang	Andrew	awang@oceannanotech.com
65	Kubovcikova	M.	The potential of BSA-modified magnetic fluids in the line of amyloid-diseases treatment	Koneracka	Martina	konerack@saske.sk
66	Kuboviva	M.	Biodistribution of Paclitaxel in the form of magnetic nanospheres	Zavisova	Vlasta	zavisova@saske.sk
67	Kucerova	Jana	PEGylation as a tool for enhancing the quality of magnetic immunosorbents used in microfluidic devices	Kucerova	Jana	jana.kucerova@upce.cz
68	Kuhnemund	Malte	Development of magnetic bead- based molecular detection assays using padlock probes for infectious disease and cancer diagnostics	Kuhnemund	Malte	malte.kuhnemund@igp.uu.se
69	Kuznetsov	Anatoly	Hydroecological applications of ultradisperse magnetic adsorbents: removal of small concentrations of pollutants from large volumes of water.	Kuznetsov	Oleg	kuznetsov_oa@yahoo.com
70	Latha	Subbiah	PREPARATION AND EVALUATION OF PREDNISOLONE MAGNETIC NANOSUSPENSION FOR POSSIBLE USE IN RHEUMATOID ARTHRITIS THERAPY	Latha	Subbiah	lathasuba2010@gmail.com
71	Latha	Subbiah	Formulation and evaluation of curcumin loaded magnetic nanoparticles for possible use in cancer therapy	Selvamani	P.	pselvamani@hotmail.com
72	Lawton	Thomas	Magnetic Characterization and Synthesis of Magnetite Nanoclusters	Mefford	Thompson	mefford@clemson.edu
73	Lim	Byunghwa	Trapping of Individual microorganisms in Magnetophoresis Channel	Kim	CheolGi	rvenu8@gmail.com
74	Lin	Yu-Zong	Preparation and biological application of magnetic ion-exchange microparticles	Lee	Wen-Chien	chmwcl@ccu.edu.tw
75	Liu	Tian	Quantification Susceptibility Mapping of Myelin Imaging Compound	Liu	Tian	tl265@cornell.edu
76	Loewa	Norbert	Cellular Uptake of Magnetic Nanoparticles Quantified by Magnetic Particle Spectroscopy	Loewa	Norbert	norbert.loewa@ptb.de
77	Loewa	Norbert	Potential of Improving MPI Performance by Magnetic Separation	Loewa	Norbert	norbert.loewa@ptb.de
78	Lopez	Jorge	Investigation of the Magnetic Properties in Fe3O4-gold Ferrofluid Functionalized by Carapa Guianensis Oil	Lopez	Jorge	vjorge0503@gmail.com
79	Love	David	Decoding Magnetically Barcoded Microcarriers in a Microfluidic System	Love	David	dml42@cam.ac.uk
80	Luke	Green	Large FePt nanoclusters: Synthesis, characterisation and elucidation of formation mechanism	Thanh	Nguyen TK	ntk.thanh@ucl.ac.uk
81	Ma	Yunn-Hwa	Manipulation of Magnetic Nanoparticle Retention and Hemodynamic Consequence in Microcirculation	Ma	Yunn-Hwa	yhma@mail.cgu.edu.tw
82	Magnet	Cécilia	Physical aspects of magnetically assisted water purification	Magnet	Cécilia	cmagnet@unice.fr
83	Mandal	Swapan	Structural and magnetic properties of Ni-Fe and Ni-Ag nanoparticles embedded in polymer	Mandal	Swapan	sk_mandal@hotmail.com
84	Mardinoglu	Adil	Optimization of 4 different SPION as potential diagnostic carriers in magnetizable stent assisted drug targeting applications.	Prina-Mello	Adriele	prinamea@tcd.ie
85	Matoussevitch	Nina	Magnetic nanoparticles coated by gold	Matoussevitch	Nina	matoussevitch@web.de
86	Mattingly	Stephanie	Dual-purpose Cancer Therapy Using Functionalized Iron Oxide Nanoparticles	Nantz	Michael	michael.nantz@louisville.edu
87	Mefford	Olin	The Stability of Poly(ethylene glycol) Stabilized Iron Oxide Nanoparticles: A study on ligand displacement under biological conditions.	Mefford	Olin	mefford@clemson.edu
88	Menezes	Hugo	Nanospheres loaded with ferrimagnetic carbon nanotube and chlorine6 for application in hyperthermia and photodynamic cancer therapy	Tedesco	Antonio	atedesco@usp.br
89	Min	Changwook	Magnetic relaxation switching biosensor: sensing mechanism and applications	Lee	Hakho	hlee@mgh.harvard.edu
90	Moore	Lee	Open gradient magnetic red blood cell sorter evaluation on model cell mixtures	Zborowski	Maciej	zborowm@ccf.org
91	Muela	A.	Biomineralization process of Fe3O4 nanoparticles by the magnetotactic bacterium Magnetospirillum gryphiswaldense	Fdez-Gubieda	Luisa	malu.gubieda@ehu.es
92	Murillo-Cremaes	Nerea	Drug impregnated magnetic nanospheres	Roig	Anna	roig@icmab.es
93	Nakamura	Kosuke	Self-heating temperature and ac hysteresis of magnetite nanoparticles and their dependence on secondary particle size	Nakamura	Kosuke	nakamura-kosuke-rn@ynu.ac.jp
94	Nguyen	KhanhVan	Magnetically modulated single particle tracking as a tool to study cytotoxicity of airborne nanoparticles	Anker	Jeffrey	janker@clemson.edu
95	Nikitin	Maxim	Quantitative Real-Time Study of Magnetic Particle Clearance from Blood and Biotransformation in Animal Organs in vivo	Nikitin	Maxim	max.nikitin@gmail.com
96	Nikolaev	B. P.	Magnetic epidermal growth factor conjugate for targeted delivery to tumor in xenograft mouse model	Soloviev	A. V.	alexei.v.soloviev@gmail.com
97	Orlov	A.	Highly Sensitive Magnetic Immunoassay Compatible with High Volume Samples	Nikitin	Petr	nikitin@kapella.gpi.ru

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98	Ota	Satoshi	Magnetofection using DNA/Polyethylenimine coated on magnetic nanoparticles	Ota	Satoshi	ota-satoshi-ty@ynu.ac.jp
99	Ott	K.-H.	A Minimally Invasive Approach to Remove Circulating Tumor Cells from the Body	Dapprich	Johannes	jdapprich@generationbiotech.com
100	Pablico	Michele	Multifunctional Magnetic Hydrogels as Cancer Nanotheranostic Agents	Pablico	Michele	mhp30@case.edu
101	Palchoudhury	Soubantika	DNA Interaction of Pt-Decorated Iron Oxide Nanoparticles	Bao	Yuping	ybao@eng.ua.edu
102	Palfreyman	Justin	Hetero-coated magnetic microcarriers for point of care diagnostics	Palfreyman	Justin	jjp38@cam.ac.uk
103	Palma	Susana	Towards an optimized preparation of negatively-charged SPION	Palma	Susana	s.palma@campus.fct.unl.pt
104	Park	Hye Hun	Magnetic Silica Composite with Satellite Ag Nanoparticles: Magnetically Recyclable Nanocatalyst and Antibacterial Material	Woo	Kyoungja	kjwoo@kist.re.kr
105	Pei-Ching	Yang	Chitosan-coated Magnetic Nanoparticles as a Carrier for Delivery of Urokinase in Targeted Thrombolysis	Chen	Jyh-Ping	jpchen1125@hotmail.com
106	Perez-Ruiz	Elena	Integrating technologies for the development of peanut allergen detection bio-assays	Perez-Ruiz	Elena	elena.perezruiz@biw.kuleuven.be
107	Phuoc	Truong	Towards a multifunctional nano-object for optical and magnetic detection of the sentinel node in early breast cancers	Pourroy	Geneviève	genevieve.pourroy@ipcms.u-strasbg.fr
108	Polikarpov	Dmitry	Biodegradation of Magnetic Nanoparticles in Rat Brain Studied by Mössbauer Spectroscopy	Polikarpov	Dmitry	polikarpov.imp@gmail.com
109	Popa	Adriana	Synthesis and Characterization of Hybrid Magnetic-Plasmonic Nanoparticles	Popa	Adriana	anp29@case.edu
110	Prabu	C.	FORMULATION DEVELOPMENT AND PHARMACEUTICAL EVALUATION OF LORNOXICAM MAGNETIC NANOEMULSION	Selvamani	P	pselvamani@hotmail.com
111	Prozorov	Tanya	Protein-Templated Magnetic Nanoparticle	Prozorov	Tanya	tprozoro@ameslab.gov
112	Pyshnyi	Michael	Specialized technique of tissue Doppler imaging for locating magnetic nanoparticles	Kuznetsov	Oleg	kuznetsov_oa@yahoo.com
113	Qi	Bin	The Effect of Ligand Molecule Length on the Size of Synthesized Iron Oxide Nanoparticles	Mefford	Thompson	mefford@clemson.edu
114	Quini	Caio	Evaluation of gastrointestinal tract absorption and distribution patterns using ACB technique associated to magnetic nanoparticles	Quini	Caio	caioquini@hotmail.com
115	Riegler	Johannes	Simulating magnetic particle interaction for targeted drug delivery	Riegler	Johannes	j.riegler@ucl.ac.uk
116	Rizal	Conrad	High Saturation Magnetization FeCo Nanostructures	Rizal	Conrad	crizal@ece.ubc.ca
117	Kong	Seon Deok	Controlled Targeting and Biodistribution of Magnetic Nanoparticles Across the Blood-Brain Barrier	Kong	Seon Deok	sdkong@ucsd.edu
118	Saber	Mohamed	Enhancing Magnetic Properties of Cyanide Based Molecular Magnetic Materials: The role of Single Ion Anisotropy	Saber	Mohamed	mohamed.saber@chem.tamu.edu
119	Sadhukha	Tanmoy	Acute Necrosis and Reactive Oxygen Species Generation by Magnetic Hyperthermia Leads to Effective Elimination of Cancer Stem Cells	Panyam	Jayanth	jpanyam@umn.edu
120	Safarik	Ivo	Magnetically responsive biocomposite materials for water technology applications	Safarik	Ivo	ivosaf@seznam.cz
121	Sandre	Olivier	Universal Scaling Law to Predict the Efficiency of Magnetic Nanoparticles as MRI T2-Contrast Agents	Sandre	Olivier	olivier.sandre@ipb.fr
122	Santos	Judes	Nanocomposite Biocompatible Magnetite-Encapsulated Hemoglobin	Santos	Judes	judesgs@gmail.com
123	Santos	Judes	Permeation Magnetic Colloid in Stratum Corneum as Model in the Permeation of Human Tissue	Santos	Judes	judesgs@gmail.com
124	Sapir	Yulia	Magnetically responsive alginate scaffolds for tissue engineering	Polyak	Boris	bpolyak@drexelmed.edu
125	Sarwar	Azeem	Optimal Permanent Magnet Halbach Designs for Deeper Tissue Targeting	Sarwar	Azeem	azeem.uiuc@gmail.com
126	Schneider	Thomas	Fractionation of Particles and Cells by Continuous Flow Magnetophoresis: Effects of Sample Concentration	Schneider	Thomas	Thomas.Schneider.81@gmx.net
127	Rahn	Helen	Tissue model for the study of heat transition during magnetic heating treatment	Odenbach	Stefan	stefan.odenbach@tu-dresden.de
128	Seino	Satoshi	Control of Thermoresponsive Protein Functionality by the Combination of Magnetic Nanoparticle and AC magnetic field	Seino	Satoshi	megane_saikou@hotmail.com
129	Seshadri	Madhav	Iron Oxide Nanoparticle-Mediated Hyperthermia of Hepatocellular Carcinoma	Liapi	Eleni	eliapi2@jhmi.edu
130	Shahraki	Reisi	Preparation and properties of magnetic ZnFe2O4-chitosan core-shell nanoparticles	Ebrahimi	Seyyed	saseyyed@ut.ac.ir
131	Shen	Z.	Fluorescence and Reflectance Co-Registered 3D Tissue Imaging System based on a Cryostat"	Shapiro	Ben	benashap@umd.edu
132	Shi	Donglu	Fluorescent, superparamagnetic nanospheres for drug storage, targeting, and imaging	Shi	Donglu	donglushi@gmail.com
133	Silveira	Luciene	Biocompativel Magnetic Colloid Based-Triterpene Polymer	Silveira	Luciene	lbatistadasilveira@gmail.com
134	Singh	Sarika	Development of Fe3O4 dendritic nanoadsorbents for water remediation	Singh	Sarika	sarikaddu@gmail.com
135	Siposova	Katarina	Magnetic fluid reverses inhibition of cell growing caused by protein amyloid fibrils	Siposova	Katarina	katkasiposova@gmail.com
136	Slabu	Ioana	Novel magnetic targeting models with superparamagnetic iron oxides	Slabu	Ioana	slabu@hia.rwth-aachen.de
137	Slabu	Ioana	Investigation of mesh implants with incorporated magnetic nanoparticles for MR visualization	Slabu	Ioana	slabu@hia.rwth-aachen.de
138	Sobik	Marten	Carbon nanotube-based magnetic nanohybrids	Ten Haken	Bennie	b.tenHaken@utwente.nl
139	Strbak	O.	Simulating of biogenic magnetite nanoparticles behaviour in external magnetic fields	Kopcansky	Peter	kopcan@saske.sk
140	Tasci	Onur	Cyclical Magnetic Field Flow Fractionation for the Separation of Magnetic Nanoparticles	Tasci	Onur	onurtasci@gmail.com
141	Tasci	Tonguc	Cyclical Electrical Field Flow Fractionation for the Separation of Magnetic Nanoparticles	Tasci	Tonguc	onurtasci@gmail.com
142	Teixeira	Maria	Biomagnetic methods in clinical practice: evaluation of influence of immunosuppressants on gastrointestinal transit	Baffa	Oswaldo	baffa@usp.br
143	Thanh	Nguyen TK	Hollow CoPt MNPs as a contrast agent for tracking transplanted neural progenitor cells in spinal cord slices	Thanh	Nguyen TK	ntk.thanh@ucl.ac.uk
144	Thanh	Nguyen TK	Core-shell gold coated magnetic nanoparticles and their interaction with thiolated DNA	Thanh	Nguyen TK	ntk.thanh@ucl.ac.uk
145	Timko	Milan	Hyperthermic effect in suspension of magnetosomes prepared by various methods	Timko	Milan	timko@saske.sk
146	Tomasovicova	Natalia	Radiation Stability of the BSA Stabilized Biocompatible Magnetic Fluid	Tomasovicova	Natalia	nhudak@saske.sk
147	Tomitaka	Asahi	Influence of surface coating on magnetic and self-heating properties of Fe3O4 nanoparticles and in vitro experiment for hyperthermia	Tomitaka	Asahi	setougoasahix@gmail.com
148	Toth	Tamara	Highly efficient DNA extraction with droplet-based microfluidics and magnetic microcarriers	Toth	Tamara	tamara.toth@biw.kuleuven.be

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150	Tserkovsky	D.	Iron-cobalt ferrite nanoparticles biocompatibility and distribution after intravenous administration to rat	Kekalo	Katsyarina	e.kekalo@gmail.com
151	Tu	Liang	Measurement of dynamic size distribution of magnetic nanoparticles	Tu	Liang	tuxxx038@umn.edu
152	Veverka	Miroslav	Co-Zn ferrite cores prepared by coprecipitation method for magnetic hyperthermia and magnetic resonance imaging	Veverka	Miroslav	veverka@fzu.cz
153	Lei	Pingyan	Thermal plasma synthesis of superparmagnetic iron oxide nanoparticles for biomedical applications	Lei	Pingyan	leixx043@umn.edu
154	Visscher	M.	Ex vivo quantification of magnetic nanoparticles in clinical samples at room temperature with high specificity and improved stability	ten Haken	Bennie	b.tenHaken@utwente.nl
155	Vitol	Elina	Ferromagnetic microdisks and polymer-encapsulated Fe3O4 nanoparticles for hyperthermia applications	Novosad	Valentyn	novosad@anl.gov
156	Vitol	Elina	Magnetomechanical actuation of single cells by ferromagnetic disks induces intercellular calcium signaling	Novosad	Valentyn	novosad@anl.gov
157	Wabler	M.	(ICP-MS)	Ivkov	Robert	rivkov1@jhmi.edu
158	Walton	Derek	Quantization of Spin Wave Levels and Ferromagnetic Resonance in Nanoparticles	Walton	Derek	waltond@mcmaster.ca
159	Wang	Andrew	Iron Oxide Nanoparticle for Cancer Therapy	Wang	Andrew	awang@oceannanotech.com
160	Wang	Yilong	One-pot Synthesis and Morphological Control of Janus Superparamagnetic Composite Nanoparticles	Wang	Yilong	yilongwang@tongji.edu.cn
161	Wang	Wei	A Robust Protein immobilization Approach onto GMR Biosensors	Wang	Jian-Ping	jpwang@umn.edu
162	Williams	P. Stephen	Magnetic field-flow fractionation using parallel-plate channels and a linear Halbach array of permanent magnets	Williams	P. Stephen	p.stephen.williams@gmail.com
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164	Xu	H.	Antibody conjugated magnetic iron oxide nanoparticles for cancer cell separation in fresh whole blood	Wang	Andrew	info@oceannanotech.com
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166	Yamazaki	Naoya	PEI-coated magnetite nanoparticles linked with anti-Fas for combination of antibody and hyperthermia therapies	Yamazaki	Naoya	yamazaki-naoya-vr@ynu.ac.jp
167	Yang	Hong-Chang	Magnetic-field enhanced relaxation rates of protons in ferrofluids characterized with high-Tc SQUID-detected nuclear magnetic resonance spectrometer	Yang	Hong-Chang	hcyang@phys.ntu.edu.tw
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169	Yuan	Yuan	Characterization of neuron interaction with magnetic nanoparticles actuated by alternating magnetic field	Yuan	Yuan	yuany3@rpi.edu
170	Zhang	Y.	Complexes of magnetic nanoparticle-adenoviral vectors encoding therapeutic gene enhanced cardiac regeneration	Li	Wenzhong	wenzhong.li@med.uni-rostock.de
171	Zhang	Qiuyu	Fabrication of Environmental Response Block Polymer Magnetic Microspheres and Their Application in Controlled Release of Drug	Zhang	Qiuyu	qyzhang@nwpu.edu.cn
172	Zhang	Qinlu	Rapid Lateral Flow Test Strips for Detection of Anti-Treponema Pallidum Antibody Using GoldMag® Nanoparticles as a Carrier	Cui	Yali	yali_cui@mail.com
173	Zhao	Lingyun	Drug-loaded magnetic nanocomposite devices for cancer magnetic thermochemotherapy	Zhao	Lingyun	nuszly@gmail.com

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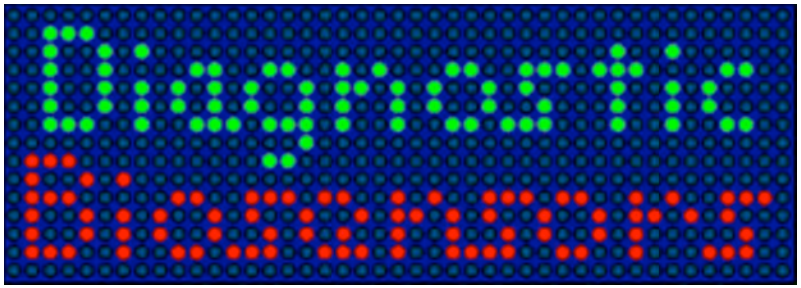

Development and application of a rapid and sensitive assay to quantify the cellular uptake of iron oxide nanoparticles

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Objective: Optimizing cellular uptake is a key aspect in the design of iron oxide nanoparticles for hyperthermia and other biomedical applications. Methods of quantifying iron, such as inductively coupled plasma mass spectrometry (ICP-MS), while very accurate, often require specialized, costly equipment and long processing time. Spectrophotometric methods, while readily available, lack consensus, and have not been validated for iron oxide nanoparticles. Here, we have developed and validated a rapid, sensitive method to quantify the cellular uptake of iron oxide nanoparticles.

Methods: A two-step colorimetric assay consisting of acidic decomposition, followed by iron detection using ferrozine, an iron(II) chelator whose complex absorbs strongly at 550nm, was developed to detect nanogram quantities of iron from iron oxide nanoparticles of different composition. Standard solutions of ferric iron were used to correlate the ferrozine assay with ICP-MS. To check the assay's sensitivity for *in vitro* applications, standard iron solutions were also tested in cellular backgrounds. We then selected a panel of breast cancer cell lines expressing varying levels of HER2/ErbB-2 (BT474: 47 copies/cell; MDA-MB-453: 11 copies/cell; MCF10A: negative) and labeled the cells with Miltenyi Biotec's anti-ErbB-2 iron oxide nanoparticles. The ferrozine assay was used alongside immunohistochemistry to provide dual characterization of the antibody-targeted particle's uptake in cells with varying antigen expression.

Results and conclusion: This modified ferrozine assay was able to detect iron concentrations of <1ppm, with a linear range between 0.2µg/mL and 4µg/mL. The assay demonstrated excellent agreement with ICP-MS and retained high sensitivity with different nanoparticles and in a background of 1 million cells. Using this method, we were able to distinguish the cellular iron content among anti-ErbB-2 iron oxide nanoparticle-labeled cells (MCF10A: <0.02 pg Fe/cell; MDA-MB-453: 0.43 pg Fe/cell; BT474: 1.8 pg Fe/cell). Overall, this assay provides a simple and sensitive method to quantify the efficiency and selectivity of the cellular uptake of iron oxide nanoparticles.

Poster 1

Biodegradable magnetic nanoparticles for *in vivo* theranostics

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Magnetic nanoparticles (MNPs) are considered highly important biomaterials due to their utilization in areas such as drug delivery, hyperthermia, imaging mechanisms, and very recently theranostics. The latter technique is based on the combination of "therapeutics" and "Diagnostics, and it encompasses the possible advances made in subjects that includes: pharmacodiagnostics, drug discovery, molecular biology and microarray chips. Considering the importance of this new biotechnology research area, the aim of current research work is the development of magnetic nanoparticles of iron oxide nanoparticles (IONPs) as theranostic agent that function to perform the delivery of anticancer drug and also as diagnostic tool and contrast agent for the in vivo applications via magnetic resonance imaging (MRI). The IONPs were prepared by a modified co-precipitation method in both aqueous and organic medium and designated as aqueous and organic ferrofluids, respectively. For nanoencapsulation of active ingredients and the IONPs, a modified method based on double emulsion evaporation technique was developed using polycaprolactone (PCL) as polymer, dichloromethane (DCM) as organic solvent and polyvinyl alcohol (PVA) as stabilizing agent. Different parameters affecting the final size, morphology and stability of the nanoparticles were studied including stirring speed, stirring time, concentration of polymer and stabilizing agent. A model drug stilbene (a fluorescent hydrophilic agent) was encapsulated by this modified method. Both types of ferrofluids were encapsulated using the developed modified double emulsion evaporation technique. Polymeric nanoparticles containing the IONPs were analyzed in vivo for the estimation of minimum detectable quantity as contrast agent using MRI. All the developed particles were well characterized before and after encapsulation to determine their size, zeta potential, stability, morphology, encapsulation efficiency, magnetic properties and chemical composition.

Poster 2

Design and evaluation of a multifunctional nano-carrier system for targeted drug delivery in gastro-intestinal cancer

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Abstract

In this project, a nano-carrier formulation for targeted drug delivery after systemic application in gastro-intestinal cancer will be developed, characterized and validated. After development and characterization, the nano-carrier will be tested *in vitro* in primary tumor cell lines derived from carcinomas of the murine mouse model and *in vivo* in genetically engineered mice bearing pancreatic ductal adenocarcinoma (PDAC).

Nano-carrier system of choice for this project are liposomes. The targeting of the liposomes towards the tumor site will be bi-functional, mechanical and biological. Efficient targeting allows focused carrier accumulation at the target site and specific drug delivery towards the tumor tissue. By encapsulation of magnetic nanoparticles (MNPs) into the liposomes, accumulation at a specific target region could be achieved mechanically by application of an external magnetic field. Surface functionalization of the liposome with a ligand molecule specifically binding a pancreatic tumor cell surface structure (Sarantopoulos et al., *submitted*) should enable biological targeting, followed by internalization of bound liposomes via endocytosis. Release of the chemotherapeutic agent from the liposomes will be induced by focused hyperthermia application (deSmet et al., 2011). Visualization of the liposome delivery and follow-up of tumor growth kinetics will be performed by magnet resonance imaging (MRI) and fluorescence molecular tomography (FMT). The results will be correlated with histology.

Presented will be first results of the *in vitro* characterization of selected MNPs and MNP-loaded liposomes. Determined physical parameters include hydrodynamic diameter, electrokinetic (or zeta-) potential and magnetophoretic mobility. Furthermore saturation curves and cell viability assays in primary murine PDAC cell lines were carried out. In addition, results of tumor tissue-mimicking MRI phantoms loaded with free and cell associated/internalized MNPs will be shown (Mykhaylyk et al., 2012).

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Poster 3

Characterization of magnetic viral vectors by MRI

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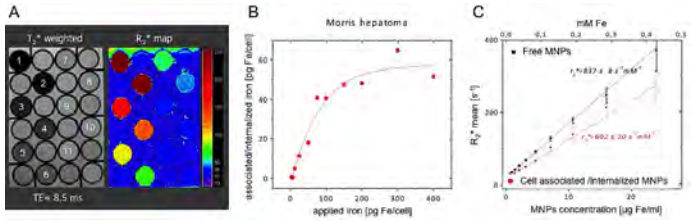
Selected core-shell magnetic nanoparticles (MNPs) and their optimized self-assembling complexes with viral vectors (magnetic vectors) are characterized for their physical properties as hydrodynamic diameter, electrokinetic (or zeta-) potential, magnetophoretic mobility in applied magnetic fields and their infectivity in various cell lines. To characterize the efficiency of the MNPs and magnetic vectors as tracers for MRI, tissue-mimicking MRI phantoms with homogeneously distributed particles and magnetic vectors were prepared. To evaluate the effect of the particle and vector assembling after internalization into cells, several cell lines were labeled with free MNPs or infected with MNP-virus-complexes. The cell loading with iron was quantified using non-heme iron analysis method. The magnetic moment of the MNP loaded cells was evaluated from magnetophoretic mobility data. Selected MNPs showed excellent r_2* relaxivities (higher than 600 s^{-1} (mM Fe)$^{-1}$) for the free and cell associated/internalized particles (Mykhaylyk et al., 2012). Assembling with viral particles resulted in slight decrease in r_2* relaxivities. Internalization into cells additionally decreased r_2* relaxivities of both, MNPs and their viral complexes. The modulation of the intracellular relaxivities of the MNPs are presumably resulting from the intracellular "clusterization" of the internalized particles and has been previously described.



(A) MNP calibration phantom; on the left the T_2* weighted image (echo time TE = 8.5 ms) and the corresponding color-coded R_2* [s^{-1}] map (right). Only the numbered wells contain the MNPs. **(B)** Saturation effect in magnetic cell labeling; the iron concentration per cell for the internalized/associated MNPs versus the applied iron doses per cell upon labeling with the nanoparticles. **(C)** Relaxivity alterations due to cell association/internalization of MNPs; dependency of the mean R_2* ± SD from the MNP or the iron concentrations in the wells and the resulting linear fit, which shows the calculated transverse relaxivity (r_2*) values for free MNPs and the MNPs associated/internalized in the homogeneously distributed cells in the calibration phantom.

Reference

Silica-Iron Oxide Magnetic Nanoparticles Modified for Gene Delivery: A Search for Optimum and Quantitative Criteria. Mykhaylyk O, Sobisch T, Almstätter I, Sanchez-Antequera Y, Brandt S, Anton M, Döblinger M, Eberbeck D, Settles M, Braren R, Lerche D, Plank C. *Pharm Res* 2012 Jan 6. [Epub ahead of print] DOI 10.1007/s11095-011-0661-9

Poster 4

A Coarse-Grain approach for the simulation of superparamagnetic dispersions under high magnetic fields

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In recent years, work in coarse grain models for the description of soft matter and biomolecular systems is experiencing a remarkable outburst [1]. The reason is that the description of these systems at experimentally relevant time and length scales requires inclusion of phenomena occurring at very different scales. The objective of coarse grain (CG) models is thus to retain sufficient molecular or nanoscale detail and yet remain amenable of simulation up to macroscopic time scales.

The aim of this work is the description of the chain formation phenomena observed in colloidal suspensions of superparamagnetic nanoparticles under high magnetic fields. We propose a new methodology based on an *on-the-fly* Coarse-Grain model [2]. Within this approach, the coarse grain objects of the simulation and their dynamic behavior are not fixed a priori at the beginning of the simulation but rather redefined on-the-fly. The motion of the CG objects (single particles or aggregates) is described by an anisotropic diffusion model and the magnetic dipole-dipole interaction is replaced by an effective short range interaction between CG objects. The new methodology correctly reproduces previous results of Langevin Dynamics simulations which fully account for the dipolar interactions whilst requiring an amount of CPU time orders of magnitude smaller. This substantial improvement in the computational requirements allows the simulation of problems in which the relevant phenomena extends to time scales inaccessible with previous simulation techniques (the MagChain software is available free of charge for academics at: `www.icmab.es/softmattertheory`.).

As a relevant example we were able to correctly predict by simulations the waiting time dependence of the relaxation time T_2 of water protons observed in magnetic resonance experiments containing dispersions of superparamagnetic colloids [3]. This success encourage us to apply this approach to other real-world applications in which the aggregation phenomena is the key factor as, for instance, the case of the cooperative magnetophoresis separation observed in superparamagnetic dispersions [4].

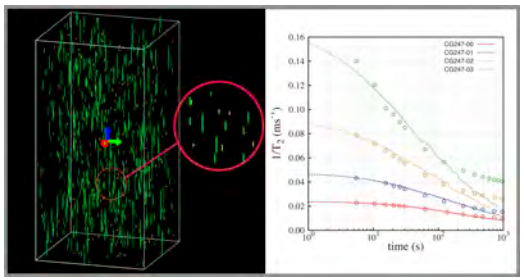

FIG. 1: **Left:** Snapshot of a simulation performed with the MagChain software corresponding to the system of 88nm superparamagnetic particles from ref. [3]. **Right:** Lines denote the corresponding relaxation time T_2 obtained from those simulations for different values of the volume fraction of the system. Symbols correspond to the experimental data reported in ref. [3].

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Poster 5

Surface functionalization effect on “protein corona” interactions for magnetite core nanoparticles

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Nanotechnologies present great opportunities but that it is also possible to foresee some hazards and toxicology, which should be taken account of by research on which regulation might be based. The nanoparticles may have unique toxic effect because of the large surface to volume ratio and other physical-chemical properties (size, shape, charge, etc.) [1].

Advancements in the synthesis and stabilization of dispersions of superparamagnetic nanoparticles (NPs), also called magnetic fluids (MFs), have promoted their use in many biomedical applications such as magnetic separation, drug delivery, hyperthermia and MRI’s contrast agents [2]. In fact, when NPs are dispersed in a relevant biological fluid their surface is modified by selective adsorption of biomolecules (such as proteins and lipids) with the formation of a biomolecular interface that has been termed “protein corona”. This “protein corona” can be thought as a biomolecular interface composed of a ‘hard’ and a ‘soft’ corona with ‘long’ and ‘short’ typical exchange times, respectively. The nature of the “protein corona” determines the biological identity of the particles and how they interact with living systems. This corona may not immediately reach equilibrium when exposed to a biological fluid. Proteins with high concentrations and high association rate constants will initially occupy the nanoparticle surface, but may also dissociate quickly to be replaced by proteins of lower concentration, slower exchange, and higher affinity [3-4].

Our project focused on expanding the approach to determination of the nature of the nanoparticle corona *in situ*, via the use of magnetic nanoparticles (stabilized with citric acid, poly(acrylic acid) or oleic acid double layer). The recovered nanoparticles were assessed using a range of approaches, including DLS and 1-D gels to identify the proteins in the corona following uptake.

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Poster 6

Nanostructuring Optical Surfaces towards FO–SPR Biosensors with Improved Performances

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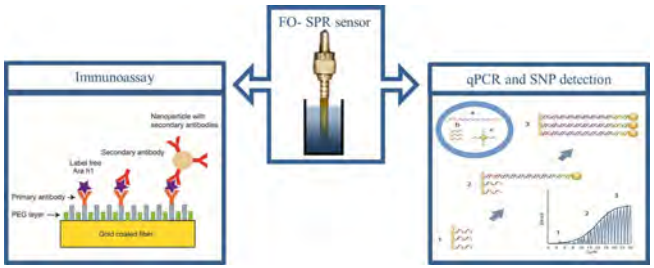
The Fiber Optic-Surface Plasmon Resonance (FO-SPR) biosensors are lately becoming a competitive tool for various applications in life science, food analysis and medical diagnostics because they allow for label free, real time monitoring of the binding reactions providing thus information on quantification and kinetics. SPR is an optical sensing technique, based on changes in the refractive index of light when guided towards a metal/dielectric interface. Our research group has developed an innovative FO-SPR platform with the light directed by an optical fiber to a thin Au layer sputtered onto the fiber surface. When the Au–coated FO is immersed in a buffer solution containing the biomolecular samples of interest, any event altering the refractive index of light at the interface will trigger a signal response.

We have successfully evaluated the FO-SPR system's applicability for diverse protein- and DNA-based bioassays with two examples depicted in the figure attached: (1) detection of peanut allergen in food samples, by using both antibody and aptamers as bioreceptors and (2) screening for single nucleotide polymorphisms (SNPs) within DNA sequences in real-time. The latter is supported by the fact that the DNA amplification/melting processes can be monitored in real-time, which has not been accomplished before with commercial SPR setups as they do not allow for fast thermocycling We demonstrated also that functionalized Au and magnetic nanoparticles can enhance the SPR detection limits when used as secondary labels, improving thus the sensitivity of our system towards the detection limits of currently used ELISA and label-free prism based SPR instruments.

Furthermore, we report various strategies for enhancing the Au layer attachment to the fiber surface and for nanostructuring the active area of the sensor in order to additionally improve the stability and sensitivity of the biosensor, respectively. Colloidal lithography approach is used for texturing the FO surface with 3D Au nanostructures, due to its simplicity and low-cost advantage. Results concerning the dispersion of various colloids and their self-assembling on optical media are presented. Furthermore, the work in progress addresses implementation of the colloidal patterning protocol within FO–SPR sensor platform.



Suitability of vitality assays for testing biological effects of coated superparamagnetic nanoparticles

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c) Center of Excellence for Polysaccharide Research, Friedrich Schiller University of Jena, Humboldtstrasse 10, D-07743 Jena, Germany
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e) chemicell GmbH, Eresburgstrasse 22-23, 12103 Berlin, Germany

Aims: The analysis of biocompatibility and nanoparticle-cell interaction is of vital interest for the evaluation of nanoparticles in medical applications, to minimize the risk of their potential cytotoxicity. It is known that nanoparticles can influence classical cytotoxicity assays (1). To establish a model test system for analysis of nanoparticles we evaluated three different viability assays and one cytotoxicity assay with the human brain microvascular endothelial cell line HBMEC, a key element of the blood-brain barrier. Furthermore we present data defining the toxic endpoint of nanoparticles.
Methods: Positively charged nanoparticles (medium core size 100 nm) with different types of shells (fluidMAG-PEI, fluidMAG-DEAE and fluidMAG-Chitosan) were provided by chemicell GmbH. HBMEC were seeded in black-walled 96-well culture plates over night with a density of $1*10^4$ cells per well. After a three hour incubation with various concentrations of nanoparticles three viability assays (CellTiter96® AQ_{ueous} One Solution Cell Proliferation Assay (MTS, Promega), PrestoBlue Cell Viability Assay (Invitrogen) and CellTiter-Glo® Luminescent Cell Viability Assay (Promega)) were tested to determine the number of viable cells. A cytotoxicity assay (CytoTox-ONE™ Homogeneous Membrane Integrity Assay, Promega) was used to estimate the number of non-viable cells by the use of the release of LDH from damaged cells. Furthermore the morphology of the cells was analyzed by phalloidin staining and fluorescence microscopy after nanoparticle addition. Four different nanoparticles with various shells provided by MagneticFluids were tested in concentrations ranging from 100 to 300 µg/cm .
Results: For defining a toxic standard particle, positively charged nanoparticles with concentrations ranging from 0.5 µg/cm² up to 368 µg/cm² were applied to HBMEC cell cultures. The viability of the cells after incubation with more than 50 µg/cm² fluidMAG-PEI nanoparticles was dramatically reduced. This could be determined with all three viability as well as with the cytotoxicity assay after consideration of assay specific controls. In contrast the fluidMAG-DEAE and fluidMAG-Chitosan nanoparticles did not show a significant reduction in cell viability or an increase in cytotoxicity even with higher nanoparticle concentrations. These data could be further confirmed by documenting the cell morphology during nanoparticle incubation based on changes of the actin cytoskeleton. Thus, fluidMAG-PEI was defined as toxic standard particle for testing the cytotoxicity of nanoparticles. After a detailed evaluation of the used vitality assays the PrestoBlue and CellTiter-Glo assays showed the highest reliability. Four core-shell nanoparticles with known cytotoxic effects, e.g. Melfalane coated nanoparticles were used to verify the cytotoxic behaviour of fluidMAG-PEI.
Conclusion: We could characterize fluidMAG-PEI as a toxic standard nanoparticle for the precise evaluation of the cytotoxicity and biocompatibility of nanoparticles with commercial vitality assays.

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Influence on cell regulation by magnetite nanoparticles (MCS-B)

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At now in Ukraine officially medicine following products nanotechnologies is applied: Micromage-B by way of biologically active additive and magnet-controlled sorbent (MCS-B) for extracorporal detoxication of biological liquids. A basis of the given preparations makes of magnetite nanoparticles (Fe_3O_4) size from 6 till 12 nm.

The results of clinic-biochemical researches by application present preparations are widely published in the central scientific - medical magazines, are submitted in the various collections materials of conferences, and are made out as the patents for the invention. However, despite of a spent complex clinic-biochemical researches, till now there is unexplored a mechanism of universality action on homeostasis system alive of organism and metabolism cells of the submitted nanotechnology preparations (NPs). In this scientific work for the first time are make an attempt investigate mechanisms action of NPs on cell regulation and metabolism as a whole.

Material: nanoparticles of magnet-controlled sorbent (MCS-B). The size of particles is from 6 to 12 nm; the total sorption surface of magnenite nanoparticles is from 800 to 1200 m^2/g; magnetisation of saturation I_s = 2.15 kA/m; volume concentration q = 0.00448; viscosity h = 1.0112 cSt; ζ - potential = - 19 mV.

Object of research: erythrocytes and leucocytes the patients of blood; the tissue organs of reticuloendothelial system (liver, kidneys, lungs) experimental animals; microorganisms (Staphylococcus aureus, Pseudomonas aeruginosa, Corynebacterium diphtheria, fungi of Candida type).

Results: after processing blood by MCS-B was discovered restoration regulator mechanisms metabolism of cell at the patients with syndrome intoxication. Normalization of a level lipid peroxidation parameters and parameters intoxication system was evidenced of blockade oxidizing stress.

On the contrary after processing blood by MCS-B, restoration of metabolism cell was not discovered at the patients terminal. It is possible this effect explainable of high level intoxication, damage metabolism apparatus of cell, substratum metabolism depletion. Also the universality of action by MCS-B on cells regulation processes was confirmed investigations enzymes action of antiradical protection at healthy persons and infectious hepatitis C patients. As a result of study AOA (SOD, catalase and glutathione) of the erythrocytes was discovered to modulation glutathione activity after processing of blood by magnetite nanoparticles (MCS-B). The influence by MCS-B was caused, on the one hand, the properties magnetite nanoparticles sorption selectively of molecule proteins from a surface membranes cell; with another, probably, change of conformation structure proteins of the superficial membranes as a result influence of magnetic field which induced by magnetite nanoparticles. The MCS-B has selective sorption properties which based on a principle magnetophoresis, and therefore as a whole does not cause destructive of structural cell membranes, and only quantitatively changes structure of molecule proteins. It was established, that extracorporally processing the blood by nanoparticles of MCS-B reliably reduces activity of Ca, Mg - ATPHese of erythrocytes.

The researches has proved that now nanoparticles of MCS-B are able not only to considerably reduce hemolysis, and thereby prolong storage time of the blood, influence activity of adenosinetriphosphateses of erythrocytes, regulate transmembrane exchange, but also to extracorporally influence cellular apoptosis.

Was management, that presence of a constant magnetic field around magnetite nanoparticles allows MCS-B to not only perform a selective adsorption of various substances like it is in magnetic phoresis, but also to actively effect intracellular biochemical processes. Activating the process of oxyhemoglobin dissociation up to 1.5-2 times and raising output of blood oxygen to tissues, MCS-B restores bioelectric potential of erythrocyte membranes, improves operation of blood cells, normalizes rheology and microcirculation.

Causing changes in hemoglobin buffer system, MCS-B exhaustively corrects pH and alkaline reserve of venous blood.

Restoration of metabolic shifts of homeostasis, of physical and chemical characteristics of tissue structures, of balance between antiradical and proradical products characterizes a direct effect of MCS-B on free radical oxidation of lipids.

Correction of balance between antiradical and proradical products provides activity of MCS-B regarding pathogenic germs and condition of cellular immunity. As a result sensitivity of pathogenic germs (Staphylococcus aureus, Pseudomonas aeruginosa, Corynebacterium diphtheria, fungi of Candida type) to antibiotics increases in 2-3 times and arises a pronounced bacteriostatic effect regarding pathogenic microflora.

At the same time, magnetite nanoparticles MCS-B do not cause changes of biological characteristics of normal flora with exception of short term slight inhibition of growth qualities.

Selective bacteriostatic and antifungal effects, correction of immunologic disorder (increase in phagocytic activity of leucocytes and in phagocytosis completeness index, liquidation of immunoregulatory cells disbalance) complete the list of biological effects of magnetite nanoparticles MCS-B.

Established, that action point of MCS-B is protein superficial cell membrane. The MCS-B has sorption activity regard to spectrin and ancirin of erythrocytes.

We have hypothesis biological action of nanotechnology preparations on protein molecules conformation and structure of cytoplasm and intracellular membranes. The change of protein a molecule structure to influences on transport substances of cell and determines intracellular metabolism processes.

Effect of magnetite nanoparticles MCS-B on microorganisms

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We have study effects of nanoparticles MCS-B on microorganisms causing various suppurative-inflammatory processes in humans. The research was made on the following objects: Staphylococcus aureus, Pseudomonas aeruginosa, Corynebacterium diphtheria, fungi from the genus of Candida.

During the study with the method of disks, we revealed increases in diameters of the areas of delayed growth of microorganism strains, treated with the above MCS-B, in comparison with "pure" strains.

Any their characteristic feature was the fact that under the effect of MCS-B the bacteria demonstrated a higher sensitivity to antibiotics. The strains of microorganisms, that were isolated from patients with pyoinflammatory diseases and possessed resistance to the majority of antibiotics, began to demonstrate sensitivity to these antibiotics, in particular to penicillin, ampicillin, tetracycline and gentamycin, after 24-hours' exposure to the above MCS-B. The minimum inhibitory concentration of antibiotics, producing the effect on pathogenic flora, was significantly decreased. The data are given in Table 1.

Antibiotic	S. aureus μg/ml		P. aeruginosa μg/ml	
	Control	After process of MCS-B	Control	After process of MCS-B
Carbenicillinum	9.0±0.6	3.0±0.4 P<0.001	≥100	60.0±10.5 P<0.05
Gentamicinum	5.0±0.8	2.0±0.9 P<0.05	12.0±1.2	4.0±1.3 P<0.05
Riphampicinum	9.0±1.3	3.0±0.7 P<0.001	14.0±1.4	5.0±1.5 P<0.001
Ofloxacinum	5.0±1.4	2.0±0.8 P>0.05	5.0±1.4	3.0±1.1 P>0.05

Table 1. Minimal depressing concentration of antibiotics regarding bacteria (mkg/ml) before and after MCS-B (M±m; n=50) exposure.

Note: P – accuracy of differences in comparison with control.

Application of magnetic alternating current hyperthermia to induce cell death in melanoma

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The use of new techniques for the treatment of cancer has been a major focus of research during the last decade. Novel methods, including heat by magnetic hyperthermia (MH) with functionalised magnetic nanoparticles (MNPs) have the potential to treat cancer cells. Herein, MH and MNPs were assessed for targeting and eliminating melanoma *in vitro* using a human cell line (DX3).

Cells were cultured and starved (12 h) to initiate cell loading with 0.5 mg/mL MNPs; fluidmag-citric acid (CT), fluidmag-carboxymethyldextran (CMX) and fluidmag-dextran (DX). Cellular uptake was studied by light microscopy with prussian blue staining and quantified by VSM-SQUID magnetometry. Subsequently, heat treatment using a patented Magnetic Alternating Current Hyperthermia (MACH) system working at a frequency of 1 MHz, was applied to induce cell death. Thereafter, the process was quantified with propidium iodide (PI) nuclear staining and flow cytometry.

Cell targeting of MNPs was found to be significantly higher for fluidmag-CT (Fig. 1a), and their perinuclear localisation indicated intracellular uptake, which was further validated by electron microscopy. SQUID magnetometry confirmed a 3-fold loading of fluidmag-CT compared to fluidmag-CMX and about 40-fold to fluidmag-DX (Fig. 1b). Upon heat treatment with the MACH system, a positive PI nuclear staining was given and indicated cellular necrosis.

To summarise, our results indicate that DX3 melanoma cells, loaded with MNPs, may be specifically targeted and treated upon application of an AC magnetic field. Such techniques show potential application in the treatment of melanoma and other cancers.

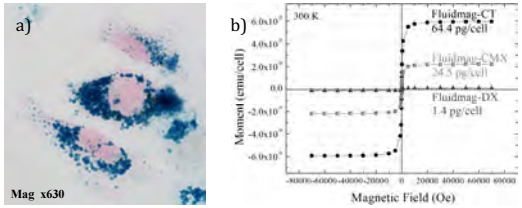

Figure 1. a) Prussian blue staining of DX3 cells treated with fluidmag-CT (0.5 mg/mL) and b) magnetization curves at 300K of DX3 cells loaded with chemicell fluidmag-CT, fluidmag-CMX and fluidmag-DX.

Unraveling the formation pathway of iron oxide nanoparticles

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Commercial iron oxide magnetic nanoparticles for biomedical applications are generally produced *via* a co-precipitation method. Their good performance in some techniques such as magnetic resonance imaging has long been proven.[1] Nonetheless, significant batch-to-batch output differences have been pointed out by studies focused on their use in magnetic hyperthermia-based treatments.[2] This limited control over the material characteristics has been a drawback for their use in biomedical applications. As a result, there is a need for a better understanding of the formation process for a reliable production of nanoparticles with long term stability.

We report on the use of sodium carbonate as a co-precipitating agent for the synthesis of uncoated iron oxide nanoparticles. Not only does this synthetic route permit the formation of magnetite nanoparticles, but more importantly it offers a benchmark for the study of the physicochemical changes that a solution of Fe(II) / Fe(III) experiences throughout the reaction. Particle size, morphology and crystal structure can be altered depending on the pH, temperature and reaction time. The simplicity of the method allows for the incorporation of coating agents and biomolecules with ease, therefore constituting suitable building blocks for biocompatible nanosystems.

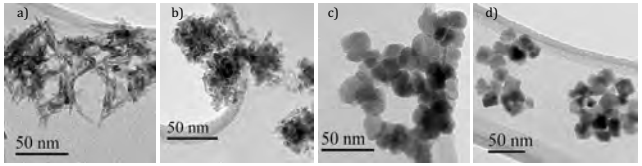

Figure 1. *Magnetite nanoparticles synthesized at room temperature and a) pH 7, b) pH 8, c) pH 9 and pH 10.*

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Magnetic Polymersomes – Multifunctional Carriers for Biomedical Applications

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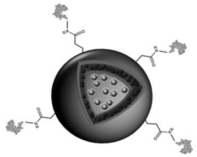

Interest in nanomedicine has dramatically increased in recent years; nevertheless, there is still a need for well-engineered and safe nanotransporters. In particular, as highly cytotoxic drugs are often applied in cancer therapy, a more specific and targeted drug delivery to the diseased tissue is desirable, allowing improved therapeutic efficacy and reduced adverse side effects.

We are engineering polymersomes (vesicles self-assembled from block copolymers) using micromixer-technology as a highly flexible and controllable tool, thus well defined polymeric vesicles as well as spherical and wormlike micelles in nanodimensions are produced in a continuous process in aqueous solution.[1,2] Furthermore, in-situ loading with drugs, dyes and even small particles as quantum dots or iron oxide nanoparticles is feasible.[3,4] The latter can be incorporated in the aqueous lumen or in the membrane core of these polymeric vesicles depending on the surface coating of the nanoparticles (hydrophobic or hydrophilic).

These magnetic polymersomes are promising tools for biomedical applications, such as magnetic separation, Magnetic Resonance Imaging (MRI), and hyperthermia. For instance, the deformation of the vesicle membrane can be induced by radio frequency magnetic hyperthermia, and a controlled drug release can be induced.[5] Furthermore, the magnetic properties of the magneto-polymersomes may allow them to be guided in a magnetic field gradient to the diseased tissue.[6]

We are also currently working on polymersomes targeting the human epidermal growth factor receptor (EGFR) as it is over-expressed in many cancer cells, particularly in cervical tumors.[7] We coupled the natural ligand epidermal growth factor (EGF) to the polymersome surface, which was verified by enzyme-linked immunosorbent assay (ELISA). Cytotoxicity tests confirmed the non-toxicity of the delivery system itself. Ligand-specific and non-specific cell binding was investigated by flow cytometry experiments and has already shown promising results. Further cell uptake studies are in progress.

Engineering polymersomes by micromixing technology allows us to control the size and features of the carrier system, leading to new possibilities for personalized medicine. Moreover, it enables the adjustment of dose and release profiles individually and a point-of-care preparation could be feasible.

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Immunomagnetic Separation of Pathogenic Bacteria for Multiplex Electrochemical Magneto Biosensing

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The increasing incidence of infectious disease pathogens is a significant public health concern for consumers worldwide. Among all food pathogens, *Escherichia coli O157:H7*, *Salmonella enterica* and *Listeria monocytogenes* are considered examples of important pathogens causing the most food-related human illnesses. [1]

In recent years, many improvements have been made in order to replace time-consuming conventional culture detection methods by rapid methodologies, such as polymerase chain reaction, immunological assays and biosensors. Moreover, the integration of magnetic particles into immunoassays provides improved analytical performances, allowing miniaturization, development of integrated systems and also the reduction of reagent and sample consumption. [2]

In the present work, a simple methodology for the simultaneous immunomagnetic separation (IMS) of different bacteria using magnetic particles modified with specific antibodies is reported. *Salmonella*, *E. coli* and *Listeria* were selected as a model.

The IMS performance, expressed as percentages of captured bacteria, was evaluated using classical culture methods and Scanning Electron Microscopy (Figure 1). In addition to this, the effects of the particles size, reaction time and bacteria concentration were also studied.

After a preconcentration step by IMS, the bacteria will be detected simultaneously with a multiplex magneto immunosensor or genosensor with electrochemical detection.

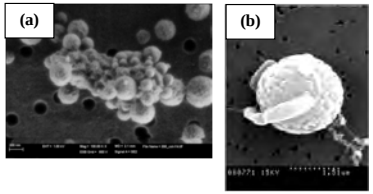

Figure 1: SEM images of the bacteria capture of: a) E.coli and b) Salmonella [2].

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Early Contrast MRI and Thermochemotherapy of Oncological Diseases

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The early revealing of proliferation by contrast MRI is an important problem in the detection of malignant tumors.

In the present work, we continued investigation of early contrast MRI and magnetohydrodynamic thermochemotherapy (MTC) of malignant tumors by BIOSPEC BC 70/30 (Bruker) and scanning electronic detector (ED) of magnetic carriers by their non-linear magnetization [1,2]. Neovascularization and early pretumoral changes of outlines of structures of biological tissues under action of malignant cells are a display of first attributes of proliferation which we revealed by contrast MRI. To reveal the early pretumoral changes of outlines of structures of biological tissues by contrast-enhanced MRI, we have tested Citric-ferrite sol (CFS). Accumulation of CFS and bracing in the healthy tissues were visualized by MRI and quantified by the ED scanning. Experiments showed that CFS appears to be a promising MRI-negative contrast agent for detection of malignant tumors.

The next step was treating the tumors by combination of chlorines (CH) with several procedures and drugs. We used Radachlorin (RCH), Cysplatin (CP), Mitoxantron (MX) and Melphalan (MP), Dacarbazine (DC), Docetaxel (DT), which are well-known chemotherapeutic drugs for treatment of the breast, lung, ovarian and other types of cancer. Besides, the effect of CP's, MX's and MP's can be increased by combining them with CFS. A complex treatment, which combines the magnetically controlled anticancer drugs such as CP and MP containing CFS, targeting them to the tumor tissue by a gradient magnetic field produced by magnetic NdFeB bandages (0.2 T) or by cryomagnet (7.0 T) [3], heating tumor by AC magnetic field with necrotic slime aspiration, and with intraperitoneal and intracavitary introduction of Cyclophosphan, was performed. The experiments were carried out for development of such complex MTCs of the rat glioma C 6, murine B 16 melanoma, mammary Ca 755 adenocarcinoma and Lewis lungs carcinoma (LLC), based on our methods for quantification of magnetic agents [2] and optimization of the treatment by 3D near-real-time MRI that controls MTC.

Application of CFS as the diagnostic agents for MRI contrast purposes, as well as heating agents combined with chemotherapeutic preparations are promising for the combined diagnosis and therapy of tumors.

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Comparative Analysis of Phosphate and Nitro-DOPA Anchoring Strength on Magnetite Nanoparticles

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Magnetic nanoparticles have recently garnered attention due to their potential applications in targeted drug delivery, diagnostics, and magnetic hyperthermia.[1] Of particular note are magnetite nanoparticles, which can be stabilized with a variety of ligands. Those modified with phosphate (PO) or nitro-3,4-dihydroxyphenylalanine (nitroDOPA) anchoring groups have been shown to have remarkable stability in solution compared with hydroxyl, amine, silane, or carboxyl groups; however a comparative study between the two is lacking.[2,3] To address this, a comparative analysis of the relative bonding strength of phosphate and nitroDOPA anchor groups onto magnetite nanoparticle surfaces was carried out and is presented herein.

Determining a ranking between phosphate and nitroDOPA anchoring group strength is especially important for biomedical applications as it can be used to predict the displacement of ligands and therefore particle stability of nanoparticle systems introduced into the human body. To compare the anchoring group strength of the two ligands, PEG-PO and PEG-nitroDOPA were synthesized, along with relatively monodispersed (7 ± 1nm diameter) magnetite nanoparticles. The nanoparticles were synthesized via thermal decomposition, and then modified with either PEG-PO or PEG-nitroDOPA. Dialysis was carried out on the modified nanoparticles, first in water to remove any free ligand, and then in phosphate buffer solution (PBS) to mimic a human body environment. Thermal gravimetric analysis (TGA) was carried out at intervals throughout the dialysis in order to monitor the displacement of both phosphate- and nitro catechol-based ligands into the surrounding media and to obtain information on the relative binding strength of these ligands.

Initial results indicate that the displacement of the nitroDOPA ligand has a logarithmic fit, which suggests that ligand loss becomes negligible once a threshold time is exceeded. Analysis of phosphate and nitroDOPA ligand displacement will include a comparison of initial rate of displacement, time taken to reach a stable ligand concentration, and final ligand concentration.

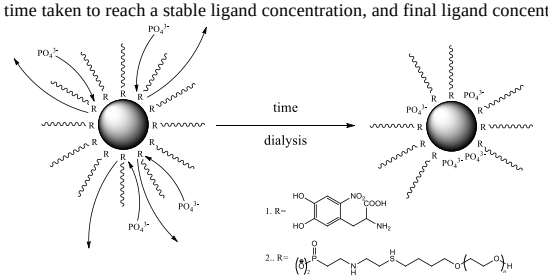

Figure 1: *Displacement of anchoring groups by phosphate in PBS*

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Study of gastric emptying and orocecal transit time in gastrectomized rats by AC biosusceptometry

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The stomach reduction surgery, gastrectomy, has been applied on treatment of several diseases related to the gastrointestinal tract (GIT), such as ulcers, stomach cancer and obesity. The gastrectomy is a surgical procedure that constitute the partial or total stomach recession, which causes modifications in some GIT physiological properties. Several studies show that one of those alterations promoted after this surgical intervention is a drastic modification on the orocecal transit time and gastric emptying. The biomagnetic methods constitute an interesting alternative for the study of these properties for they are potentially non-invasive, free from radiation and safe. The Alternate Current Biosusceptometry (ACB) is a magnetic method that uses inductions coils on the acquisition of magnetic flux variation obtained in response to the magnetic material ingested or fixed in the tract. This technique was validated as a standard method for gastrointestinal motility studies presenting high accuracy on the evaluation of gastric emptying. This paper proposes the partial gastrectomy influences and consequences analysis in gastric emptying time (GET) and orocecal transit time (OCTT) by the ACB technique using a test meal magnetically marked with Ferrite (Fe_3O_4). This work was developed and divided in two steps, before and after surgery. Each stage consisted of monitoring the magnetic intensity values on both stomach and cecum projections on the abdominal surface by the single-sensor ACB in a group of six male Wistar rats. All raw signals were analyzed in MatLab (Mathworks, Inc., USA) by visual inspection and the statistical moment was calculated. Using this approach, it is appropriate to quantify the following parameters: Mean Gastric Emptying Time (MGET) and Mean Cecum Arrival Time (MCAT). With the next stage, after surgery, was possible to compare these data and obtain a relation between these steps. Figure 1 shows a comparison between the gastric emptying **(a)** and cecum arrival **(b)** for both stages, before and after surgery. It also possible to observe the alteration on the MGET and MCAT values (before surgery: 182.0 and 327.9 minutes and after surgery: 121.3 and 246.5 minutes, respectively). The partial gastrectomy is characterized by the stomach fundus resection, where occurs the major part of the food accommodation. The comparison between gastric emptying before and after surgery showed a significant difference, which can be better observed by the MGET and MCAT values obtained in each stage. This gastric emptying time reduction is related to the physiological consequences of the gastrectomy. This time variation affects directly both the digestion and absorption process. Different kind of surgery should cause different effects on the gastric emptying and orocecal transit time, keeping a large whole of applications for the gastrectomy technique. The association of these obtained parameters to other properties as gastric contraction activity frequency and intensity should provide a better understanding of these procedure consequences.

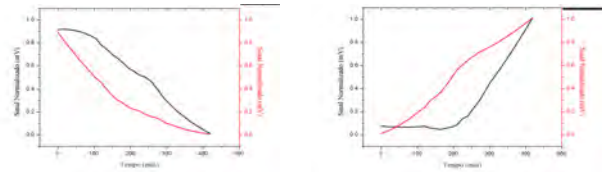

Fig. 1: Normalized magnetic signal intensity through time for each stage, black line: before surgery; red line: after surgery. **A**– Stomach region**; B** – Cecum region.

Magnetically Labeled Endothelial Progenitor Cells for Cellular Therapy in Brain Ischemia Treatment

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Endothelial progenitor cells (EPCs) show stemness characteristics with the ability of differentiating into endothelial cells (1). These cells constitute a new model for angiogenesis, endothelial regeneration and vessels repair (2). In recent years stem cell labeling with superparamagnetic iron oxide nanoparticles (SPIONPs) has been used as strategy for cellular therapy and tissue repair, as for instance in central nervous system diseases (3).

Our final objective is to enhance angiogenesis and tissue repair in peri-infarcted brain areas by engrafting functional EPCs using an external magnetic field. For that, citrate coated SPIONPs were synthesized through thermal decomposition route (4) yielding a γ-Fe_2O_3 core of 6 ±1 nm in diameter and were subsequently transferred to water by using anionic surfactants. Stable aqueous dispersion at pH= 7.5 imaged by Cryo-TEM showed nanoparticles aggregates with hydrodynamic sizes of 50 nm and 30% polydispersity. Magnetic measurement at room temperature confirmed the absence of remnant magnetization and a high saturation magnetization value (54 emu/g Fe_2O_3). Early EPCs from mouse were successfully labeled with the SPIONPs after 24h of co-incubation with a non-toxic iron concentration of 50 µg/ml. Labeled cells show an uptake of 24 pg Fe/cell. TEM images established cellular uptake and the storing of SPIONPs into endosomal compartments.

Furthermore, we observed that magnetized outgrowth EPCs were fully functional since they shaped vessel-like structures as non-magnetized cells and we have found that magnetized human and mouse EPCs secrete more VEGF and FGF than control cells. Finally, a preliminary *in vivo* cell tracking experiment demonstrates that magnetized EPCs can be guided to cortical areas of the brain by an external magnetic field as confirmed by MRI images.

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Magneto immunosensor for the enumeration of CD4$^+$ T lymphocytes in HIV diagnosis

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The incidence of human immunodeficiency virus (HIV) infection and clinical disease continues to increase rapidly in underdeveloped and developing countries.

In a patient with HIV infection, CD4 counts help determine the stage of infection, guide drug choices and indicate the response of the patients to the treatment as well as disease progression. Moreover, this indicator is also recommended for immune disorders, after an organ transplant or a graft. In developed countries, CD4 counts for patient with HIV infection are usually determined every 3–6 months. Flow cytometry is the standard method for CD4$^+$ cells counts, but the high investment of the instrument and costly reagents make it unaffordable to most of the centres in a developing country.

To solve the urgent need for improved diagnostic tools of HIV, a magneto immunosensor with electrochemical detection is presented, as a simple, rapid and inexpensive strategy for CD4$^+$ T cells counting.

In this strategy, CD4$^+$ T cells were successfully separated from the sample and preconcentrated using one-step immunomagnetic separation based on the CD3 receptor and using magnetic particles modified with antiCD3 antibody. The optimization of the immunomagnetic separation was performed using optical microscopy as well as flow cytometry.

After the immunomagnetic separation, the captured cells were then labelled by a biotinylated antiCD4 antibody, followed by the reaction with the streptavidin-peroxidase conjugate. Finally, the electrochemical detection was performed using a magneto electrode based on graphite epoxy composite and compared with the optical detection in a magneto-ELISA procedure.

Preliminary results indicated that the LOD was as low as 20 CD4$^+$ T cells per μL of human serum being the linear range between 0 to 1000 cells/μL, involving the whole medical interest range for counts for HIV-1-infected patients. Future work will be focused on the evaluation of this new strategy in both healthy volunteers as well as HIV infected patients. Moreover, the immunomagnetic separation will be also evaluated coupled with a genosensing strategy for detection of virus transcripts for HIV confirmatory diagnosis.

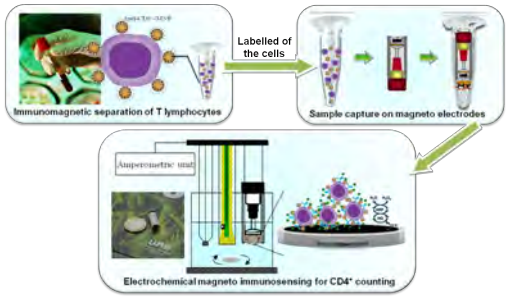

Figure 1. Schematic representation of the bioassay for the rapid CD4$^+$ cell counting in peripheral blood by using magnetic particles.

Characterization and Quantification of the intrinsic magnetization of a number of cell types

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Intrinsic magnetic susceptibility. Whereas it is generally understood that most biological material is diamagnetic (repulsed by a magnetic gradient), it has been known since early work by Linus Pauling and coworkers in 1936 and 1937 that the chemical bonds between the Fe atom and the porphyrin ring changes from an ionic bond in the deoxygenated state to a covalent bond in the oxygenated state. However, the relatively low level of magnetic susceptibility of the deoxygenated Hb has traditionally limited applications of this property. A similar discussion can be made on the magnetic properties of various states of manganese, another common, magnetic element in cells.

However, the development of ultra-high power, low cost, neodymium magnets and modern computer aided magnetic field designs and imaging technology has facilitated the development of instruments that can track the movement, on a cell-by-cell basis, of large numbers of cells and particles, including deoxyHb, oxyHb, and metHb containing red blood cells (RBCs) in specifically designed, and extremely powerful magnetic energy gradients. This instrument is referred to as a **Cell Tracking Velocimeter, CTV** .

Using a combination of CTV, X-Ray Photoelectron Spectroscopy, XPS, and IPC-mass spectroscopy, IPC-MS, we have begun to characterize both the amount and oxidation state of Fe and Mn in a number of different cells and cell types, including human red blood cells, RBCs, plaques from coronary artery disease, algae, and human sperm. In this presentation we will not only summarize our methodology to make these measurements, but also discuss the sensitivity and potential limits of this approach.

Multifunctional magnetic nanoparticles for deep-tissue manipulation and imaging

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Magnetic fluorescence nanocomposites have been extensively studied for cell labeling, tumor targeting, drug delivery, and hyperthermia. However, the fluorescent components in these magnetic nanoparticles have the limited application in deep tissue due to their excitation source of UV light. Herein, we report a facile method to prepare magnetic radioluminesent nanoparticle for deep tissue imaging high resolution. This novel magnetic radioluminesent nanoparticles provides a crucial tool for manipulation and tracking nanoparticles used for tumor targeting, drug delivery, and hyperthermia in deep tissue.

Poster 21

Protein Denaturation During Magnetic Fluid Hyperthermia at a Constant Thermal Dose: Does it Matter How the Magnetic Field is Applied?

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Hyperthermia treatments for cancer are currently based on an equivalent thermal dose or Cumulative Equivalent Minutes under a reference temperature of 43ºC ($CEM_{43ºC}$). Although the exact pathways leading to cell killing during hyperthermia are still not fully understood, it appears that protein denaturation is crucial in such process. To determine if the manner in which the magnetic field is applied has an effect on the amount of protein denaturation induced, three different magnetic field profiles were applied during Magnetic Fluid Hyperthermia (MFH). Conformational changes induced by thermal denaturation of Bovine serum albumin (BSA) protein were monitored by changes in fluorescence of 8-anilinonaphthalene-1-sulfonic acid (1,8-ANS) bound to the protein's hydrophobic sites. Iron oxide nanoparticles coated with covalently attached carboxymethyl-dextran (IO-covCMDx) synthesized in our laboratory were added to samples at a concentration of 0.6 mg IO core/ml. Real-time fluorescence measurements of the ANS-BSA complex emission spectra were performed during treatment. MFH under the different conditions and HWH were applied to ANS-BSA samples until an equivalent thermal dose of 60 $CEM_{43ºC}$ was achieved. For MFH, average induction heater power values of 48% (36.58 kA/m) for 43ºC and 61% (40.11 kA/m) for 45º were used, with the following schedule: A - constant power, B - On/Off power with duty cycle of 33%, and C - On/Off power with duty cycle of 67%.

Figure 1 shows that ANS-BSA exposed to MFH at 43ºC exhibited greater denaturation than standard HWH treatment under equal temperature set points and $CEM_{43ºC}$ of 60 minutes. Moreover, for a temperature set point of 45ºC and equal $CEM_{43ºC}$, fluorescence recovery of ANS-BSA observed between MFH and HWH showed similar behavior to the temperature set point of 43ºC. Between the different MFH conditions applied, results showed statistically similar denaturation was achieved with Condition A versus Condition B, whereas Condition C achieved greater denaturation than the previous two conditions ($p<0.05$, $n=3$). Importantly, the results demonstrate that under same conditions of $CEM_{43ºC}$ MFH results in greater irreversible protein denaturation, compared to HWH. Furthermore, the results demonstrate that even under conditions of equal average power and equal medium temperature, alternating magnetic field duty cycles with higher power application result in greater irreversible protein denaturation contrary to expectations based on continuum heat transfer arguments.

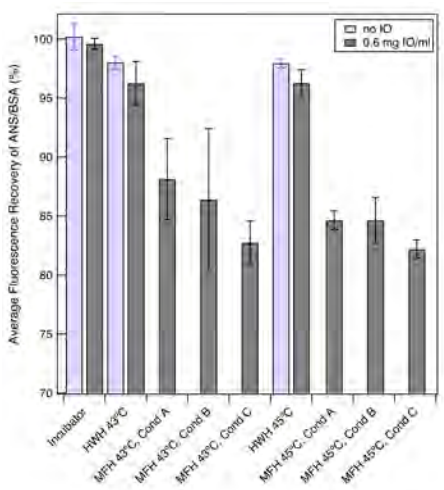

Figure 1. Summary of results on average conformation recovery of BSA from ANS fluorescence measurements performed during different hyperthermia treatments of HWH and three different MFH schedules, for temperature set points of 43ºC and 45ºC.

Poster 22

The Dynamic Simulation and Applications of the Magnetotactic Bacteria

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The strain of MO-1 is one type of magnetotactic bacteria (MTB), which is characterized by the presence of the magnetosomes and the ability to swim downward along an inclined geomagnetic field in both hemispheres. It is interesting to research swimming mechanism of MTB under magnetic field and develop their special applications by their special swim behavior.

In this work, the six-degrees-of-freedom dynamic model of MO-1 was built on the Newton–Euler dynamic equations, and the interaction between the flagellum and fluid was considered by the resistive force theory. The simulated motion trajectory of MO-1 was found to consist of two kinds of helices: small helices resulting from the imbalance of force due to flagellar rotation, and large helices arising from the different directions of the rotation axis of the cell body and the propulsion axis of the flagellum. The motion behaviors of MTB in various magnetic fields were studied, and the simulation results agree well with the experiment results. In addition, the rotation frequency of the flagella was estimated at 1100 Hz and the included angle of the magnetosome chain was predicted at 40˚ that is located within 20˚ to 60˚ range of the observed results by the simulation.

Besides the simulation, it is the critical point if the strain MO-1 was coupled with functional loads to form a MTB-microrobots. In our lab, MTB-microrobots were constructed by attaching 2 m microbeads to MO-1 cells using immunoreactions and were controlled using a special control and tracking system. The results show the attachment efficiency could be improved by ~30% via immunoreaction. The results show that MO-1 transported one microbead at a velocity of ~21 m/s. And the MTB-microrobots could be stopped by trapping them as they swim within a circular field with a controllable size under rotating magnetic fields. The magnetic-guided, auto-propelled MTB-microrobots have potential application in target molecule separation and detection, drug delivery, and target cell screening in a microfluidic chip.

These results would provide useful information to research the swimming mechanism of MTB and the application of MTB-microrobot.

Novel optomagnetic platform for particle detection in DNA assays

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Magnetic particles are often employed in biological experiments to isolate specific macromolecules (e.g. proteins, nucleic acids, etc) from complex matrices. Less frequently, the same magnetic particles are used as labels for detection of macromolecules. Still, using magnetic particles for both manipulation and detection makes them ideally suited for use in point of care diagnostic applications. The dual use of these particles facilitates system integration, keeping the final detection instrument inexpensive and small.

Most often the detection of bound magnetic particles is achieved by using sensors that measure the particles magnetic stray fields. Examples of such devices include giant magneto resistance sensors, spin-valves, magnetic tunnel junctions, etc. Although such sensors have proven to be very sensitive, their use in point of care diagnostic devices is hampered mainly because of their large production costs.

In an attempt to design a cost-effective and sufficiently small device, we propose the detection of magnetic particles by means of their holographic footprint. More specifically, diffraction of light around bound particles generates an interference pattern that is recorded by a digital camera. The mathematical description of this phenomenon enables reconstruction of the particles distribution present at the surface.

To verify this technology, functionalized magnetic particles were used to specifically capture single stranded DNA fragments. After capturing, the particle-DNA complexes were hybridized to receptor probes immobilized on a surface. The hybridization time was significantly reduced, from around 50 minutes to a mere 5 minutes, by applying a magnetic force directed towards the binding surface; while a magnetic force directed away from the surface was used to remove unbound particles and hence can be regarded as a magnetic washing step. The resulting bound particles were illuminated by partially coherent light and a holographic image was recorded. Successful reconstruction of the bound particles was confirmed by optical microscopy.

Our data show that the presented method of detecting magnetic particles through their holographic footprint enables rapid and successful detection of DNA fragments within an inexpensive system.

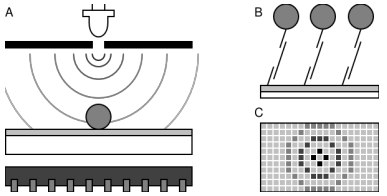

Figure 1: schematic representation of different aspects of the performed experiments: detection system (A), DNA assay (B), holographic image (C).

Core/Shell Magnetization in NiO Nanoparticles

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Magnetic nanoparticles have been attracting great interest due to their applications in healthcare and medical diagnostics, such as magnetic resonance imaging, targeted drug delivery, treatment of solid tumors by hyperthermia and magnetic biosensors [1,2]. For many applications of magnetic nanoparticles an accurate determination of their moment and active magnetic volume is required. However, previous studies [3] have found that using SQUID magnetometry or similar techniques to estimate the particle size distribution gives magnetic sizes significantly smaller than the structural sizes found by transmission electron microscopy (TEM).

Here we present a new approach to unambiguously prove and quantify the existence of a surface, magnetically disordered, layer. Large amounts of uncoated NiO nanoparticles were prepared by hydrothermal synthesis [4] and first characterized by X-ray diffraction (XRD), thermogravimetric analysis, SQUID magnetometry and TEM, Fig. 1 (left). Finally, neutron diffraction techniques were employed above and below the magnetic ordering (Néel) temperature, T_N = 523 K, Fig. 1 (center). By using antiferromagnetic NiO and neutron powder diffraction we were able to completely separate the magnetic from the structural information. The results confirm bulk structural properties of NiO, crystallizing in a fcc NaCl structure with the space group Fm3m, and a lattice parameter of a = 4.168(6) Å above T_N. At 5 K the pattern has an additional Bragg peak, solely due to the antiferromagnetic order, which is also broadened. Refinement of the magnetic structure leads to a solution identical to that of the bulk, *i.e.* fcc type 2 with a moment per Ni atom ~ 1.9 μB. The magnetic (½½½) reflection was used to obtain the magnetic particle size 'core' as 51 Å, while all the nuclear peaks were used to obtain the structural size, yielding 65 Å in good agreement with XRD results. SQUID magnetometry results showing the existence of a superparamagnetic surface layer will also be discussed.

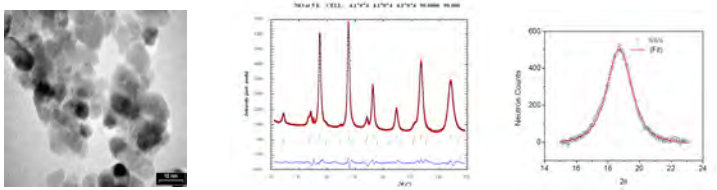

Figure 1: (Left) TEM image of bare 6 nm spherical NiO nanoparticles. (Center) High resolution neutron powder diffraction scan at 5 K and fit. (Right) The purely antiferromagnetic (½½½) reflection at 5 K and fit.

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Balancing Magnetic Dipolar and Viscous Forces on Superparamagnetic Beads for Improving the Specificity of Microfluidic Surface-based Immunoassays

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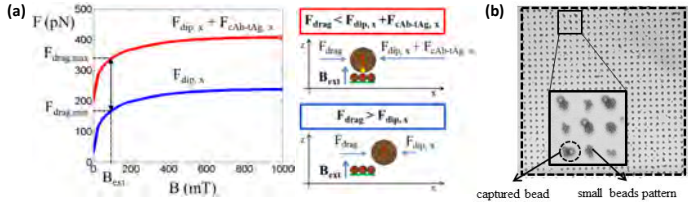
Different superparamagnetic bead-based immunoassays have been proposed in recent years exploiting fluorescence, magnetic or optical detection methods. Analyte detection at concentrations down to the attomolar range has been demonstrated. One of the main issues is to be able to clearly discriminate between the proper signal generated via specific analyte binding and the background signal, which is mainly due to unspecific interactions and adsorption.

We propose here a novel bead counting-based detection method, which relies on dipolar interactions between superparamagnetic beads of different size. Our microfluidic chip consists of a polydimethylsiloxane (PDMS) half-channel that is fixed on top of a glass substrate. The glass substrate features a dot pattern of small capture antibody (cAb)-coated superparamagnetic beads (diameter 1.0 μm), which are electrostatically fixed on the surface. Larger cAb-coated magnetic beads (diameter 2.8 μm) are used for specifically capturing target antigens (tAgs) from a sample solution. A sandwich immunoassay is subsequently realized by magnetophoretically exposing the small-bead pattern to the larger beads by placing the chip in the field of a permanent magnet, while flowing through the solution of large beads. The small magnetic bead pattern on the chip leads to local magnetic field maxima, enhancing interaction between the immobilized small beads and the moving larger ones. The improved selectivity of the small bead-large bead binding is based on the fine-tuning of the balance between magnetic dipolar interactions and drag forces, thus allowing removing selectively nearly all non-specifically bound beads (i.e. beads that are not linked via tAg). Accurate modelling of the governing forces has been pursued by interfacing 3D Finite Element Method simulations with numerical calculations. Different working regimes for bead detection could be identified, proving the key role of magnetic dipolar interactions in enhancing the magnetic bead capture specificity.

The presented work opens up novel perspectives with regards to the development of fast, integrated and extremely specific immunoassays, with the potential for improving rare protein analyses for early disease detection.



(a) Schematic representation and (b) experimental implementation of the dipolar interaction-mediated detection technique. Proper combinations of external magnetic field distribution (magnetic dipole force) and flow rate (drag force) will ensure the specific capture of large superparamagnetic beads carrying tAgs onto a dot pattern of small superparamagnetic beads, while preventing unspecific adsorption of the large beads. Simply counting the number of large beads provides the detection signal (∝ t-Ag concentration) of the immunoassay.

Magnetic nanoparticles penetration and transport *in-planta*

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Magnetic nanoparticles are very suitable for a broad range of applications, like those involving synthesis and use of ferrofluids for bio-applications in general. In medicine the aim is to use them in diagnosis as well as in therapy. The ongoing research and results obtained up to now in these fields open a wide range of possibilities for using magnetic nanoparticles in other disciplines, for example in general plant research and agronomy. To study the use of nanoparticles in agriculture the first stage is to work out the penetration and transport into living plants and plant cells. We present here an overview of the research carried out within the scope of an interdisciplinary collaboration, on how inorganic nanoparticles interact with plant cells and tissues[1,2,3].

We have used iron/iron-oxides carbon-coated nanoparticles, synthesized by a gas-phase condensation method, in an arc-discharge furnace. Biocompatible suspensions of the nanoparticles have been synthesized and injected into pumpkin (*Cucurbita pepo*) living plants. The graphitic shell of our nanoparticles made possible their visualization into plant cells and tissues, using different microscopy techniques (fluorescence, confocal, light and electron microscopy). Moreover, their magnetic character allowed the nanoparticles to be positioned in the desired plant tissue by applying magnetic field gradients (produced by small permanent magnets). We have also observed that in the absence of magnetic fields, the nanoparticles can travel as well along the vascular systems, reaching different cell and tissues. Nanoparticles have been found both in the cytoplasm and in the extracellular space between cells. A size-based selection mechanism seems to be operating, probably involving cell walls and waxes acting as a barrier. With respect to cytotoxicity, it has been observed that cells containing nanoparticle agglomerates exhibited a cytoplasm denser than that of cells containing few nanoparticles. Damage at the plant level was not macroscopically evident.. However, further detailed studies are needed to evaluate the cytotoxicity and phytotoxicity of more intense treatments than those carried out in this study.
We have also studied the absorption and translocation of our nanoparticles into plants of different families (wheat, tomato, sunflower and pea) after their administration through the roots and by spraying the nanoparticles suspension on the leaves. It has been observed that nanoparticles reach the aerial part of the plant after 24 hours. In wheat plants accumulation of nanoparticles was detected in leaf trichomes, suggesting a way for nanoparticles excretion/detoxification.
Although more studies are necessary to unveil the nanoparticles penetration and translocation mechanisms as well as their cyto- and phyto-toxicity, our results open a wide range of possibilities for using magnetic nanoparticles in general plant research and agronomy.

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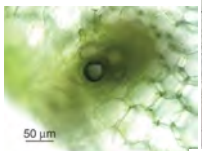
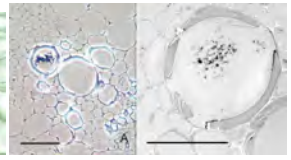


Light microscopy image of a petiole tissue at the magnet application point.



Nanoparticles inside xylem vessels: A) Phase contrast image B) TEM image of an ultrathin consecutive section. Bar: 30 µm

Magneto-capillary valve for integrated biological sample preparation using magnetic microcarriers

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A key challenge in point-of-care diagnostics is the integration of biological sample preparation. Magnetic microcarriers are very convenient for sample preparation, but it is difficult to miniaturize and integrate the required sequences of processes in a device technology.

We report a novel microcarrier-based microtechnology in which magnetic particles are transported by magnetic forces through several stationary aqueous liquids separated by a capillary structure. The device consists of two microscope slides; the bottom one is completely hydrophobic, while the top substrate is patterned in hydrophilic and hydrophobic regions. In this way, fluid chambers and valve regions are defined. Since the transport of microcarriers between the liquids is based on a balance between magnetic and capillary forces, we have named it the magneto-capillary valve (MCV) [1].

In this presentation we will demonstrate that magnetic particles can be transported reproducibly between the aqueous liquids by magnetic forces. We will show that we can determine the magnetic forces applied on the magnetic particles and we will characterize the behavior of the valve in a model that balances magnetic forces, capillary forces and friction forces [2]. Furthermore, we have investigated the valving efficiency of the MCV by monitoring the concentration of a fluorescent tracer in a purification procedure. For each crossing of particles over a MCV the dye concentration decreases by two orders of magnitude, which demonstrates very efficient purification. In addition, we have studied integrated nucleic-acid sample preparation and compared it to a standard manual procedure using Eppendorf tubes and a magnetic rack [3]. The results demonstrate that the purification of DNA in MCV cartridges has a performance comparable to standard manual purification in tubes. We conclude that the MCV microtechnology opens new opportunities for integration and miniaturization of automated biological sample preparation and assays based on magnetic microcarriers.

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The role of interactions in systems of single domain ferrimagnetic iron oxide nanoparticles

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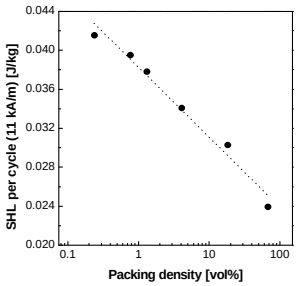
Magnetic nanoparticles are interesting materials for a lot of medical and technical applications. Each application requires particles with adapted magnetic properties. A less experimentally investigated question is the influence of packing density of magnetic nanoparticles on their magnetic properties due to magnetic interactions between single particles. In the literature a strong influence of the packing density on the magnetic properties is described theoretically [1].

Aim of this study is the experimental investigation of the correlation between packing density and magnetic properties, e.g. coercivity, relative remanence, and specific hysteresis losses.

For this aim, magnetic nanoparticles of iron oxides prepared as fine dry powder by laser deposition [2] are investigated with respect to their structural and magnetic properties as function of packing density. The particles are nearly spherically shaped single crystals in the magnetic single domain size range with a mean diameter of 21 nm occasionally exhibiting spinel growth facets. Samples of these particles were prepared in a range of volume concentrations from 0.2% up to 68% of the bulk density of magnetite by diluting with nonmagnetic silicon oxide particles and pressing in an uniaxial press. For the investigation of the magnetic properties the hysteresis curves at saturation field amplitude (1275 kA/m) as well as at lower field amplitude (11 kA/m) were measured by vibrating sample magnetometry. The specific hysteresis losses of the samples were calculated by integrating the areas of the measured hysteresis curves.



Dependence of the mass weighted specific hysteresis losses (SHL) on the packing density of magnetic nanoparticles measured at low field amplitude (11 kA/m).

Main result of the study is the fact that with decreasing packing density (which means a decrease of the magnetic interactions) the values for the magnetic parameters coercivity, relative remanence, and specific hysteresis losses increase. The found concentration dependence of these parameters may be understood in terms of magnetic interactions between neighbouring particles. In the Henkel-Plot an increase of the magnetic interactions between the single particles of the nanoparticle powder for increasing packing densities was found. The results of the magnetic characterization are in good agreement with the theoretical estimations [1] and may be understood in terms of the cubic anisotropy of magnetite distorted by a small uniaxial shape contribution.

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Ultra-sensitive detection of proteins and infectious agents using magnetic particle-based Proximity Ligation Assay

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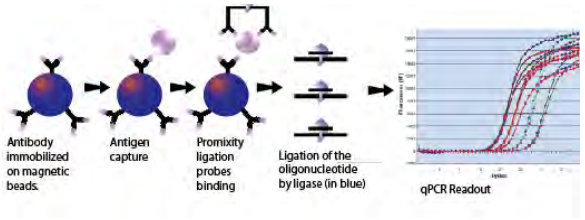
Sufficiently sensitive and specific techniques for protein detection will expand the range of molecules that can be targeted for analysis to include e.g. tissue-specific proteins present in trace amounts in blood, complexes of interacting proteins, and very low copy numbers of specific infectious agents. Accordingly, improved assay techniques can greatly improve the prospects for diagnostics in complex diseases such as cancer, infectious and neurological diseases.

We have recently developed a proximity ligation assay, where very high specificity and sensitivity of target molecule detection results from the requirement for multiple recognition events, combined with extremely high efficiency of signal detection due to amplification of reporter DNA molecules that specifically form in the detection reactions. A multiplex version of the assay allows parallel analyses of panels of proteins in minute amounts of samples, while other forms of the assay facilitate detection of high-order biological complexes. Magnetic particles greatly improve assay performance by significantly reducing the background of the assay. A schematic of the assay is shown in the figure below.

This assay represents a versatile analytical protein detection technology and it has been adapted to detect Aβ aggregation in Alzheimer's disease, for detection of exosomes in blood in prostate cancer, and for detection of viruses and bacteria in liquid samples and in situ. The combination with magnetic particles improves signal to background, supports multiplexing, and can form the basis for an integrated biosensor device for rapid diagnostics.



Schematic of the Proximity Ligation Assay.

Magnetic Anisotropy and its Role in Magnetic Particle Imaging

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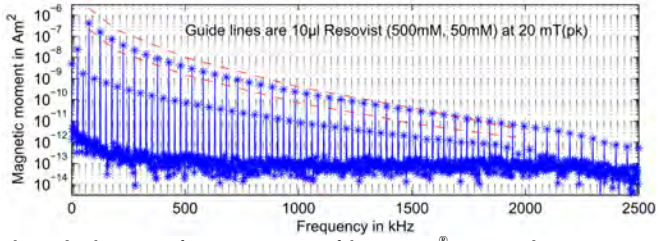
Biocompatible magnetic nanoparticles are interesting tracers for diagnostic imaging techniques, including magnetic resonance imaging (MRI) and magnetic particle imaging (MPI). Here, we will present our studies of the physical, chemical, and especially magnetic properties of magnetic iron oxide dextran nanoparticles, which show promising tracer signals in MPI. The MPI spectrum of nanomag®-MIP particles (50 mM iron concentration) with a hydrodynamic diameter of 100 nm was compared with the MPI spectrum of Resovist® (50 mM and 500 mM iron concentration):



The results show a significant improvement of the nanomag®-MIP particles, i.e. an increase of the signal amplitude by a factor of about 2 at the 3rd harmonic, as compared to Resovist®. In particular, the signal improves progressively with the order of the harmonic, a prerequisite for better spatial resolution. To understand this behaviour, we also investigated the samples by quasistatic magnetisation measurements, M(H), and by magnetorelaxometry (MRX). While M(H) provides the magnetic size distribution (roughly equivalent to the core sizes), MRX, which measures the change in the magnetisation of immobilised (freeze dried) nanoparticles after a field pulse, yields the magnetic anisotropy constant. The mean volume diameter (d_V) of the nearly monomodal lognormal size distribution of nanomag®-MIP is 19 nm and the dispersion parameter (σ) is 0.3. For Resovist®, d_V=22 nm and σ=0.25. However, about 80% of magnetic signal for the nanomag®-MIP is attributed to this one mode in the size distribution; whereas in Resovist®, only 30% of the magnetic signal originates from the mentioned mode in the size distribution. The remaining Resovist® particles are smaller, and, in practice, do not contribute to the MPI spectrum. This explains the spectrum shown above and is in good agreement with AC susceptometry data (as interpreted with the Yoshida approach[1]).

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Nanoparticle induced Cell Magneto-Rotation: Monitoring Morphology, Stress and Drug Sensitivity of a Suspended Single Cancer Cell.

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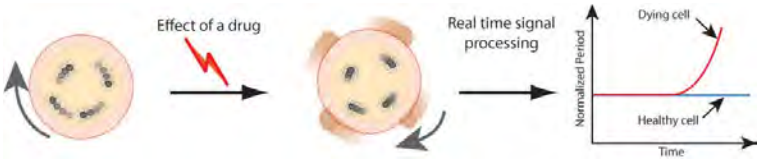
Single cell analysis has allowed critical discoveries in drug testing, immunobiology and stem cell research. In addition, a change from two to three dimensional growth conditions radically affects cell behavior. This already resulted in new observations on gene expression and communication networks and in better predictions of cell responses to their environment. However, it is still difficult to study the size and shape of single cells that are freely suspended, where morphological changes are highly significant.

Described here is a new method for quantitative real time monitoring of cell size and morphology, on single live suspended cancer cells, unconfined in three dimensions. The precision is comparable to that of the best optical microscopes, but, in contrast, there is no need for confining the cell to the imaging plane. The here first introduced *cell magnetorotation* (CM) method is made possible by *nanoparticle induced cell magnetization*. By using a rotating magnetic field, the magnetically labeled cell is actively rotated, and the rotational period is measured in real-time. A change in morphology induces a change in the rotational period of the suspended cell (e.g. when the cell gets bigger it rotates slower). In the presence of an effective drug or in cytotoxic conditions, blebbs are formed at the surface of the cell in the early stages of cell death. This phenomenon slows down the rotating cell. The unaffected cell will keep a steady rotation rate over time.

The ability to monitor, in real time, cell swelling or death, at the single cell level, is demonstrated. Our new technique is also compatible with fluorescence imaging. This method could thus be used for multiplexed real time single cell morphology analysis, with implications for drug testing, drug discovery, genomics and three-dimensional culturing.



Schematic of the principle of the Cell Magneto-rotation method.

Selol-loaded magnetic nanocapsules for cancer treatment by hyperthermia

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The development of drug delivery systems (DDS) has been largely used in antitumor research. In general, DDS represent an important strategy to improve the efficacy of drugs by increasing their bioavailability in the targeted areas, enhancing the drug solubility, while decreasing the side effects. When associated with magnetic nanoparticles, DDS also have the potential to carry out hyperthermia and to be site-guided to a specific target tissue by extenal magnetic field.

A magnetic drug delivery system composed by nanocapsules of poly(lactic-co-glycolic acid) loading both selol and maghemite nanoparticles (SL-MNC) (Fig. 1) was recently synthesized. Selol is a hydrophobic mixture contaning 5% of selenium that present chemopreventive and chemotherapeutic effect. The aim of the present research was to evaluate through *in vitro* tests the SL-MNC biocompatibility, antitumor effects, and hyperthermia induction efficacy.

The investigation was performed using murine (4T1) and human (MCF-7) mammary carcinoma cells and human normal breast cell line (MCF-10A). Viability analysis by MTT assay showed that the cytotoxicity induced by SL-MNC is dose and time dependent. In low doses, SL-MNC reduced significantly the cell viability in tumor cells (about 35%) with no effects on normal cells. This antitumor effect of SL-MNC was increased when cells were exposed to an alternating magnetic field. Morphological analysis by light microscopy revealed major uptake of SL-MNC by tumor cells. Also, strong evidences of apoptosis and DNA fragmentation were found by flow cytometry analysis.

Our results showed that SL-MNC is able to induce apoptosis to cancer cells, especially when exposed to an alternating magnetic field thus performing the magnetothermocytolysis process. We conclude that SL-MNC has a high potential as an antineoplastic DDS.

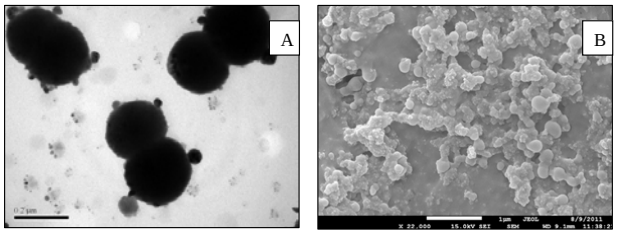

Fig. 1 - Morphological characterization of Selol-loaded magnetic nanocapsules (SL-MNC) by transmission (A) and scanning (B) electron microscopy, showing nanocapsules with average diameter of 244 nm and monodisperse feature.

Efficient one-pot synthesis of polymer-coated USPIOs for image-guided nanoparticle-mediated cancer gene therapy

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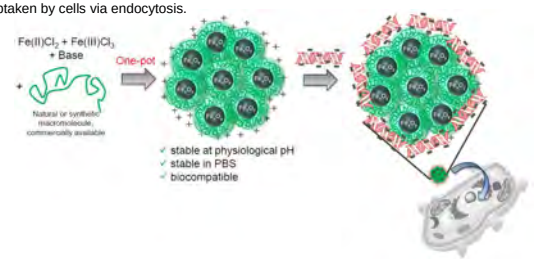
Non-viral gene therapy holds great promises for treating diseases such as cancer, by correcting the expression of defective genes.[1] However, in order for this technology to be improved, there is a critical need for safe, reproducible administration of genetic material (plasmid DNA, siRNA), and consequently determination of its *in vivo* fate. To this end, imaging reporters such as MRI contrast agents in the form of nanoparticles, superparamagnetic iron oxide nanoparticles (USPIOs), can be coupled to the genetic material in order to track the movement of the nanotransporters in real time (image-guided gene therapy).[2] These USPIOs can be also considered as model systems for nanoparticles-based gene delivery systems.[3]

Nanoparticle-mediated gene therapy provides many advantages compared to direct injection of naked DNA or siRNA: stealth nanoparticles prolongs the stay of the genetic material in the blood pool and the genetic material benefits from the favorable biodistribution of nanoparticles. Indeed, nanoparticles can reach cancer cells by benefiting from the Enhanced Permeability and Retention (EPR) effect, which takes into account the defective tumour vasculature, as well as the low lymphatic drainage of the tumour tissue. Moreover, nanoparticles are passively uptaken by cells via endocytosis.



Pristine USPIOs are easily synthesized by the well-known coprecipitation method using iron (II) and iron (III) salts in presence of a base. These USPIOs that are electrostatically stabilized in aqueous solutions, despite their biocompatibility, do not fulfil the requirements for biomedical applications: due to potential destabilization in biological media, it is necessary to coat them with stabilizing molecules or polymers. The polymeric coating should introduce sufficient steric stabilization in aqueous media so that USPIOs dispersions are stable under physiological conditions, at pH close to the pH of blood (7.4) and to the plasmatic isoosmolarity, while preserving USPIOs biocompatibility and limiting their aggregation state, of prime importance when nanoparticles are administered via intravenous injection. Moreover, in order to complex the negatively charged genetic material, the polymer coated USPIOs have to be positively charged at physiological pH.

A further challenge concerns their synthesis: with the perspective of producing reasonable quantities of final product at relatively low cost, a one pot procedure starting with commercially available low priced reagents is necessary. Until now, examples of efficient one pot synthesis at room temperature of polymer-coated USPIOs that are stable under physiological conditions are scarce, due to the difficulty of finding appropriate synthetic conditions and stabilisators. This type of synthesis brings also the problem of preserving appropriate magnetic properties by controlling the homogeneous growth of the iron oxide crystals, which is influenced in many cases by the presence of these (macro)molecules.

In this contribution, we are presenting an efficient one-pot synthesis of polymer-coated USPIOs, stable in water at physiological pH, positively charged (characterization by zeta potential ζ>+30mV), also stable in PBS, with a limited aggregation state (diameter<100 nm) and a narrow size distribution (characterization by DLS and TEM). Some preliminary results reporting on their magnetic properties, as well as their biocompatibility (cytotoxicity tests), will also be presented. This constitutes the first step towards polymer-coated USPIOs for image-guided nanoparticle-mediated cancer gene therapy.

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Novel Magnetic Particles for Bioassays

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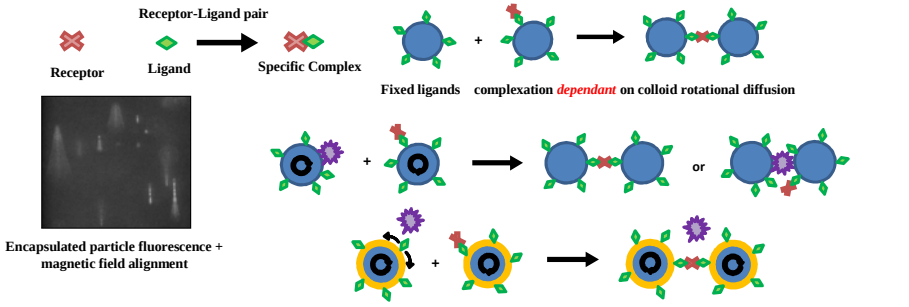
The colloid based Agglutination assay has long been amongst the most simple and efficient of biological assay techniques. Agglutinating colloids combined with measurement of optical density allow rapid determination of low concentrations of analyte. More recently magnetic agglutination assays (incorporating superparamagnetic materials into the colloid) have overcome the problem of reduced particle interaction at low particle concentration as particles may be aligned under a magnetic field.

Agglutination assays are once again at their limits of sensitivity; ligands in fixed positions at the colloid surface ensure doublet formation is the limiting step, dependent on the rate of Brownian rotational diffusion. Furthermore, non-specific interactions give rise to false signals as material binding non-specifically to the colloid surface may also result in particle aggregation.

In this project we explore the ability for fluid interfaces to overcome the limits of rotational diffusion and to protect the surface from non-specific aggregation. Ligands embedded in a fluid interface are free to move independently of Brownian diffusion of the entire colloid particle, effectively negating the restraint of rotational diffusion. The interface may also be developed in such a way as to reduce interactions with species other than the target. Two distinct routes are utilized; Phospholipid bilayers adsorbed to magnetic core silica colloids and ferrofluid-based emulsions. Particles can be visualized using fluorescence microscopy and the fluid layer can be differentiated from unencapsualted particles using fluorescent membrane components. Ligand and receptor functionality will be modeled using the biotin-streptavidin specific complex.

A range of techniques will be used to probe interface fluidity, including Fluorescence recover after photobleaching (FRAP) and magnetic chaining techniques combined with measurements of optical density. Non-specific binding can be measured using fluorescently labeled proteins or more simply turbidimetric measurements.



Poster 35

Investigations on a branched tube model in magnetic drug targeting – systematic measurements and simulation

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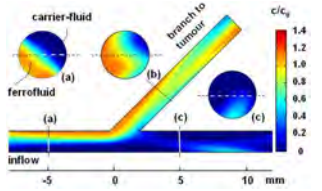
Magnetic drug targeting has been established as a promising technique for tumour treatment. Due to its high targeting efficiency unwanted side effects are considerably reduced, since drug-loaded nanoparticles are concentrated within a target region due to the influence of a magnetic field.

In order to contribute to the understanding of basic phenomena experiments on a half-Y-branched glass tube model as a model-system for a blood vessel supplying a tumour were performed. As a result of measurements, novel drug targeting maps, combining e.g. the magnetic volume force, the position of the magnet and the net amount of targeted nanoparticles were presented. In a first targeting-map [1], which summarizes results for 63 magnet positions, the concentration of the injected ferrofluid is 2.95vol%. Up to 97% of the nanoparticles were successfully targeted into the chosen branch; however, the region where yield was considerable is rather small. A high concentration of injected ferrofluid brings the danger of accretion in the tube. It is shown that an increase in magnetic volume force does not necessarily lead to a higher amount of targeted nanoparticles. In a second targeting-map [2] the concentration of injected ferrofluid is reduced to 0.14vol%. At a first glance the result with low concentration is promising, since the danger of accretion is avoided. Nevertheless, one has to consider, that, unless the magnetic volume force in the branch-point was provided in the necessary strength, an application would not be successful.

The current focus is a finite-element simulation based on the considered setup and artery-model. The fluid flow is described by the Navier-Stokes equations, the magnetic field is derived from Maxwell's equations and mass flux is given by the advection-diffusion equation. The magnetic volume force acting on a volume of magnetic fluid combines the magnet and the ferrofluid data and is proportional to the field dependent magnetisation and the gradient of the field strength. The diffusion equation additionally allows the implementation of a concentration-dependent magnetic volume force.
Our experimental investigations mentioned above and [3] have shown that the miscibility of even the water-based ferrofluid and water is low. Since in medical applications the ferrofluid will be injected close to an appropriate junction one cannot assume a homogeneous mixture approaching the branch-region.



Therefore, the main focus of the presented simulation is a model where at the point of injection the ferrofluid and the carrier-fluid are initially separated and further mixture occurs due to diffusion and the velocity-field.
The figure shows the dimensionless concentration c/c_0 in a cut-plane through the centre of the 3D artery model. Three profiles that are close to the branch show that due to the magnetic volume force the ferrofluid is attracted into the branch leading to the target region.

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Poster 36

Susceptibility and hyperthermia studies of fine magnetic particles

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The origin of the heating for magnetic hyperthermia in nanoparticles is still not clear [1, 2]. There are reports in the literature that the effect at frequencies around 100kHz is due to Néel relaxation of the nanoparticles [1] and also reports that the heating is due to Brownian rotation of the particles [2]. Either of these effects can give rise to a susceptibility loss which will generate heating in the system. Recent studies have shown that in the low fields used hysteresis loss is likely to make a small, if any, contribution to the heating [3].

In this paper we consider the heating mechanism by comparison of the heat generated with AC susceptibility studies as a function of frequency. From these studies shown in figure 1 we conclude that the heating effect arises from a combination of Néel and Brownian relaxation. The interpretation of the data is complex but it is clear that for certain systems a single mechanism is responsible for the heat generation whereas in others two separate

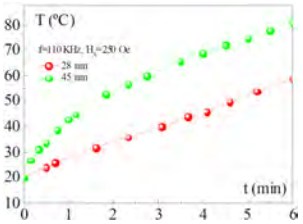

Figure 1. Variation of temperature with time for samples of different sizes at a set frequency of 110kHz and a field amplitude of 250 Oe.

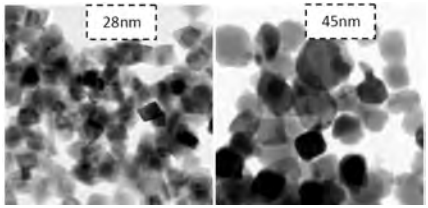

Figure 2. Typical TEM images for the samples studied in this work.

mechanisms are evident. For the sample with median diameter 28nm a linear heating effect is observed. For the sample with D = 45nm the non-linear behaviour indicates two mechanisms. We believe this is due to the nature of the relaxation which gives rise to the heating effect.

This effect is not as expected from initial basic studies [1]. However it is often not realised that particles having diameter greater than about 15nm are invariably clustered together in either primary aggregates or secondary aggregates. Such aggregates are known to reverse by Brownian relaxation hence distorting the results from what is expected from simple calculations. In the full paper data will be presented for identical systems in both solid and liquid form where the reversal mechanism can be identified clearly. Examples of the typical aggregated systems which have been studied are shown in the TEM images in figure 2.

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Magnetic Nanoparticles and Self-Assembled Magnetic Microspheres for Magnetic Separation: Preparation, Characterization, and Application

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Hybrid nanocomposites, e.g., silica-encapsulated nanomagnets have attracted a lot of interest for magnetic separation processes, e.g., in biomedicine or catalysis. As compared to the conventional iron oxide particles, reports on silica-encapsulated superparamagnetic metal particles like Co, Fe, or Ni are more 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 control particle nucleation and growth but also to provide reactive siloxane groups on the particle surface as functional interface for further deposition of oxides (e.g., SiO_2 and TiO_2).[1] Co nanoparticles with short-chained aminoalkyl siloxanes (e.g., 3-aminopropyl triethoxysilane) initially assemble into mesoscale spherical particles (up to 0.7 µm, Fig.1), maintaining the superparamagnetic character of the individual nanoparticles. Individual nanoparticles could be stabilized colloidally by adding further surfactants. Size, crystal structure, and magnetic properties of the particles were characterized by TEM, REM/EDX, DLS, XRD, AES-ICP, and magnetic measurements. The influence of the reaction parameters such as reaction time, temperature, nature of siloxane on the assembly of mesoscale particles was further investigated by REM and DLS. The assembled mesoscale particles were additionally coated with a 30 nm-thick SiO_2 layer by using a sol-gel technique. The resulting superparamagnetic nanocomposite microspheres Co@SiO_2 are remarkably stable in air and water and reveal beneficial magnetic properties, thus opening up interesting possibilities for biomedical applications (e.g., for magnetic separation). We further show the application of Co@SiO_2 microspheres for the magnetic separation of catalysts with respect to sustainable process management. Therefore, a catalytically active compound, in this case a homogeneous Rh catalyst, was immobilized on the superparamagnetic microspheres. The activity, selectivity, and magnetic recycling of the immobilized Rh complex are shown for hydroformylation reactions using 1-octene as model substrate.

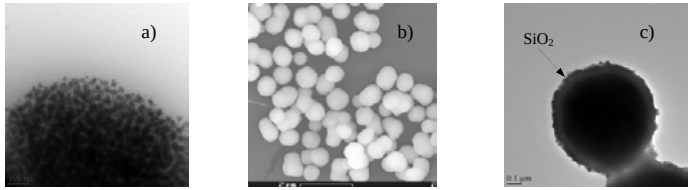

Fig. 1: TEM (a) and REM (b) images of self-assembled Co nanoparticles. TEM image of a Co@SiO_2 microsphere (c).

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An Easy Assay for Measuring the Sheelding of Iron Oxide Cores by the Nanoparticle Shell

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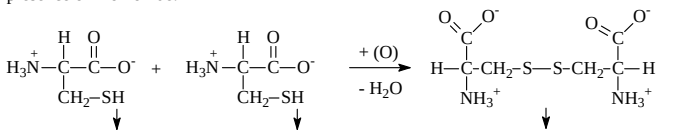
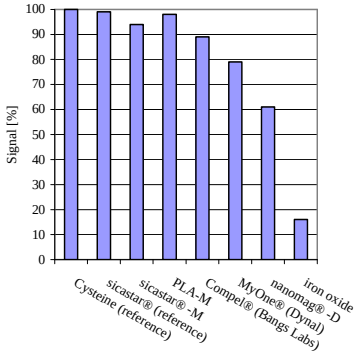
Many applications of magnetic nanoparticles require an intact coating of the iron oxide core to prevent any redox-sensitive interactions with the biochemical environment. Especially the activity of enzymes can be significantly influenced by conjugation to magnetic nanoparticles with an incomplete coating of the iron oxide. Gao et al. described this oxidation activity of Fe_3O_4 nanoparticles with different coatings and used its peroxidase-like effect for the design of an immunoassay.[1]
Several methods for the characterization of the particle surface are based on redox reactions, that are influenced by free iron oxide surfaces. Thus the protein binding capacity of magnetic nanoparticles is very often measured with the BCA or Bradford assay, that can easily be disturbed by non-sheelded iron oxide.
To measure the coating quality of magnetic particles we have developed a quick semi-quantitative assay to compare the oxidative action of different particle types. This assay is based on the formation of disulfide bridges between thiol groups of cysteine molecules in the presence of iron oxide:



The better the iron oxide core is sheelded by the coating material, the lower is the degree of this transformation. The cysteine concentration is measured before and after interaction with a standard amount of magnetic particles with Ellman's reagent. The cysteine concentration is nearly not influenced by incubation with "white" silica particles (sicastar®), magnetic silica particles (sicastar®-M) and poly(lactic acid) particles with encapsulated iron oxide (PLA-M). The oxidation power increases from magnetic polystyrene particles (Compel® and MyOne®) to dextran coated iron oxide (nanomag®-D) and leads to > 80% disulfide formation for pure iron oxide.
Finally this easy assay can be used to develop an intact coating of iron oxide nanoparticles and to asses the potential of magnetic particles for conjugation of redox-sensitive molecules.



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Antibody Conjugation to Magnetic Nanoparticles by Strain-Promoted Alkyne-Azide Cycloaddition

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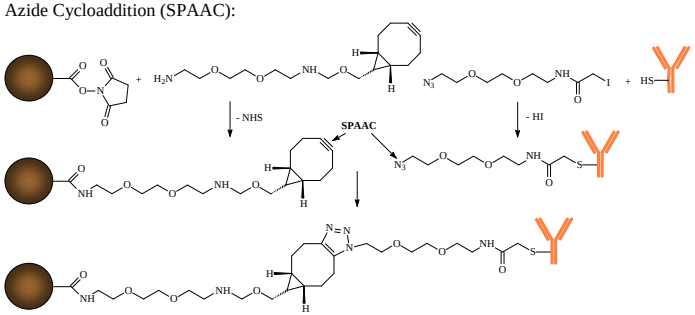
The site-specific conjugation of antibodies to the surface of magnetic nanoparticles is the key for particle-targeting applications in diagnosis and therapy. It was shown that the reaction of maleimide or SPDP functionalized magnetic nanoparticles with thiolated antibodies leads to an efficient binding of antibodies without a significant loss of antibody activity.[1]
A new powerful strategy is the protein conjugation with bioorthogonal chemistry. This method is based on the introduction of an 1,3-dipole, e.g. an azide group, at the antibody molecule and of a bicyclononyne (BCN) on the particle surface for Strain-Promoted Alkyne Azide Cycloaddition (SPAAC):



In contrast to the well-known click reaction, that involves the copper(I)-catalyzed (3+2) cycloaddition of azides and terminal alkynes, the new SPAAC strategy works under mild conditions without any metal catalysis. This is an important advantage, because the presence of copper (I) can severely compromise the bioconjugation of magnetic nanoparticles with biomolecules by denaturation and/or redox processes.
The potential of SPAAC for antibody conjugation on the surface of magnetic nanoparticles was compared with the established conjugation reaction between maleimide modified nanoparticles and thiolated antibodies. Therefore the conjugation efficiency of rabbit anti-goat IgG as model antibody to BNF-starch particles was studied in an already described immunoassay by measuring the binding of goat anti-mouse IgG-HRP in comparison to rabbit anti-goat IgG-HRP.[1] To address this technique for applications in stem cell research, a CD29 antibody was conjugated to BNF-starch particles by both methods. CD29 antibodies bind to the β1-integrin receptor which plays a functional role in many cell types including mesenchymal stem cells. Binding of nanoparticles to integrins allows tracking or functional manipulation of mesenchymal stem cells.

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Effect of the anesthesia on the magnetic nanoparticle biodistribution after intravenous injection

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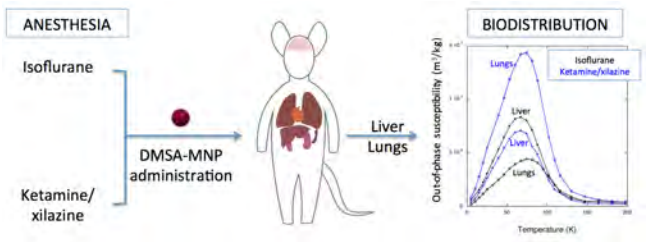
Several factors can influence the biodistribution of magnetic nanoparticles throughout the organism after systemic administration, such as the particle size and composition or the particle coating. We have found that particle biodistribution differs depending on the anaesthesia administered to the animals during the magnetic nanoparticle treatment.

In this project, we compare the amount of particles that reach the liver and the lungs using two different anesthesias: isoflurane (inhaled, 0,5 % in oxygen) and a mixture of ketamine and xilazine (injected). The same amount of monodisperse dimercaptosuccinic acid-coated magnetite nanoparticles (DMSA-MNPs) was injected to the mice. Animals were sacrificed 30 minutes after the particle administration, and the tissues were collected and freezed-dried overnight. The quantitative determination of the magnetic nanoparticles in the tissues was performed by the measurement of alternating current (AC) magnetic susceptibility. The out-of-phase susceptibility component was used as an indicator of the amount of particles in a tissue, allowing the differentiation from other biogenic iron-containing species such as ferritin.

We have found that while the amount of particles that reaches the liver remains similar in both treatments, there is a 3-fold difference between the particles that reach the lungs. It is known that isoflurane produces systemic vasodilation and lowers the cardiac output, although it is still not clear why it changes the particle accumulation just in the lungs and not in the liver tissues.

These results show that there is a clear influence of the anaesthesia used during magnetic nanoparticle administration in the particle biodistribution. Therefore, it will be fundamental to take this parameter into account when comparing different studies on the biodistribution of magnetic nanoparticles.



Poster 41

The torsional properties of proteins studied by magnetic particle actuation

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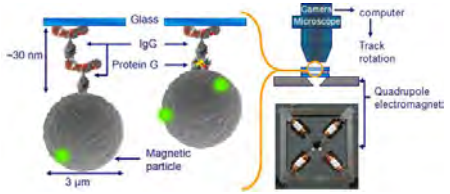
In this study we explore the application of magnetic particles for rotational deformation of proteins and protein pairs, in order to extract their response to the application of torque. In literature, the mechanical properties of proteins have been studied by applying linear forces with AFM, optical tweezers and magnetic tweezers.[1] The application of torque to biological systems has been focused on studying the mechanical properties of nucleic acids and the activity of enzymes attached to DNA.[2]

To study the twisting of proteins, a novel technique based on magnetic particle actuation is employed.[3] The technique uses an electromagnet that generates an in-plane rotating magnetic field to control the torque upon superparamagnetic beads. In order to visualize the angular orientation of the magnetic beads, small fluorescent particles are attached to the surface of the magnetic bead. The rotation of the magnetic beads is observed with an optical microscope and recorded with a camera. See Figure 1. Single molecule resolution is reached by diluting the protein surface coverage of both particle and substrate.



We have investigated two different protein complexes, namely the protein G — (mouse) IgG antibody complex and an (anti-mouse goat)IgG — (mouse)IgG complex. Upon rotational actuation, we observe that the protein complexes behave as a torsional spring and inhibit the rotation of the magnetic particles in the magnetic field. From these experiments we obtain the stiffness of the molecular complexes. The torsional spring constant of the protein G-IgG complex was found to be $3.5\pm1.5\times10^{-18}$ Nm and for the IgG-IgG complex the torsional spring constant is $5.5\pm2.5\times10^{-19}$ Nm (a factor six lower). The differences between these values are attributed to the structural properties of the protein complexes.

Our results pave the way for investigations on the biological activity of proteins upon conformation changes induced by rotational deformation. Furthermore rotational deformation can be investigated as a method to differentiate between bond types in biosensing assays.

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Poster 42

Cytotoxicity of intracellular nanoparticle-mediated hyperthermia depends on cell number

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Heat generated by magnetic nanoparticles in alternating magnetic fields (AMF) shows great promise for cancer therapy. It has been hypothesized that intracellular nanoparticle heating may enable successful micro-scale metastatic cancer treatment. Here, we use human DU145 prostate cancer cells containing intracellular magnetic nanoparticles as an experimental model to evaluate this hypothesis. We measure the dynamic surface temperature of AMF-treated pellets containing variable numbers of cells. We compare maximum measured surface temperatures to those predicted by a simple heat diffusion model that does not account for intercellular thermal barriers. The measured temperatures were consistent with those predicted by this model. For a given intracellular iron concentration, a critical minimum number of cells was required for cytotoxic hyperthermia. Above this threshold, cytotoxicity increased with increasing cell number. These results confirm the prediction that effective heating of a single magnetic nanoparticle containing cell is presently unachievable and suggest a minimum tumor volume threshold (~ 1 mm^3) below which nanoparticle-mediated heating is unlikely to be effective as the sole cytotoxic agent.

Surface charges influences the protein adsorption kinetics, colloidal stability and subsequent cell interaction of polymer coated SPIONs *in vitro*

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Recently, it has been reported that the adsorption pattern of proteins to the surface of nanoparticles (NPs) is strongly dependent upon the specific physicochemical characteristics of NPs (*e.g.* size, shape and charge). In the case of systemically applied NPs, it is hypothesized that any cellular response is a direct reflection of the adsorbed biomolecule layer to the surface of the NP, rather than to the NP itself. Furthermore the NPs may aggregate and change their physicochemical characteristics when suspended in biological fluid, which additionally impacts upon their interaction with cells.
The aim of this study therefore was to investigate the influence of surface charge of neutral, positively and negatively charged polyvinyl alcohol (PVA) coated superparamagnetic iron oxide NPs (SPIONs) on the specific protein adsorption pattern and kinetics and their colloidal stability and how this determines subsequent cellular interaction *in vitro*. To study the interaction with the proteins present within biological fluid all PVA-SPIONs were incubated with a fetal bovine serum (FBS) solution for both 1h and 16h in a cell-free environment. Adsorbed proteins were eluted using a novel magnetic fixed bed reactor and identified using sodium dodecyl sulphate polyacrylamide gel electrophoresis (1D SDS-PAGE) and mass spectrometry (LC-MS/MS) techniques. Colloidal Stability of the different surface charged PVA-SPION within biological fluid was investigated via turbidity measurements by means of UV/Vis. The ability for each PVA-SPION type to enter HeLa cells was assessed after 1, 6 and 24h by Fe quantification and confocal laser scanning microscopy (CLSM). 1D SDS-PAGE showed that the kinetics and amount of adsorbed proteins change dependent upon the specific surface charge of the PVA-SPIONs, but also revealed that albumin and other abundant serum proteins have a high affinity towards all PVA-SPION surfaces, irrespective of surface charge. Due to the high affinity of albumin, LC-MS/MS detected only minor differences in level of other adsorbed proteins, such as complement proteins or coagulation factors. In contrast to the similar proteins adsorbed to the different charged PVA-SPIONs, the cellular Fe content revealed a higher cell up take of the positive charged PVA-SPIONs than for the neutrally and negatively charged PVA-SPOINs. Additionally Static Light Scattering (SLS) measurements within the tested fluid showed a lower colloidal stability for the positively charged PVA-SPIONs compared to the neutrally and negatively charged PVA-SPIONs. The results of this study clearly show that a difference in surface charge can cause a modification in the protein adsorption kinetics and colloidal stability of PVA-SPIONs in biological fluid and affects subsequent cellular interaction *in vitro*.

An Easy and Simple method in manipulating temperatures for various sizes of magnetic nanoparticles on hyperthermia applications

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In this report, an easy and simple method was developed to manipulate the temperatures for magnetic nanoparticles. This method includes a design of circuit to generate an ac magnetic field and the way to manipulate the temperature by applying such ac magnetic field during hyperthermia operation. For electronics, a tunable capacitor was introduced to reduce the total impedance of the circuit system, and hence a magnetic field of hundreds Oe at hundreds of kHz could be easily obtained. On the other hand, the raising rate of the temperature could be changed by varying the amplitude of the magnetic fields. In ours system, the heat treatment temperature for hyperthermia could be easily reached from 37 ^{o}C to a fixed temperature ~50 ^{o}C within 4 minutes, rather than several tens of minutes for conventional method. For better control of the operational temperature, the investigation of heating for a series of different sizes of magnetite particles was also performed.

Patterned Nano-magnet on-Chip for Screening Circulating Tumor Cells in Blood

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Circulating Tumor Cells (CTCs) are considered as potential indicators for prognosis and clinical management. However, detection of CTCs has become very challenging due to its rare number (1:10^{7}-10^{9}). We have previously developed a microchip-based immunomagnetic screening system for the isolation of CTCs from whole blood [1,2]. Here we report patterned metallic nanoscale magnetic thin-film on the substrate that locally enhances the magnetic field and further improve the system's capture capability.

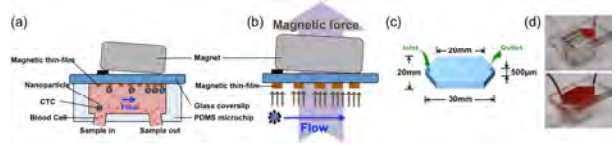

Figure 1. (a) Device schematic. (b) Locally enhanced magnetic field by thin-film magnets, which is magnetized by permanent magnets placed on top of the microchannel. (c) Dimension of the microchannel. (d) Experimental setup of blood filling the microchannel.

Figure 1(a) illustrates the CTC screening system. A polydimethysiloxane (PDMS)-based microchannel is fixed on a 150μm thick glass cover slip. The glass slide serves as a substrate to capture target cancer cells. CTCs are labeled with magnetic nanoparticles (100nm in diameter, Ferrofluid^{TM}, Veridex, LLC), which are conjugated with anti-EpCAM that are able to specifically bind to the target CTCs. As the blood flows through the microchannel, nanoparticles-labeled CTCs are captured because of the magnetic force induced by the designed permanent magnets and thin-film magnets (Figure 1(b)). Figure 1(c) shows the dimension of the microchannel. Figure 1(d) shows the experimental setup of the blood flowing into the microchannel.

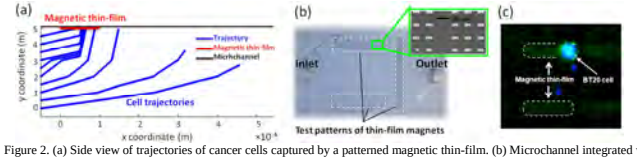

Figure 2. (a) Side view of trajectories of cancer cells captured by a patterned magnetic thin-film. (b) Microchannel integrated with test patterns of thin-film magnets. (c) Cancer cell BT20 captured directly on one of the patterned thin-film magnet.

According to the simulation results, the magnetic field intensity is increased by 10 times locally near a single thin-film magnet. Using an algorithm we have developed, we are able to calculate the CTC trajectories under the influence of the magnetic field introduced by the coating and magnets (Figure 2(a)). The cells undergo stronger capture forces when they come close to the thin-film, and are captured around it. Figure 2(b) shows top view of the PDMS microchip bonded with cover slip patterned with thin-film magnets. Arrays of thin-film magnets are photolithography patterned and thermally deposited on the cover slips. Dimension of a thin-film magnet is 8μm by 24μm by 200nm. BT20 (human breast cancer cell line) is used to test out new design. Figure 2(c) shows an example that a cancer cell is captured on a patterned thin-film magnet. Metallic thin-film magnets uniformly distribute cancer cells and free nanoparticles on the substrate and achieve high capture rate of 85% for the sample spiked with Colo205 (Colon cancer cell line).

In conclusion, we developed a unique design utilizing nanofabrication techniques to pattern the microchannel with metallic thin film. After being magnetized, the thin film can enhance the local magnetic field, providing stronger capture force for CTCs. The consistency of the simulation and experimental results show great promise of such microchip for high throughput screening systems for cancer prognosis and personalized therapy.

Reference

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Magnetic nanoparticle hyperthermia: Magnetic requirements for non-invasive cancer therapy

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Magnetic iron oxide nanoparticles (MIONs) can generate heat when exposed to alternating magnetic fields (AMF), making them a promising platform for cell-specific cancer therapy. The amount of heat deposited in tissue depends upon AMF conditions, and on MION concentration and their magnetic structure. Non-specific tissue heating constrains the choice of AMF amplitude and frequency. MIONs having mean diameter ≤100 nm that produce >80 Watts/g Fe at low amplitude (<20 kA/m) and low frequency (<200 kHz) are needed. Methods to accurately measure heat output are also required.

We describe methods to synthesize magnetic iron oxide nanoparticles having magnetic properties that are tuned for various hyperthermia applications. Also described are methods to accurately measure heat output using a custom four-turn, copper, helical solenoid (7.5 cm OD × 13.5 cm L) that produces uniform (± 10%) AMF (≤100 kA/m) in a cylindrical volume (Figure 1). Particle heating, or specific loss power (SLP) of four nanoparticle samples was estimated from sample specific heat capacity, iron concentration, and measured heating rate. Three commercially available dextran-MION solutions having mean diameter <100 nm were identified and tested: Ferridex® (an MR contrast agent), Nanomag-D® SPIO (SPIO), and Bionized NanoFerrite W1(BNF-W1). A custom 70-nm citrate-stabilized MION (JHU-MION) formulation was also tested. SLP data are reported to 95% C.L.

SLP of the MIONs at 16 kA/m and ~150 kHz was: JHU-MION 113 ± 2 W/g Fe; BNF-W1 22.6 ± 0.3 W/g Fe; SPIO 60.5 ± 0.4 W/g Fe; and, Feridex® 2 ± 1 W/g Fe.

We document the results of heating experiments using both cell culture and animal models of human cancers using the different formulations.

Devices for generating uniform alternating magnetic fields for non-invasive cancer therapy

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The potential benefits of hyperthermia for cancer therapy, particularly metastatic cancers, may be realized by heating systemically delivered targeted magnetic nanoparticles in alternating magnetic fields (AMF). The total heat deposited depends upon the concentration of nanoparticles and their heat output, which in turn depends upon AMF conditions. Thus non-invasive hyperthermia with targeted magnetic nanoparticles requires development of devices that produce homogeneous fields capable to treat significant regions in order to ensure predictable and consistent heating throughout the treatment volume. This requirement presents challenges to the design and manufacture of induction systems because a homogeneous and high flux density AMF at high frequency must be created in a relatively large volume. Additional challenges are presented by the requirement that the inductor must maintain an operating temperature between 35 °C and 39 °C with continuous duty operation for more than one hour. We present results of simulations and tests of a) a modified Helmholtz inductor for cell culture; b) a modified solenoid for small animals; and, c) a co-planar loop scale clinical prototype.

Finite-element analysis simulation methods were used to optimize electrical and thermal parameters of each inductor by comparison with desired field specifications. The target volume of maximum homogeneous AMF for each inductor was: A) modified Helmholtz – max. field of 32 kA/m ± 10% with rectangular volume 80 mm width × 120 mm length × 20 mm height, for standard cell-culture plates (e.g. 96-well); B) modified solenoid – max. field of 100 kA/m ± 10% with cylindrical volume 50 mm diameter × 60 mm length; and, C) Co-planar loop – max. field of ~40 kA/m ± 10% with cylindrical volume 200 mm diameter × 100 mm length. All inductors were constructed from oxygen-free, high-conductivity copper by AMF Life Systems, LLC. Both Helmholtz and solenoid coils incorporated high permeability material (Fluxtrol®, Fluxtrol, Inc., Auburn Hills, MI) to enhance field uniformity in the target volume. Water cooling paths were designed to optimize cooling of inductor components, with additional temperature regulation of sample environments. Magnetic fields produced by the each inductor were measured using a commercial magnetic field probe (AMF Life Systems).

All coils demonstrated max. AMF flux intensity to within 10% of desired specifications, and maintained flux homogeneity as specified with temperature control of both environment and components permitting continuous operation. Presented also are results obtained from cell culture and animal experiments validating inductor performance with magnetic nanoparticle formulations.

Composition- and Phase- Controlled High-Magnetic-Moment Fe1-xCox Nanoparticles for Biomedical Applications

Ying Jing, Shihai He and Jian-Ping Wang

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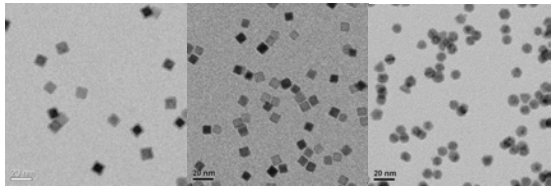
There are growing interests in high-magnetic-moment nanoparticles for biomedical applications because they are able to provide larger signal than traditionally used ferrite nanoparticles[1,2]. Fe-Co occupies the high end of saturation magnetization among alloy materials. When FeCo nanoparticles are used in biosensing for example, it is predicated that they can provide 6-10 times higher signal than that of iron oxide nanoparticles at small field[3,4]. The awareness of the benefits encourages people to explore high-magnetic-moment nanoparticles. In this work, Fe-Co nanoparticles were fabricated and investigated with varying composition and phase.

Physical gas condensation method was employed to synthesize Fe-Co nanoparticles of atomic composition ratio 70:30, 40:60 and 10:90. Optimal uniformity was given by controlling magnetic field on the surface of the target and the sputtering gas pressure. Crystal structure of nanoparticles evolves from BCC to FCC as the concentration of Co increases. Magnetic characterization by SQUID revealed correlation between stoichiometry and anisotropy and exchange bias. It was also found that magnetic behavior at room temperature was much influenced by interactions among nanoparticles arising from this particular way of deposition.



TEM images of $Fe_{70}Co_{30}$, $Fe_{40}Co_{60}$ and $Fe_{10}Co_{90}$ Nanoparticles

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Magnetic Properties of Magnetic Multi-Core Particles

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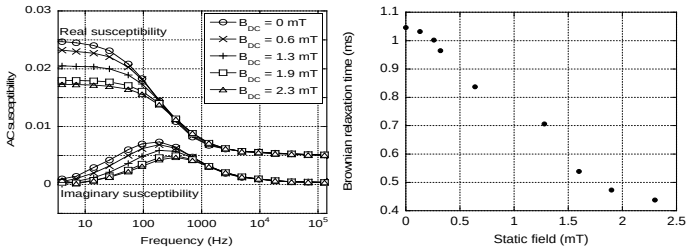
Magnetic multi-core particles consist of several single-domain magnetic nanocrystals geometrically positioned in different types of configurations, for instance densely or loosely packed inside the particle volume, or on the surface of the particles. Dependent on the size distribution of the magnetic nanocrystals inside the particle as well as the geometrical configuration of the nanocrystals, different types of magnetic behavior can be obtained [1]. Magnetic multi-core particles are used in many applications, for instance in magnetic separation of biomolecules and in several magnetic biodetection schemes [2, 3]. In this study, we will investigate the behavior of magnetic multi-core particles when varying the average size of the particles (and thereby the number of single-domain nanocrystals inside each particle) and compare the results with results obtained for particles consisting of only one single-domain magnetic nanocrystal, i.e. single-core particles. In detail we will focus our study on the effective magnetic moment of the particles, which is a quantity of significant importance for many low-field magnetic biodetection methods using magnetic multi-core particles. We will also address the dynamic magnetic properties of the magnetic multi-core particle systems (using the DynoMag system developed at Imego) as well as the static magnetic properties (using a PPMS system from Quantum Design) in the whole field range up to magnetic saturation.



(Left) AC susceptibility versus frequency of a magnetic multi-core particle system (130 nm particle diameter from Micromod, containing magnetite single-domain nanocrystals). The different curves represent AC susceptibility at different superimposed DC magnetic fields applied perpendicular to the AC field. The peak in the imaginary susceptibility is due to Brownian relaxation. (Right) Median values of the Brownian relaxation time versus the superimposed DC magnetic fields. The results resemble well the theoretical predictions presented in ref [4] and will be used to determine the effective magnetic moment of the multi-core particles.

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Thermal properties of magnetic nanoparticles modified with polyethylene glycol

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Magnetic fluids used in biomedicine have to be biocompatible and therefore the magnetic nanoparticles are modified by different biocompatible materials. In the work the magnetic nanoparticles Fe_3O_4 sterically stabilized by sodium oleate were prepared by coprecipitation method. Consequently they were modified with polyethylene glycol (PEG) with different PEG to magnetite Fe_3O_4 weight ratio *x* varying from 0.5 to 30 to produce biocompatible magnetic fluid (MFPEG). The addition of PEG to an oleate-stabilized magnetic fluid may cause considerable structural changes. The micelle formation involving free sodium oleate was observed in the magnetic fluids [1]. *In vitro* toxicity of the magnetic fluids using cells of skin cancer of mice B16 was also tested. It was found that a magnetic fluid containing PEG partially inhibited the growth of cancerous B16 cells at the highest tested dose (2.1 mg/ml of Fe_3O_4 in MFPEG). Morphology and particles size distribution were observed by scanning electron microscopy. Mean hydrodynamic diameter of prepared coated nanoparticles was found to be at 73 nm whereas the unmodified nanoparticles had the diameter of 58 nm. Thermoanalytical methods (differential scanning calorimetry (DSC) and thermogravimetric analysis (TGA)) were used to study the adsorption of PEG on magnetic nanoparticles and determine the optimal concentration of PEG needed to modify magnetic nanoparticles.

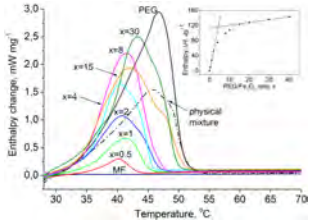

Fig. 1. DSC traces of PEG-modified magnetic fluid MFPEG with different PEG/ Fe_3O_4 weight ratio *x* and of pure components as well as. The insert shows the relationship between the weight ratio *x* and the melting enthalpy of PEG.

Typical DSC traces can be seen in Fig. 1. The melting temperature of pure PEG (M_w = 1 000 g.mol^{-1}) was about 47°C and the temperature was not changed for the physical mixture consisting of the same components of PEG and MF. However, in the system of adsorbed PEG on MF, the melting temperature was shifted to a lower temperature of about 41°C. In the case of higher amounts of PEG in MF (weight ratio PEG/Fe_3O_4>8), not all PEG was adsorbed on magnetic nanoparticles and as a consequence, the split melting peak was appeared indicating the presence of non-adsorbed PEG on magnetic particles. The optimal concentration of PEG needed to modify magnetic nanoparticles was found to be 4.5 determined from the relationship between the PEG/Fe_3O_4 weight ratio and the melting enthalphy of MFPEG (as the insert of Fig. 1. shows). Adsorption isotherms for the coverage of PEG on magnetite nanoparticles were constructed. The results indicate that the prepared biocompatible sodium oleate–magnetite nanoparticles are coated by PEG and could be effective in magnetic drug targeting.

[1] M. V. Avdeev, A. V. Feoktystov, P. Kopcansky et al.: J. Appl. Cryst. 43, 959 (2010)

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Efficient Transfection method using the Magnetic nanoparticles and Episomal vector

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Magnetic nanoparticles (MNPs) are great potential for cell reprograming technology as a gene delivery system, such as the induction of induced pluripotent stem cell (iPSC). Recently, the researchers reported the iPSC production by nonviral cell reprogramming method using episomal vector. An episomal vector is the nonviral autonomous replicon that was constructed for mammalian cells. However, the transfection efficient of episomal vector is usually low using transfection reagent such as cationic polymer, polyethylenimine (PEI).

To solve this problem, we attempted the development of the MNPs (gamma-Fe_2O_3) for high efficient gene delivery sytem using episomal vector and MNPs. MNPs were well coated with deacylated PEI (PEI max, Polysciences, Inc.) and were highly dispersed in PEI solution (1 mg/ml) or deionized water. The characteristics of PEI coated MNP (PEI-MNP) were as follow; the secondary size: 121.32 ± 27.36 nm, the zeta-potential: +45.53 mV in PEI solution (1 mg/ml), and +30.05 mV in deionized water. Furthermore, the PEI-MNPs were able to introduce episomal vectors and express the mRNA of the target gene (enhanced green fluorescent protein, EGFP) with high efficiency and long term (for 7 days) in mammalian cells (mouse embryonic fibroblast, MEF) compare with conventional methods using transfection reagent and transient force expression vector.

To optimize transfection condition, we show some factors for higher transfection efficiency. This result suggests the magnetic force applied to the MNPs can enhance the transfection efficiency.

Magnetic Heating of Fe-Co Ferrites: Experiments and Modeling

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Magnetic nanoparticles (MNPs) of Fe-Co ferrites ($Fe^{II}_{1-x}Co_xFe^{III}_2O_4$ with x = 0 ÷ 1) were synthesized using a classical precipitation method. Subsequently, heat treatments were performed at 100-600 °C for 1 hour in air. The average size of the nanoparticles was 8 nm, which did not change significantly when Fe(II) was replaced by Co. The saturation magnetization of the MNPs is ~30-40 emu/g for most samples. The influence of the amount of Co and the heat treatment temperature was non-linear. The specific absorption rate (SAR) was calculated based on calorimetrical data. The sample with 10% Co treated at 400 °C had the highest SAR (Figures 1 and 2).

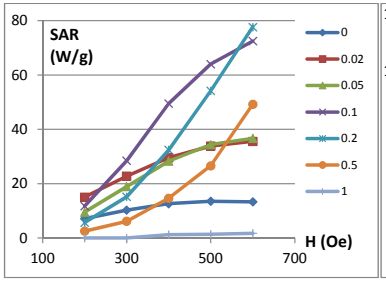

Figure 1. SAR of $Fe^{II}_{1-x}Co_xFe^{III}_2O_4$ MNP with x = 0, 0.02, 0.05, 0.1, 0.2, 0.5 and 1.

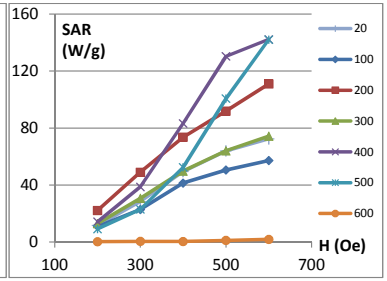

Figure 2. SAR of $Fe^{II}_{1-x}Co_xFe^{III}_2O_4$ MNP with x = 0.1, treated with 20 - 600 °C.

Recent studies have demonstrated that aggregated single domain, superparamagnetic MNPs exhibit multi-domain behavior under an applied magnetic field. In a multi-domain ferromagnet, the domains are strongly couplinged.. To understand the underlying physics of the single domain aggregated MNPs in this work we used three approaches. First, we investigated the interaction between MNPs using a full, three-dimensional electromagnetic numerical model called the "method of auxiliary sources", developed at Dartmouth to solve low-frequency EM induction problems. Inter-MNP coupling constants are determined for differently shaped and sized MNPs at different separations; then the effective magnetic field is estimated as a superposition of the applied and coupling magnetic fields. Second, the magnetization of aggregated MNP is modeled using both a modified Langevin expression and the effective magnetic field. Third, the specific absorption rates are calculated for the aggregated MNPs, and the comparisons between the modeled and observed magnetizations and SARs are analyzed.

This research was supported by National Institute of Health (NIH) grant 1U54CA151662-01.

AUTOMATED MEASUREMENT OF MAGNETOPHORETIC MOBILITY: METHOD AND APPLICATIONS

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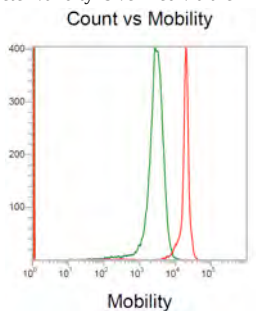
The measurement of magnetophoretic mobility has several practical uses including: Determination of magnetic susceptibility of nanosize and microsize objects, quality control and standardization of magnetic microspheres, evaluation of magnetic resonance imaging contrast agents, prediction and analysis of magnetic bioseparations, and analysis of intrinsically paramagnetic biological cells. Measurements were performed using the Hyperflux™ Velocimeter product of IKOTECH, LLC, which accepts samples in the 0.1 – 10 mL volume range containing 10^3 to 10^6 particles/mL and automatically draws them into a thin stopped-flow cell. Particle migration in a constant-force magnetic field is monitored by dark-field illumination at 30 frames/s using a High Definition monochrome video camera with a low-magnification telecentric lens. A user selectable number of frames is recorded and stored as video data with up to 2,000 events per frame. Frame data are analyzed using proprietary software and stored in list-mode files with 23 parameters per event. The list-mode files are exportable to FCS (Flow Cytometry Standard) datasets containing all stored parameters for up to one million events for display and analysis using standard flow cytometry tools, creating single-parameter histograms of magnetophoretic mobility and 2-D scatter plots of parameters such as size vs. magnetophoretic mobility. All parameters are available for gating the data, and especially useful are aspect ratio, size, track length, and optical intensity. Parameter validity is verified via the software package Magex™, which provides "instant replay" of stored video and graphically correlates the data to the tracked events. Magnetophoretic mobility distributions were determined automatically for 33 commercial magnetic reagents (two shown in the figure) used in bioseparations; mobilities ranged from 10^{-14} to $2x10^{-10}$ $m^3T^{-1}A^{-1}s^{-1}$ with CV's ranging from 25% to 150%. Lot-to-lot variability of magnetophoretic mobility was determined for a small number of commercial reagents. Nanoparticles down to 250 nm diameter were evaluated. Living cells were labeled with biomarker-specific magnetic reagents, and mobilities were determined for the purpose of magnetic sorting. The data have been shown useful for evaluating and optimizing magnetic separations.



This research was supported by U.S. Army SBIR contract # W81XWH-11-C-0017, NIH SBIR grants 5R44HL074606, 3R44DK072647, the State of Ohio Third Frontier Fund, the State of Indiana 21st Century Fund.

Temperature Responsive Hydrogel-Coated Magnetic Nanoparticles

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Abstract

The incorporation of magnetic nanoparticles into hydrogels forming hybrid systems gives additional functionalities to the system and widens the field of potential applications in biomedicine, biotechnology, and other fields. We have developed an effective synthetic route to produce stable, well-dispersed core-shell magnetic nanoparticles-hydrogel for good potential in targeted drug-delivery systems. The miniemulsion polymerization method was adopted to prepare nanospheres of magnetic nanoparticles, Mag NPs, encapsulated in poly-*N*-isopropylacrylamide based hydrogel. In this approach, ~ 9 nm diameter Mag NPs were first synthesized via the thermal-decomposition method using oleic acid as a capping agent. Subsequently, the core-shell structures of aggregated Mag NPs-cores within poly-*N*-isopropylacrylamide-shells were successfully formed by the polymerization of *N*-isopropylacrylamide in the presence of Mag NPs, i.e. via the miniemulsion polymerization method. The morphology, shape, size, and elemental composition of these as-prepared Mag NPs-poly-*N*-isopropylacrylamide composite particles were determined by field-emission scanning electron microscopy, X-ray diffraction,and Fourier-transform infrared spectroscopy. The encapsulation of aggregated Mag NPs within the polymer matrix to form core-shell structures was further confirmed by transmission electron microscopy.

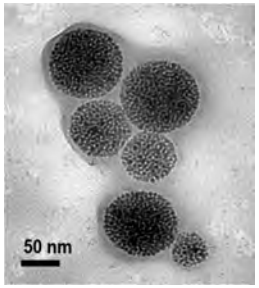

Fig. 1. TEM image of hydrogel-coated magnetic nanoparticles

High Magnetic Moment Nanoparticles for MRI Contrast Enhancement

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Magnetic nanoparticles surface functionalized with biomolecules have been shown to be very promising for clinical applications such as, imaging, therapy, biomarker, sensors as well as targeted drug delivery [1]. For clinical trials, all these applications rely on the magnetization of nanoparticles used so an important challenge is to come up with nanoparticles with high magnetic moments and to be able to limit their quantity that can be used in the body. Nanoparticles of iron and cobalt-iron alloy exhibit much higher magnetic moments, than those of iron-oxide of comparable size, due to their intrinsic properties.

In this work, nanocubes of iron-cobalt alloy have been synthesized using high temperature reduction of organometallic compounds [2]. Oleic acid, oleylamine and tri-octyle phosphine were used to coat particles surface, and to obtain monodispersity and stability. The size and composition can be tuned by varying reaction parameters (time, temperature, reactant and concentrations). The structural and micro-structural properties were characterized using X-ray diffraction and transmission electron microscopy and magnetic properties were studied using a Physical Properties Measurement System (PPMS). To make the nanoparticles biocompatible and water dispersible, pluronic-F127 (PEO-PPO-PEO) triblock copolymer was used for surface modification of nanoparticles via physical adsorption [3] which is known to give a robust double-layer structure over the particle surface where the inner layer is still the original oleate and the outer layer being the hydrophilic copolymer. The hydrodynamic particle size measured using dynamic light scattering technique showed an average increase of 20 nm in the particle size. In-vitro T_2 contrast studies were performed using a 7 Tesla Agilent ASR 310 MRI scanner. Changes in T_2-contrast are evident in the T_2-weighted image (TR=3196 ms and TE=8.31 ms), and show the potential utility of these nanoparticles as negative contrast agents. In vivo studies will be performed to demonstrate tumor contrast and overall biocompatibility.
This work has been supported by USAMRMC-W81XWH10201001.

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Synthesis of $La_{0.75}Sr_{0.25}MnO_3$ Fine Particles for Magnetic Hyperthermia by Ultrasonic Spray Pyrolysis

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Manganese perovskite $La_{1-x}Sr_xMnO_3$ have been receiving attention as a self-regulating hyperthermia heating source because of the ability to adjust its Curie temperature from room temperature to about 100 ºC. Its Curie temperature decreases with decreasing the crystallite size owing to destabilization of the magnetic ordering. We have synthesized $La_{0.75}Sr_{0.25}MnO_3$ fine particles by an ultrasonic spray pyrolysis technique employing nitrate salt. The spray pyrolysis method is regarded as a possible solution to these problems. Its benefits include the fact that (1) it is a relatively short, low-temperature, single-step synthesis process, (2) highly crystalline particles can be produced, (3) uniform control of the particle size on a micron to submicron scale is possible and (4) it involves low contamination levels.

The aqueous solution of lanthanum, strontium and manganese nitrate nitrates was contained in the ultrasonic spray generator. The generated microdroplets were sent to the furnace reactor by air flow at 5.0 L/min and droplets start the evaporation in the reactor. In droplets, metal nitrates concentrate, deposit, thermally decompose and finally form the crystalline particles. The synthesized particles were collected with an electrostatic precipitator. Furnace temperatures were changed from 800 to 1200 ºC.

The synthesized particles were polycrystalline spheres with 529-620 nm in the average diameter. The crystallite sizes increased with the synthesis temperature, and Curie temperature increased with the crystallite size (Fig.1). Figure 2 shows the temporal change of the temperature of the particles suspension when an alternating magnetic field (40 Oe, 1 MHz) was applied. The suspension temperature increased with time and attained to constant values of around Curie temperature of $La_{0.75}Sr_{0.25}MnO_3$.

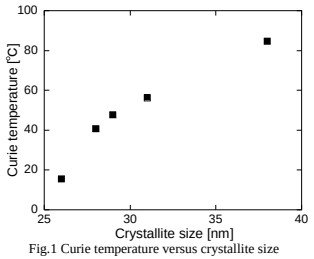

Fig.1 Curie temperature versus crystallite size for the synthesized particles.

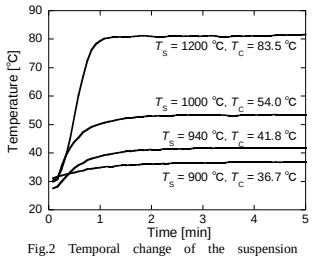

Fig.2 Temporal change of the suspension temperature under AC magnetic field.

Quantification of Magnetic Nanoparticle Uptake in Cells by Temperature Dependent Magnetorelaxometry

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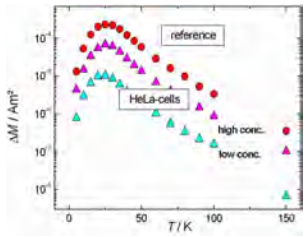
Magnetorelaxometry (MRX) is proven to be a powerful tool for the quantitative detection of magnetic nanoparticles (MNP) utilized in novel medical therapy modalities like magnetic drug targeting or magnetic hyperthermia. So far we adopted MRX measurements at room temperature for in-vitro quantification of MNP in blood and tissue samples. Since the MRX relaxation time crucially depends on MNP size, particles with less than about 17 nm in diameter decay too fast at room temperature and are not detectable within the recording time window of present MRX devices. To circumvent this limitation we performed temperature dependent MRX measurements to demonstrate the applicability of MRX for quantifying the uptake of small MNP in tumor cells.

Two tumor cell lines (HeLa and Jurkat) were incubated with small sized iron oxide MNP, Feraheme (ferumoxytol, AMAG pharmaceuticals, $d \sim 5$ nm) and CD021110 (carboxy dextran coated preclinical MNP, Charité, $d \sim 4$ nm) at varying concentrations for about 30 h, after which samples of about 10^6 cells were harvested. Additionally, reference samples of known iron amount were prepared (dilution series). A conventional SQUID magnetometer (MPMS-XL, Quantum Design) was utilized for temperature dependent relaxation measurements between 5 K and 300 K. First a magnetizing field of 2 mT is applied for 8 min to magnetize the MNP in the sample followed by the recording of the relaxation signals for about 40 min. By normalization of the reference relaxation amplitudes to the amplitudes of the cell samples (see figure) a straightforward quantification was carried out. For cross validation the cell samples and references were analyzed by $M(H)$ measurements using the same device and by non-linear ac-susceptometry using an MPS spectrometer (Bruker BioSpin).



The cell samples incubated with CD021110 displayed a relaxation at decreased temperatures with a maximum relaxation amplitude at about 25 K. For both cell types we quantified an iron uptake in the microgram range scaling with the concentration of MNP during the incubation. In contrast, for Feraheme, no uptake was detected for either of both cell types. These results were confirmed by the $M(H)$ and MPS measurements. From the temperature dependent MRX measurements of the reference samples a detection limit of about 100 ng iron (absolute) was estimated.

The extension of MRX measurements to lower temperatures allows the in-vitro quantification of MNP of smaller sizes, which at room temperature cannot be detected due to the short relaxation times. Furthermore, changes in the particle size distribution during the uptake of the MNP by a biological system can be resolved as a shift in the temperature dependence of the relaxation amplitude. A size specific cellular uptake of MNP can be quantified.

SYNTHESIS AND RELAXIVITY PROPERTIES OF SUPERPARAMAGNETIC IRON OXIDES WITH VARIABLE SIZE AND OXIDATION STATE

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Application of superparamagnetic iron oxide nanoparticles in biology and medicine finds its rapidly developing emphasis on contrast agents for MRI. Superparamagnetic particles shorten the proton relaxation rate via the outer-sphere mechanism due to their high magnetic moment, and they are better known as negative (T2) contrast agents. In so-called positive (T1) contrast agents, the inner-sphere relaxation mechanism is utilized, due to interaction of protons with the high-spin d^5 transition metal ions such as Mn^{II} and Fe^{III}, or more commonly, the f^7 Gd^{III} ions.

There is a noticeably growing effort on the development of T1 contrast agents, based on superparamagnetic iron oxide particles. The motivation is that most of the existing positive contrast agents based on gadolinium chelates, raise substantial toxicity issues. In addition, high mobility of the gadolinium chelates shortens their presence in the vascular system. The best currently known blood pool MRI agents are based on iron oxides and considered non-toxic. It is documented that reducing the particle size below 5 nm causes lowering of their magnetic moment, and therefore lowering the outer-sphere relaxivity $r2$. At the same time larger surface-to-volume ratio of these small particles causes the greater involvement of iron atoms in the spin-lattice relaxation process. Consequently particle size reduction can be the way to obtain better T1 contrast agent.

The goal of this work was to refine the synthesis conditions for superparamagnetic iron oxides, obtain samples with one property changed at a time, and to study how the particle morphology and the oxidation state affect their proton relaxivity properties. We used the same chemistry for synthesis of all nanoparticles, and changed only the reaction conditions such as temperature, time, and concentrations. Reactions of controlled high-temperature hydrolysis on iron(II) and iron(III) chelated alkoxides in solutions of highly-polar parent alcohol (diethylene glycol or tetraethylene glycol) (reaction 1) were performed. Even though no capping ligands or surfactants were used, the obtained nanoparticles remained in colloidal solution stabilized by electrical double-layers of solvated ions. Obtained colloids were characterized by the Dynamic Light Scattering (DLS) and zeta-potential techniques. Relaxivity studies were performed by NMR. Isolated nanoscale solids were characterized by X-ray diffractometry, TEM, TGA and elemental analysis.

$$[Fe(Hdeg)_2] + 2[Fe(Hdeg)_2Cl] + 2H_2O + 2OH^- \rightarrow Fe_3O_4 + 6H_2deg + 2Cl^- \quad (1)$$

$$4Fe_3O_4(H_2deg) + O_2 \rightarrow 4Fe_3O_4(Hdeg) + 2H_2O \quad (2)$$

We synthesized several batches of magnetite colloids with particle size ranging from 3 to 6 nm and tested their relaxivity on OH and CH_2 protons of diethylene glycol. We also synthesized and tested the oxidized iron oxide particles, which was done by oxygenation of the magnetite colloids' aliquots at room temperature with dry oxygen (reaction 2). It was found that proton relaxation time is affected by both, the particle morphology and the iron oxidation state. Details of these studies will be presented.

Asynchronous Magnetorotation Based Biomedical Platforms: From Biomarker Analysis to Rapid Testing for Microbial and Cancer Drug Sensitivity

Raoul Kopelman

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Asynchronous magnetic bead rotation (AMBR) is a new principle for biomedical tests and instrumentation. It utilizes low level magnetic fields, low cost optics, low cost magnetic beads, and works in a droplet of water at room or body temperature. It is also free of fluorescent or other molecular labels. Growth anomalies in single cells are important for biological studies and can also have significant biomedical impacts. For instance, the identification of the proper antibiotics and its optimized dose can be determined by monitoring bacterial cell growth over less than an hour. Similarly, cancer drug chemotherapy effectivity can be determined rapidly by monitoring cancer cell growth anomalies, with embedded magnetic nanoparticles. Sample size requirements are on the order of a single cell. Also, ELISA type determinations can be carried out with no fluorescent labels. AMBR is based on classical chaos phenomena first observed (and avoided) by Faraday. The magnetorotation method utilizes micrometer scale magnetic particles that are rotated by an external magnetic field, or, alternatively, non-magnetic particles, or cells, that are magnetized by magnetic nano-beads. When rotated beyond the "critical slipping frequency", they are asynchronous with the driving field frequency, due to drag. For instance, when bacteria attach to the particle and keep growing there are significant changes in the drag, and thus in the frequency of the asynchronous rotation (e.g. the particle rotation slows down with increasing cell size). Single bacterial cell growth can be monitored, with minimal perturbation, in real time with nanometer precision, i.e. well below the diffraction limit of optical microscopy. In this way, cell growth can be rapidly detected and the time needed to obtain bacterial susceptibility to a given antibiotic can be dramatically reduced, compared to current methods. In contrast to genotypic methods, this method is highly sensitive to drug resistant mutants.

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Monodisperse cobalt-zinc ferrite nanoparticles prepared by thermal decomposition and coated with silica in reverse microemulsion

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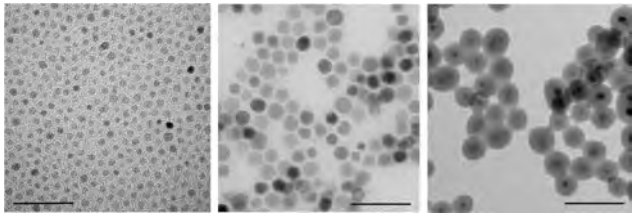
[a]Institute of Physics AS CR, v.v.i., Cukrovarnická 10, 162 53 Prague 6, Czech Republic; [b]Department of Cell Biology, Faculty of Science, Charles University in Prague, Albertov 6, 128 43 Prague 2, Czech Republic; [c]Institute of Clinical and Experimental Medicine, Vídeňská 1958/9, 140 21 Prague 4, Czech Republic; *E-mail: veverkap@fzu.cz

The nanoparticles of the cobalt-zinc ferrite have attracted attention due to their possible use in biomedicine and biological research. Their magnetic properties can be well adjusted, varying their composition and their size, thus reaching the required parameters for a particular application. The precise control of the magnetic behavior can be facilitated employing strictly monodisperse cores, whereas the colloidal stability and applicability in biological environment can be resolved by a suitable shell. In the present contribution, such monodisperse ferrite cores are reported and their silica coated products are presented.

The magnetic nanoparticles of the $Co_{1-x-y}Zn_xFe_{2+y}O_4$ stoichiometry were synthesized by the thermal decomposition of the corresponding acetylacetonates in a high boiling solvent. The shape and size control of the particles was achieved by surfactants and influenced by both the temperature program and concentration of reaction constituents. The silica coating was carried out by the reverse microemulsion method, typically using cyclohexane as the oil phase and tetraethoxysilane as the silica precursor. Final products exhibiting colloidal stability in water were obtained by purification and size fractionation.

According to XRD the prepared spinel phase products possess the mean size of crystallites d_{XRD} = 7 - 21 nm. The comparable size observed by TEM indicated their single crystalline nature. The chemical composition of the cores was analyzed by means of XRF. SQUID measurements showed high magnetization of the samples (e.g. $Co_{0.42}Zn_{0.22}Fe_{2.36}O_4$ with d_{XRD} = 14 nm has $M_{2000kA/m}$ higher than 72 Am^2/kg) and the ZFC-FC study confirmed the narrow size distribution of their cores. For the coated products TEM revealed well dispersed particles with the shell thickness ≈ 10 nm while its silica structure was probed by IR spectroscopy. Further, DLS data were in agreement with the observed morphology. Finally, basic viability tests were carried out using HeLa cells and preliminary relaxometric studies were focused on the possible use of the particles as T_2 contrast and labeling agents for MRI.

The support by the project of the Czech Science Foundation No P108/11/0807 is gratefully acknowledged.



Examples of bare and silica coated cobalt-zinc ferrite nanoparticles possessing different size, the scale bar is 50 nm.

Measuring the Behaviour of Antibody-Functionalised Magnetic Nanoparticles using Quartz Crystal Microbalance

B. Kozissnik[1,2,3], K. L. Vigor[1], B. Tolner[1], G. Bhavsar[1], S. Diez-Posada[1], J. Dobson[3], Q. A. Pankhurst[2] and K. A. Chester[1]

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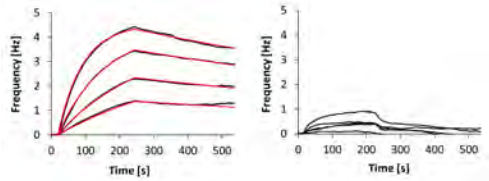
In recent years the focus in functionalising nanoparticles was mainly on the feasibility of specifically targeting them *in vitro* and *in vivo*. Conjugation chemistries and purification techniques have been improved, but still little is known about the conjugates themselves.

We conjugated a recombinant single chain Fv (scFv) specific to the carcinoembryonic antigen (CEA) to Ferucarbotran, also known as MRI contrast agent Resovist®. The conjugates were purified and protein functionality evaluated by ELISA. Taking characterization of the conjugate even further we used quartz crystal microbalance (QCM) - a technique, widely used in material sciences and molecular biology - to measure the interaction between a ligand immobilized on a quartz crystal embedded within a chip and an a corresponding or cognate analyte.

As a non-optical technique, QCM in contrast to surface plasmon resonance (SPR), allows measurement of the binding affinity of the scFv-functionalized nanoparticles in real-time and provides the first measure for the behaviour of the conjugates.

Current and future studies are focusing on applying the knowledge obtained through this technique to target antibody-functionalised magnetic nanoparticles to activate specific biomolecular pathways in cells for tissue engineering & regenerative medicine.



Ferucarbotran-Antibody conjugate binding to the cognate antigen. Interaction profiles of conjugates functionalized with a CEA-binding scFv (A) and a non-CEA binding scFv variant (B). On/off rates were determined against concentrations of antigen ranging from 3.1 - 50 ng/ml using an Attana A100 Biosensor (Attana, SE). Black lines indicate the measured data; red lines represent the best fit to binding curve.

Kozissnik B, Green LAW, Chester KA, and Nguyen TKT. Functionalisation of Magnetic Iron Oxide Nanoparticles. In: Magnetic Nanoparticles: From Fabrication to Clinical Applications (2012). Edited by Nguyen TK Thanh. Publisher: CRC Press Inc., ISBN-13: 9781439869321.

Kozissnik B et al. Characterizing the Target Ability of Antibody Functionalized Magnetic Nanoparticles by Quartz Crystal Microbalance. Submitted to Biomaterials.

Magnetic nanoparticles targeting into specific cells using epidermal growth factor as affinity ligand

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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.

In this work, different types of binding of epidermal growth factor (EGF) 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 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 N-(2-aminoethyl)-3-aminopropylmethyl-dimethoxysilane (APMS) 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, NHS-fluorescein was attached to the nanoparticles' surface. The carboxyl functionalization of the nanoparticles was provided by a ring-opening elongation reaction of the non-reacted surface amines of fluorescent amino-functionalized nanoparticles with succinic anhydride. Finally, epidermal growth factor (EGF) peptides were bound to the fluorescent amino- or carboxyl-functionalized nanoparticles in five different procedures: (i.) using 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC)chemistry (Figure 1), (ii.) using crosslinker molecule bis(sulfosuccinimidyl) suberate (BS3), (iii) using crosslinker molecule bis(succinimidyl)penta(ethylene glykol) (BS(PEG)5), (iv.) using absorption on the amino-functionalized nanoparticles, and (v.) using absorption on the carboxyl-functionalized nanoparticles. The cytotoxicity/viability of the fluorescently labeled nanoparticles was performed using flow cytometry. The size of the functionalized nanoparticles was determined with dynamic light scattering (DLS). Recognition of the epidermal growth factor receptor (EGFR) over expressed on the A431 cells by the EGF-marked fluorescent nanoparticles was studied using flow cytometry. HeLa cells were used for comparison of cellular uptake efficiency since they express low level of the EGFR. The cellular uptake specificity was determined by competitive blocking of EGFR using free EGF. In procedure, where EDC chemistry was used for conjugation of EGF peptides onto the nanoparticles' surface the targeting specificity was significantly improved.

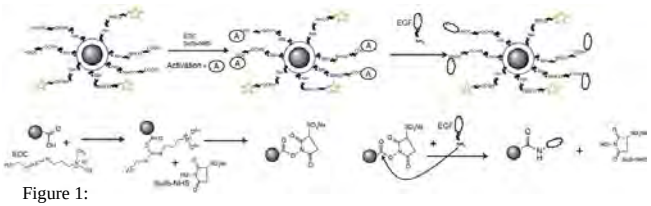

Figure 1:

Poster 63

Inorganic nanoparticles for biomedical applications.

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Attribute to their unique size-dependent optical, electronic, and/or magnetic properties as well as their superior stability under various environmental conditions, nanocrystals are considered ideal for different medical applications. Most nanoparticles with well defined size are prepared by solution based method, in which surface coating serves an important role in stabilizing nanoparticles in biological fluid and in linking affinity ligands or drug molecules for imaging, sensing and therapy. The inorganic nanoparticles very popular in biomedicine, including fluorescent quantum dots and magnetic iron oxide nanoparticles are produced in organic solvent, and then converted to water soluble and biolinkable nanoparticles for the bio-applications. Different surface coating strategies have been developed, such as ligand exchange, hydrophobic interaction and in-situ polymerization. In the meantime, bio-affinity of the affinity ligand on the surface of nanoparticles is also affected by conjugation method, density and orientation of the ligand. This presentation will summarize our recent progress in the synthesis, characterization, surface coating and bioconjugation of inorganic nanoparticles, as well as their biomedical applications as bioimaging contrast agents, sensing probes, and vaccine adjuvant. The technical challenges and possible solutions in these areas are also going to be discussed.

Poster 64

The potential of BSA-modified magnetic fluids in the line of amyloid-diseases treatment

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Magnetic fluids have attracted great attention due to application in many areas such as medical treatment, biosensor techniques or magnetic separations. Recent works report ability of nanoparticles (NPs) to affect protein amyloid aggregation which is associated with amyloid-related diseases. In these applications the magnetic nanoparticles need to be stable in aqueous solutions and the colloidal system should have maximum biocompatibility and biodegradability. This made us prepare and characterize magnetic fluid (MF) modified by bovine serum albumin (BSA) in more details and exam their effect on amyloid aggregation as well.

BSA adsorption on the magnetic nanoparticles was tested as to the function of weight ratio BSA/ Fe_3O_4, temperature and time. Magnetic nanoparticles were prepared by co-precipitation method and stabilized by sodium oleate as surfactant to prevent them clustering. MF modification by BSA (MFBSA) was done by incubating a given concentration of BSA and the MF (30mg Fe_3O_4/ml) to obtain different MFBSAs . The effect of both temperature and time on the BSA adsorption was studied by means of dynamic light scattering (DLS). The results showed that adsorption was rapid and that the reached plateau values depended on the temperature applied during adsorption process (Fig.1). Characterization of the prepared modified nanoparticles was carried out also by scanning electron micrography (SEM) to determine the particle shape, size and morphology. As it is shown in Fig. 2, the particles have roughly spherical shape. DLS technique was used as an additional method to determine the particle size distribution (inset in Fig.2). The data showed significant difference in diameter between the unmodified and BSA coated nanoparticles. To monitor the MFBSAs colloidal stability the temperature transitions were studied through a combination of DLS and AC susceptibility measurements. For all the MFBSA samples in the range of 20-90°C there were no sharp changes in hydrodynamic diameter or in the high frequency susceptibility indicating high stability of the BSA modified magnetic fluid.

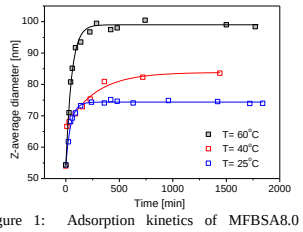

Figure 1: Adsorption kinetics of MFBSA8.0 (BSA/Fe_3O_4 = 8.0w/w) at various temperatures.

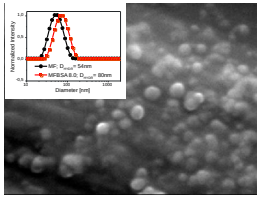

Figure 2: SEM image of MFBSA8.0. Insert: DLS size distribution of MF and MFBSA8.

Our recent experiments indicate [1] that MFBSA has an ability to depolymerise protein amyloid associated diabetes mellitus type II.

[1] K. Siposova, M. Kubovcikova, Z. Bednarikova et al.: Nanotechnology 23 (2012) 05510.

Acknowledgments
This work was supported within the projects Nos. 26110230061, 26220220005, 2622012033 in frame of Structural Funds of European Union, Centre of Excellence of SAS Nanofluid, VEGA 0041, 0077, 0079, Slovak Research and Development Agency – contracts No. APVV-0742-10 and SKRO-0012-10.

Biodistribution of Paclitaxel in the form of magnetic nanospheres

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The aim of this work was to study the time dependence distribution of anticancer drug Paclitaxel (Taxol, TAX) applied in the form of magnetic nanospheres (NPs) and to examine tumour reduction in comparison with non-targeted delivery. Paclitaxel loaded magnetic NPs were prepared and tested *in vivo* in mice. The polymer matrix for the encapsulation of the drug and magnetic Fe_3O_4 particles was created by poly(lactide *co* glycolide) (PLGA).

Paclitaxel loaded in magnetic NPs at applied an external field had a different biodistribution than paclitaxel administered in Cremophor based injection formulation (Fig. 1). Biodistribution of TAX was determined in the plasma, selected organs (liver, lung, spleen, kidney, heart) and the target tissue (placed in magnet pole during the treatment). The organ of the highest TAX content after i.v. administration of its injection form was the liver. The following organs of high affinity were the spleen and the kidney. Concerning to the target tissue biodistribution, an important observation was that the maximum determined concentration of TAX was not during the application of magnetic field (28 ± 6 ng/g at the end of 0.5 h exposition), but 10 hours after the administration (311 ± 53 ng/g). An explanation of this fact can be slow release of TAX from the PLGA matrix. It is assumed, that the TAX loaded magnetic NPs were collected at the target site to form a deposit, but TAX molecules remained dissolved or dispersed in PLGA. Later, the deposit of TMNPs slowly released its TAX content into the surrounding tissue. For the external field, a C-like construction of Nd-Fe-B magnets was made with flux density 0.44 T and gradient ca. 15 Tm^{-1} at target area.

For studying of tumour treatment efficiency several compositions of NPs with different magnetite/drug ratio were tested. The drug loaded magnetically targeted NPs caused a reduction of tumour weight. The therapeutic effect at four doses of 5 mg/kg in tumour bearing mice C 57BL/6 (B16 melanoma) kept in magnetic field for 30 minutes after intravenous administration was comparable with the effect of injection paclitaxel formulation. A better result was achieved using the composition with higher magnetite/drug weight ratio than in the sample with the smaller ratio what is due to fact that the samples with higher magnetite content were trapped more effectively in the target site (tumour) by the external field. The tumour was destroyed in both cases.

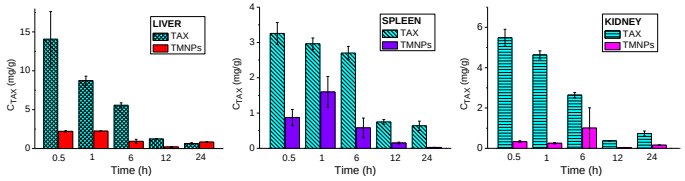

Fig. 1 Biodistribution of TAX in selected organs (content in one gram) as a function of time after i.v. administration. Comparison of TAX injection formulation (with Cremophor) and of TMNPs (TAX:PLGA:Fe_3O_4 10:100:50) using an external magnetic field for 30 min.

Acknowledgment
This work was supported within the projects 26220220005, 26110230061 and 2622012033 in the framework of the SF of EU, CEX of SAS Nanofluid, VEGA 2/0041/12, 0077, 2/0051/09, APVV 99-026505, 0742-10 and 0171-10.

PEGylation as a tool for enhancing the quality of magnetic immunosorbents used in microfluidic devices

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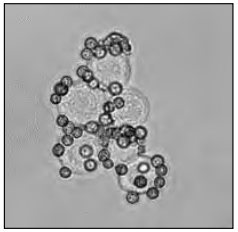
Combination of magnetic microspheres with microfluidic devices has been commonly used in various bioapplications for more than 10 years (1,2). Nevertheless even in this well described area problems with microspheres aggregation and unspecific sorption to the inner space of microchannels can occur, especially when newly developed non-commercial microspheres are used. One of the most promising strategies is to cover the microspheres surface with a poly-(ethylene glycol) layer (PEG) to prevent these complications. This poster deals with the problematics of microspheres´ PEGylation, PEG detection and subsequent biofunctionalization with specific monoclonal antibodies with the aim to capture the antigen of interest in the batchwise arrangement and in the PDMS microchip.

Monodisperse magnetic poly-(glycidyl methacrylate) microspheres with terminal functional carboxyl group were prepared by multistep swelling and polymerization method. The prepared microbeads were porous, 3.9 µm in size and carboxyl content was 0.2 mmol.g^{-1}. Despite their highly hydrophilic character they strongly adhered to the PDMS microchannel. The surface of the microspheres was therefore modified with heterobifunctional PEG. The presence of PEG was confirmed by biotin-streptavidin-based sandwich assay and by zeta potential measurements. PEG served simultaneously as a spacer for subsequent antibodies conjugation, which could increase their steric accessibility and affinity of immobilized antibody to the surface antigen (3,4).

After that, preliminary optimization tests with immobilization of human unspecific IgG onto PEGylated microspheres were carried out. This was followed by immobilization of specific monoclonal anti-EpCAM antibodies. Affinity of the prepared anti-EpCAM immunosorbent was tested by rosette test in batch arrangement with EpCAM positive MCF7 cell line. Finally, the immunosorbent was tested in PDMS microchip where the low non-specific sorption onto the channel and highly specific capture of MCF7 cells was confirmed.



Rosette of MCF7 cells and PEGylated anti-EpCAM immunosorbent.

References:

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Acknowledgment: The work leading to this invention has received funding from the European Community ´s 7th FP (2007-2013) under grant agreement n°228980, CAMINEMS.

Development of magnetic bead- based molecular detection assays using padlock probes for infectious disease and cancer diagnostics

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Diagnostic assays are fundamentally limited by the molecular analysis technique deployed in an assay. Padlock probe-based techniques allow highly specific and sensitive DNA target recognition and amplification. After ligation- dependent circularization the padlocks can be amplified in an isothermal Rolling circle amplification (RCA) and even further amplified by Circle-to-circle amplification (C2CA).

We develop multiplex C2CA assays for cancer and infectious disease diagnostics employing magnetic particles as solid support. DNA or cDNA from pathogens or DNA of a patient is isolated from a sample by specific capture on magnetic beads. Target specific padlock probes and Circle-to-circle amplification are used to detect pathogens or DNA point mutations within oncogenes. We are currently applying this assay onto an isothermal microfluidic chip for automated assay performance.

The assay efficiency with different orders of hybridization and bead capture before the first RCA was initially compared in reaction tubes. Hybridization and ligation before bead coupling yielded the highest detection efficiency, whereas the efficiency strongly decreased when performing hybridization, ligation and coupling simultaneously. Initial conjugation of capture oligos to the beads and subsequent hybridization and ligation results in relatively little signal loss and therefor is a good alternative in order to perform all assay steps on chip.

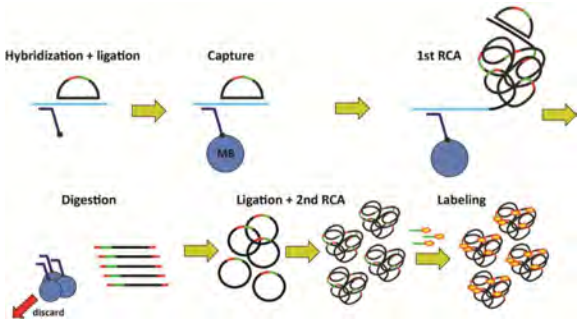

Fig. 1 Circle to circle amplification (C2CA): Padlock probes are ligated upon specific target recognition. A biotinylated oligo captures the complex on magnetic beads. After RCA the products are enzymatically cleaved, the fragments recircularized and amplified in a second RCA reaction leading to a total of 10^6 fold amplification. RCA products are fluorescently labeled and detected.

Hydroecological applications of ultradisperse magnetic adsorbents: removal of small concentrations of pollutants from large volumes of water.

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Pollution of natural bodies of water (rivers, lakes, ground water, etc) is unfortunately very common, both from natural sources like volcanic activity; and, even more importantly, from human activity, including disposal of industrial and municipal waste, mining, etc. Many toxic substances are harmful for humans and other organisms even in very low concentrations (e.g., less than 1 µg/L of cadmium is harmful, for Hg it is 0.5 µg/L, for phenol - 1 µg/L), and can remain in water for decades or longer. Cleaning large volumes of water even from low concentrations of pollutants is a challenging technological task and is very expensive.

We propose to use suspension of ultradisperse magnetic adsorbents, for example, nanostructured ferro-carbon particles, produced by plasmachemical technique, for removing small concentrations of pollutants from large volumes of water. The suspension is introduced into the water. Due to their small sizes and densities similar to water (we measured the density of FC-4 ferro-carbon to be about 1 g/cm^3; presumably due to porosity) the particles do not sediment for a long time (hours, days or longer), move due to Brownian motion and adsorb a variety of substances from the water. The particle surface can be modified to provide selectivity of the adsorption. Sorption capacities of ferro-carbon adsorbents is in dozens of percents. So, to collect 1 kg of a pollutant, 2 to 20 kg of the adsorbents is required. Then the particles with the adsorbed contaminant can be collected (e.g., downstream of the river) using a variety of magnetic traps. The traps can consist of ferromagnetic wires and permanent magnets, a variety of simple and inexpensive designs are available.

This approach was tested using a model system. Kinetics of adsorption of a highly diluted (0.002 mg/ml) aqueous solution of a low molecular weight compound (toluidine blue, F.W. 305.8) by a small concentration of the ferro-carbon powder was studied by spectrophotometry (Specord UV, Fig. 1). Before each measurement the particles with the adsorbed toluidine blue were removed from the solution by magnetic separation. These experiments demonstrated the validity of the method, where a small concentration of a pollutant was successfully collected from a large volume of water. By varying the ratio of the sorbent/pollutant, it is possible to optimize the sorbent use and the time required to adsorb all pollutant.

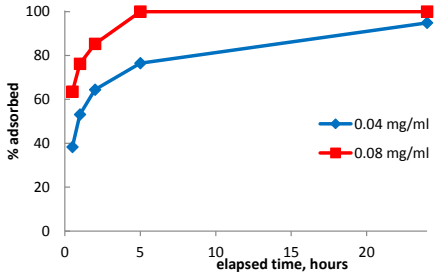

Figure 1. Adsorption of toluidine blue (0.002 mg/ml aqueous solution) on ferro-carbon powder. Two concentrations of the adsorbent (0.04 and 0.08 mg/ml) were tested.

Poster 69

PREPARATION AND EVALUATION OF PREDNISOLONE MAGNETIC NANOSUSPENSION FOR POSSIBLE USE IN RHEUMATOID ARTHRITIS THERAPY

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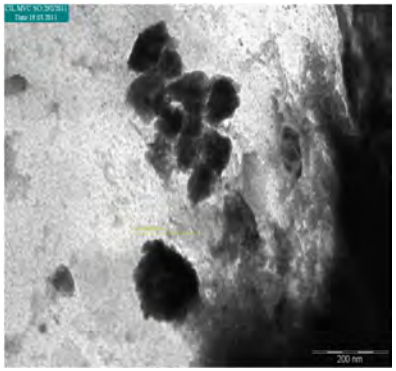
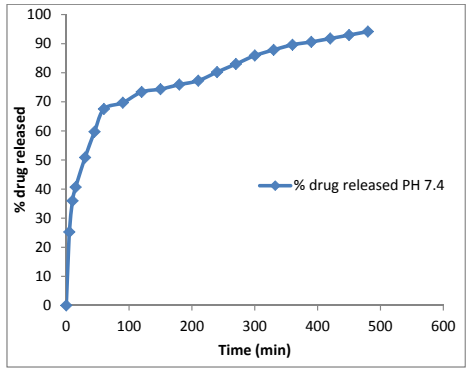
There is an increasing interest in use of bio-compatible magnetic nanoparticles for targeted drug delivery. Prednisolone, an immunosuppressant used in therapy of rheumatoid arthritis, but it has a short half-life, i.e., 3-4 hours. Magnetic Prednisolone nanosuspension was prepared in order to enhance the bio-availability by sustained release and to achieve targeted drug delivery. The developed formulation may minimize the severe associated side effects such as acne, osteoporosis by reduction of drug concentration at non target sites. Magnetic ferrofluid was synthesized by co-precipitation method using ferric and ferrous salts at (2:1) in alkaline medium. Prednisolone powder (1% w/v) was dispersed in an aqueous solution containing polysorbate 80 (0.8%), Poly ethylene Glycol 4000 (0.5%), Hydroxylpropyl methyl cellulose (0.2%) as suspending agent, magnetite and glycerol (2.5 ml) was added and mixed for 10 minutes. Then the resultant formulation was subjected to ultrasonication for particle size reduction. FT-IR spectrum reveals that there is no such interaction between the drug and the excipients used in the formulation, peak at 2920 cm^{-1} and 3436 cm^{-1} indicates drug was capped with the polymer and magnetite via hydrogen bonding. Prepared nanoparticles particle sizes were in uniform size range of 240nm with smooth surface are shown in TEM images. Zeta potential value with -9.12 mV indicates the good stability of the formulation. Magnetic susceptibility 24×10^{-6} shows better superparamagnetism by the external applied magnetic field. *In-vitro* drug release was evaluated in the physiological pH 7.4 indicates the formulation follows the zero order drug release. From all the results, it is concluded that the prepared prednisolone magnetic nano suspension can be effectively used for the treatment of rheumatoid arthritis as targeted manner.

TEM Image



Drug release pattern



Reference

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Poster 70

Formulation and evaluation of curcumin loaded magnetic nanoparticles for possible use in cancer therapy

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Deviations in the drug delivery to the target site lead to adverse effects due to poor specificity and may require higher dose / dosing frequency for rational drug therapy. The combination of developing drug delivery module utilizing both controlled release technology and drug targeting technology may provide a more efficient and reduce the related adverse effects. In this study, the anticancer drug curcumin was encapsulated in a polymeric magnetic nanoparticle complex which was synthesized with polymers i.e., β-cyclodextrin cross linked with epichlorhydrin, hydrophobically modified dextran by oleoyl chloride and magnetite as magnetic material. Particle size, surface morphology, zeta potential and magnetic measurements were determined. The developed curcumin-iron conjugated nanoparticles by varying drug:polymer ratios were found to be within the size range of 100nm with excellent negative surface charge (>-30eV) and spherical in shape. The magnetic susceptibility and magnetization curve substantiate the super paramagnetic property of the developed curcumin magnetic nanoparticles (CMN). Furthermore the drug content and encapsulation efficiency found was directly proportional to epichlorhydrin β-cyclodextrin concentration in the developed formulations. The *in-vitro* release profile of curcumin loaded magnetic nanoparticles exhibited biphasic initial release first 24 hours and release extended upto 72 hours. The drug release kinetics indicated that drug release from CMN were best explained by Higuchi's equation, as these plots showed the highest linearity but a close relationship was also noted with first-order kinetics.

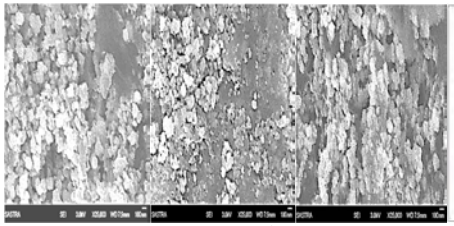

Fig 1. SEM micrographs of CMN

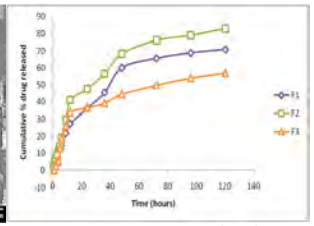

Fig 2. In-vitro drug release profile of CMN

Reference

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Magnetic Characterization and Synthesis of Magnetite Nanoclusters

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Magnetite nanoparticles and nanoclusters have great potential for development and application in the biomedical field. The facts that they are magnetically responsive and biologically compatible make magnetite nanoparticles ideal materials for magnetically induced hyperthermia and for use as Magnetic Resonance Imaging contrast agents. It has been established through prior research that nanoparticles made from magnetite display superparamagnetic properties which vary based on particle size (radius). The purpose of the preceding months of research has been to determine whether or not nanoclusters of a given size will behave differently in the presence of an identical magnetic field than would discrete, monodispersed nanoparticles of the same radius.

Thus far, iron oxide nanoclusters have been synthesized in high-pressure reactors with the use of DHCA (3,4-dihydroxyhydroxycinnamic acid) as a ligand. These nanoclusters are being characterized using dynamic light scattering (DLS) in order to determine hydrodynamic cluster radius, TEM to confirm the clustered structure of the particles and magnetic susceptibility to ascertain specific response to different magnetic fields. The expressed objective of this group is to accurately characterize any relationship between cluster size/particle size and magnetic susceptibility.

Trapping of Individual microorganisms in Magnetophoresis Channel

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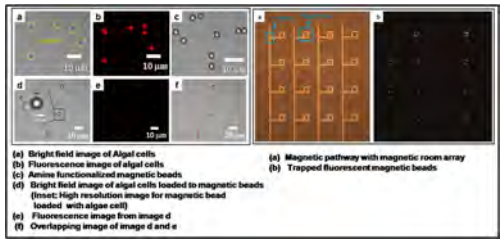
A single cell separation and analysis technique is a new frontier in the cell biology. The cellular heterogneneity within an isogenic cell population is well-known and analyzing ensembles of individually cells with high spatiotemporal resolution will lead to a more accurate representation of cell-to-cell variations instead of the average of bulk measurements for new discoveries. Here, we demonstrate the trapping of individual algae cell attached with superparamagnetic beads onto magnetic room array in the microfludic channels using magnetophoresis technique. The algal cells (*Synechocystis*sp.PCC 6803) attach to superparamagnetic beads (Dynabead® M-270 Amine) was used 1-ethyl-3-(dimethylaminopropyl)carbodiimide (EDC) – N-hydroxysulfosuccinimide (Sulfo-NHS) coupling chemistry. The confocal microscope image shows the algal cells attached with bead.

The magnetic pathways were patterned photo-lithographically such that half-cut disk $Ni_{80}Fe_{20}$ elements were arranged for translocation and addressing of bead carrier. An external rotating magnetic field was used to drive translational forces on the magnetic beads that were proportional to the product of the field strength and its gradient. The integration of T-junctions in the magnetic pathway provides selective movement of beads for individual addressing function of magnetic beads by changing the external magnetic field direction. When beads attached with algae cells enter into the isolated room surrounded by magnetic patterns they cannot come out irrespective of the field rotating direction, as a result the algae cells are trapping in the room array. In this work, we will discuss the magnetic force for cell translocation along the magnetic patterns, addressing function and trapping results of the biological cells.



Poster 73

Preparation and biological application of magnetic ion-exchange microparticles

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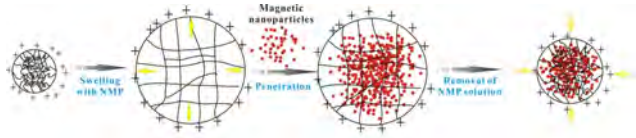
Abstract

Magnetic microparticles have a wide range of biological applications, including enzyme immobilization, nucleic acid sequencing, and separation of biomolecules (DNA, RNA, protein) and cells. The use of magnetic microparticles takes advantages of quick and one-step purification of biological substances without centrifugation. In this work, magnetic microspheres with ionic exchange feature were prepared by a swelling and penetration process, by which poly(styrene-divinyl benzene) (PS-DVB) ion exchange resins were swelled with an aqueous solution of N-methyl-2-pyrrolidone, followed by incubation with superparamagnetic iron oxide nanoparticles for allowing them to penetrate into the swollen particles. The amount of iron oxide entrapped within the ion-exchange microspheres increased with the concentration of iron oxide incubated with the ion-exchange particles. Using the 1% crosslinked PS-DVB anion-exchange particles (100-200 mesh) with diisoproplyamine functionality as the starting material, the resultant magnetic anion-exchange microspheres were shown to be very effective for DNA isolation. Batch adsorption experiments yielded a DNA adsorption capacity that one gram of magnetic anion exchange microspheres could bind 8.76 ± 0.94 μg purified plasmid DNA. With the magnetic anion-exchange microspheres the plasmid (pEGFP-C1) in cell lysate was selectively captured and purified to an A260/A280 ratio of 1.80. These magnetic anion-exchange microparticles were easily be collected in few seconds in a magnetic field.



Poster 74

Quantification Susceptibility Mapping of Myelin Imaging Compound

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Introduction. A gadolinium [Gd] based myelin imaging compound (MIC) that binds to myelin with high specificity has been reported to having promising relaxometric properties. T1 weighted magnetic resonance (MR) images have qualitatively demonstrated that MIC distributed preferentially in highly myelinated regions, but it was difficult to provide an absolute quantification. In this study, we aim to measure the amount of MIC non-invasively using a novel MRI method, quantitative susceptibility mapping (QSM).

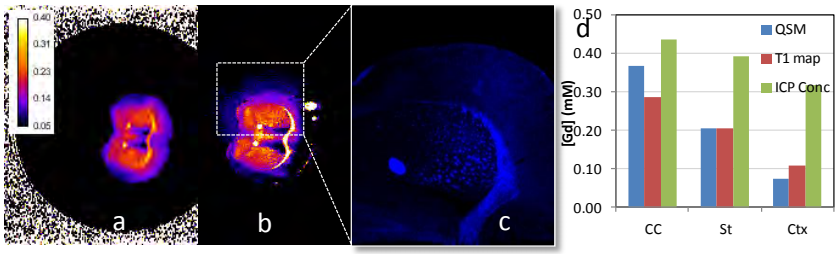
QSM does not quantify the relaxation properties of the contrast agent, which is sensitive to flip angle error and erroneous when localized Gd has limited access to water, leading to the well-known T1 quenching effect. Instead, QSM exploits the susceptibility property of the Gd. Field inhomogeneity reflected in MR phase images is utilized to calculate the susceptibility, which linearly correlates with the concentration of Gd [Gd].

Sample preparation and MR imaging. Two-month-old C57BL/6 mice were perfused with 4% paraformaldehyde (PFA) and the brain was extracted and fixed in 4% PFA. A 2 mm thick axial section was incubated with a 1 mM MIC solution for 24 h and then extensively washed with saline to remove unbound MIC. Before imaging, the brain tissue was embedded in 1% agar. T1 maps were generated using a spin-echo multiple TR saturation recovery with at least 10 TRs (TE = 6.99 ms, TR = 300−9500 ms, spatial resolution = 0.1×0.1×0.4 mm^3). QSM were generated using a 3D gradient echo (1st TE = 7.79 ms, TR = 50.0 ms, flip angle = 15°, spatial resolution = 0.1×0.1×0.1mm^3, 7 averages).

Fluorescent imaging and inductively coupled plasma mass spectrometry (ICP-MS). Following MR imaging, the brain tissue was divided sagittally into two symmetrical parts. The first was embedded in OCT and used to prepare 20 µm-thick frozen sections, which were then examined using epifluorescence microscopy with a Leica DM5000B microscope equipped with an HCX PL FLUOTAR 1.25x/0.04 objective and using the A4 filter (360/40 nm band pass excitation, 400 nm dichromatic mirror, 470/40 nm band pass suppression). The second part was subdivided into three regions corresponding to i) corpus callosum (CC), ii) striatum(St) iii) cortex(Ctx). These three portions were digested in concentrated nitric acid and used to prepare samples for ICP analysis.

Results. The [Gd] map generated by T1 mapping (a) and QSM (b) demonstrated similar pattern of MIC distribution, lowest in cortex and mild in striatum. However, [Gd] measured by QSM was markedly higher in corpus callosum, which may be explained by the theory that QSM is not subject to the quenching effect. The QSM [MIC] map also correlated well with the fluorescence image. The [Gd] measured by T1 map, QSM and ICP were on the same order of magnitude (~0.3mM) and demonstrated a similar trend. However, the values measured by ICP were substantially higher than the other two methods, especially in cortex, although this finding was not supported by visual inspection in any of the images (a-c).

Summary. In this study, we showed the feasibility of using QSM to quantify MIC, and obtained a [Gd] map qualitatively agreeing with the ones generated by T1 mapping and fluorescence imaging. Further experiments are planned to image phantoms with known [Gd] to reconcile the quantitative differences between QSM and ICP.



[Gd] by T1 mapping [Gd] by QSM Fluorescence image [Gd] concentration

Poster 75

Cellular Uptake of Magnetic Nanoparticles Quantified by Magnetic Particle Spectroscopy

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Introduction

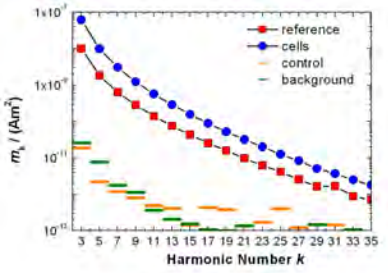
The quantification of magnetic iron-oxide nanoparticles in biological systems (cells, tissues and organs) is of vital importance in the development of novel biomedical applications such as magnetofection, drug targeting or hyperthermia. Among several techniques established to detect iron in tissue [1], magnetic particle spectroscopy (MPS) provides signals specific for magnetic nanoparticles [2] [3]. We demonstrate the feasibility of this technique to quantify nanoparticle uptake in cells using a commercial magnetic particle spectrometer (Bruker BioSpin). This instrument detects the harmonic spectrum generated by a sample exposed to a sinusoidal excitation field of 25 kHz and up to 25 mT amplitude.

Methods

HeLa and Jurkat cells were incubated with various magnetic nanoparticles concentrations and types for one day (4° C). Samples of about 10^6 cells were harvested and the MPS spectrum was measured. Additionally, stock reference material of each nanoparticle type was used to prepare serial dilutions in deionized water to assess the detection limit. The uncertainty of quantification due to sample alteration in the cells was determined by measuring the reference material in different states (e.g. immobilized, aggregated). For comparison, these samples were measured by conventional magnetometry (MPMS-XL, Quantum Design) and inductively coupled plasma optical emission spectrometry (ICP-OES).

Results

The resulting spectra are specific for the nanoparticles and, unlike linear susceptibility data, show no interference with any cell material or with the sample holder. From measurements of the reference serial dilution we determined detection limits between 10 ng and 100 ng depending on the nanoparticle type.



Conclusion

The MPS spectrum provides imformation to quantify the amount of magnetic nanoparticles in cells or in biological tissue. We found that the ratio of the amplitudes of the harmonics is influenced by nanoparticle type and diameter. Using these signatures we were able to identify type and size specific cellular uptake. The fast and easy sample preparation together with the measurement duration of only a few seconds for an individual sample makes the magnetic particle spectroscopy a favorable method for high-throughput demands.

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Poster 76

Potential of Improving MPI Performance by Magnetic Separation

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Introduction

We report on the experimental results of applying magnetic separation to Resovist®, a magnetic fluid with superior MPI performance and attested bimodal distribution of magnetic core sizes [1]. Larger particle diameters are assumed to perform better in MPI signal generation [2]. Thus particle size fractionation is expected to result in significant MPI signal enhancement. We separated Resovist® using magnetic separation at different field strengths. In the following we investigated the particle size distribution of the fractions by magnetization measurements and magnetic particle spectroscopy (MPS).

Methods

We performed magnetic fractionation using a commercially available separation column (MS column, Miltenyi Biotec) and separated at 12 mT, 36 mT and 500 mT starting with the original sample and in the following using the eluate of the previous step. Following, the static magnetization of the samples was measured to determine the size distribution of the separated fractions. Therefor we used the moment superposition model (MSM) [3] assuming a bimodal distribution of particles magnetic core sizes as shown in literature [1]. Additionally, MPI signal performance of the samples was analyzed using a magnetic particle spectrometer (MPS3, Bruker, Germany).

Results

We found good agreement of the bimodal model of the size distribution to the measurement data of Resovist®. In the fraction of 12 mT the proportion of larger particles significantly increased and mounted to about 73%. With increasing separation field strength we gradually decreased the larger fraction. The sample containing no larger particles (eluate 500 mT) showed a considerably smaller signal than the original sample, the MPS data of the 12 mT fraction yielded a mean increase about 2 times of signal amplitude.

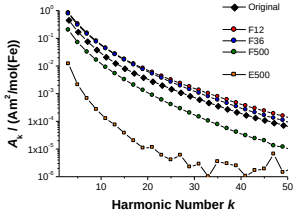

Figure 1 | MPS spectra of fractions of Resovist® separated at 12 mT (F12), 36 mT (F36), 500 mT (F500, E500). F12 increased by factor 2 whereas E500 showed only 1 % of original sample signal.

Conclusion

We demonstrated the potential of improving MPI performance by magnetic separation of Resovist®. As it was expected low signal amplitudes were obtained for the isolated smaller fraction of the discussed samples, since these particles do not meet ideal parameters for MPI. However, increasing the larger fraction up to 73 % by magnetic separation, improves MPI signal about a factor of two. By superposition of the resulted fraction signals a mean signal increas by factor 3 can be assumed. Note that only a 3 % fraction of Resovist® is suggested to contribute to the overall signal [2].

References

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Investigation of the Magnetic Properties in Fe_3O_4-gold Ferrofluid Functionalized by Carapa Guianensis Oil

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ABSTRACT

A ferrofluid based on Fe_3O_4 has been synthesized using the condensation method by coprecipitating aqueous solutions of $FeCl_2$, HCl and $FeCl_3$ mixtures in NaOH at 323K. In the next step magnetite-gold core-shell nanoparticles were synthesized from $HAuCl_4$ using an ethanol as a reducing agent. After second step the precipitated was treated with $Fe(NO_3)_3$ 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 ~5 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 4.2 emu/g and coercivity to 230 Oe. 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 1.3×10^4 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.

Decoding Magnetically Barcoded Microcarriers in a Microfluidic System

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We are working towards a novel lab-on-a-chip technology based on suspended magnetically encoded microcarriers [1]. The new generation of microcarriers *{figure 1a-b}* consist of up to seven individual magnetic elements encapsulated within a SU8 polymer and gold layer [2], which both offer routes to bio-functionalization through surface epoxide groups [3] and thiol chemistry. For the epoxide groups, carbodiimide chemistry is used to add spacer molecules and thereby increase accessibility for large probe molecules. Whilst mixed self assembled monolayers of 11-mercaptoundecanoic acid ($HS(CH_2)_{10}CO_2H$) and 6-mercapto-1-hexanol ($HS(CH_2)_6OH$) are utilized on the gold surface of the microcarriers to optimize binding coverage. The writing/reading of the digital magnetic codes is achieved through coercivity engineered magnetic elements. The possibility of attaching different fluorescent labels to each side of the microcarriers enables a positive control in binding assays. Potential applications for this platform range from DNA/protein analysis for genotyping and point-of-care diagnostics to drug development and combinatorial chemistry.

The coercivity engineering of the magnetic elements allows global addressability and is achieved by utilizing shape anisotropy. Wider magnetic elements, whilst giving lower coercivity, form domain walls *{1c}* which reduces the element's overall stray field and detection sensitivity. Instead we have developed composite elements *{1d}*, with feature sizes of 50-1200nm, to generate larger stray fields with less domain walls while maintaining tuned coercivities.

A microfluidic platform has been developed that incorporates both magnetic and fluorescence detection in-flow as well as microcarrier manipulation to form a multiplexed screening assay *{1e-f}*. The microfluidic channels are 50μm wide, moulded in Polydimethylsiloxane (PDMS) and direct the microcarriers over an integrated tunnelling magnetoresistive (TMR) sensor buried directly beneath the flow path [4]. Fluorescing microcarriers that indicate successful binding can be used to trigger magnetic detection and are identified through their magnetic elements' stray fields.

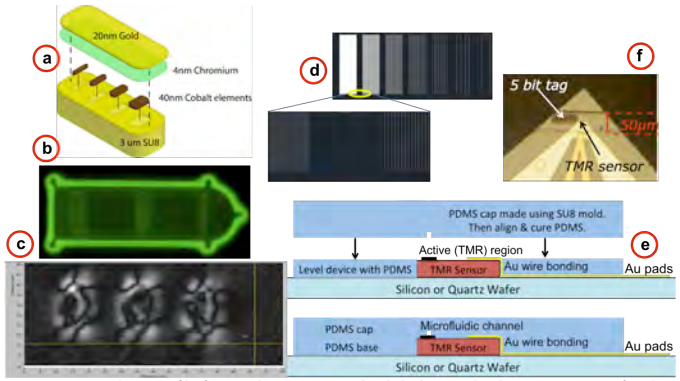

Figure 1: **a,** Schematic of bi-functional microcarrier with cobalt elements. **b,** Fluorescence image of a 3-bit microcarrier. **c,** 2D TMR scan of domain structure in bits, leading to reduced stray field and global writing capabilities. **d,** New bit design of composite elements with feature sizes of 50-1200nm to generate larger stray fields and maintain global writing capabilities. **e,** Schematic of PDMS based fabrication procedure for microfluidic device incorporating a TMR sensor directly under the microcarriers' flow path. **f,** Photo of in-flow detection of 5-bit microcarrier.

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Large FePt nanoclusters: Synthesis, characterisation and elucidation of formation mechanism

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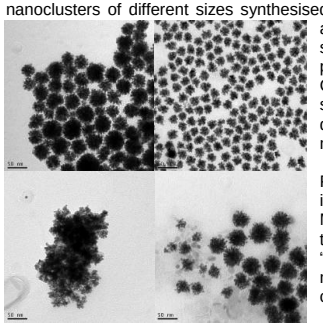
Due to its high Curie temperature, high saturation magnetization, and high chemical stability, FePt NPs are a promising candidate for many applications [1]. Here we present FePt nanoclusters of different sizes synthesised via the pyrolysis of iron ethoxide with platinum acetyl acetonate [2]. The ratio of precursors to stabilisers was studied; the lower the ratio of precursor to stabiliser, the larger the clusters. Changes in stabiliser:stabiliser ratio, precursor : stabiliser ratio, and solvent were also studied. In dioctyl ether, increasing amount of OLA results in more monodisperse and larger clusters.



TEM images of FePt nanoclusters, left dioctyl ether, right dibenzyl ether, top and bottom high and low ratio precursor : stabilizer respectively.

Preliminary SQUID data of 25 – 50 nm clusters indicate superparamagnetism at room temp with M_S ~ 25 $emug^{-1}$, this value is significantly lower than expected and may be due to the "polycrystalline" nature of the clusters; magnetic moments may 'cancel' each other out. XRD crystallography allowed facile identification of the most prominent FePt fcc phase, however, with changing conditions, peak shifts of this phase were observed, alongside evidence of the cubic L12 $FePt_3$ and magnetite phases. TEM imaging determined size and morphology of different clusters and STEM allowed elemental mapping of select samples. A discussion of cluster formation is presented to explain the relationship between synthetic conditions and size, shape, magnetic properties and crystallographic data. HRTEM indicates fusion of small crystals (4-6 nm) into larger clusters.

High magnetic moment NPs may be better for example, for MPI, magnetic hyperthermia, MRI and drug targeting via magnetic actuation [3]. Elucidation of cluster formation will help in designing better MNPs for applications. By changing the phase from fcc to face centred tetragonal (fct), these particles could potentially be used in memory storage.

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Manipulation of Magnetic Nanoparticle Retention and Hemodynamic Consequence in Microcirculation

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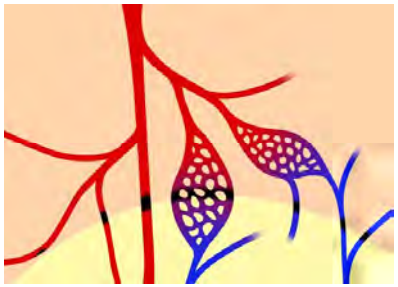
Magnetic nanoparticles (MNP) have been proposed for targeting or embolization therapeutics. How MNP retention occurs in circulation may critically determine local hemodynamics, tissue distribution of MNP, and the therapeutic efficacy. We attempted to establish a microcirculation model for study of magnetic capture of MNP in small vessels and determine parameters modifying MNP retention. Two-dimensional hemodynamic changes in response to magnet-induced MNP retention in micro-vessels of cremaster muscle *in vivo* was observed in a real-time manner using a laser speckle imaging technique. Changes in tissue perfusion of the cremaster muscle appeared to be closely correlated with the location of the magnet placement underneath the muscle piece in response to intra-arterial administration of dextran-coated MNP; magnet-caused retention was observed along the edge of the magnet, as corroborated with results from histology analysis and micro computed tomography. In these preparations, tissue iron content almost doubled, as revealed by inductively coupled plasma optical emission spectroscopy. In addition, MNP retention was associated with reduced downstream flow in a dose-dependent manner. Dissipation of MNP (5 mg/kg) occurred shortly after removal of the magnet, which was associated with significant recovery of tissue flow. However, MNP dissipation did not easily occur after administration of higher MNP dose (10 mg/kg) or prolonged exposure to the magnetic field; application of ultrasound after magnet removal may induce partial dispersion of MNP and thus partially improved hemodynamics. In conclusion, our results revealed important correlation of local MNP retention and hemodynamic changes in microcirculation.



Schematic diagram of MNP retention along the edge of the magnet. From left to right, MNP retention may occur in the lumen of the 2nd arteries, the major artery (in red), the capillaries, and the venules (in blue) in the cremaster muscle preparation. MNP deposit (in black) may occur at the margin of the magnet, which may completely or partially block flow in the microvessels. Lower part of the figure with light background indicates the location of an NdFeB magnet underneath the muscle layer.

Physical aspects of magnetically assisted water purification

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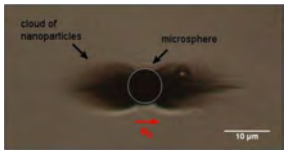
Capture of micron-sized magnetic particles on high gradient magnetic separators has been studied for decades in view of broad applications to ore benefication and to magnetic separation of biological cells. Filtration of magnetic nanoparticles, as small as 10-20 nm in diameter, is often considered as practically irrelevant because of the weakness of magnetic interactions as compared to Brownian effects.

In this work, we propose to use a bimodal ferrocolloid composed of labeled magnetic nanoparticles and non-labeled magnetic microparticles to remove pollutants from water. The advantages of a such system are : 1) large specific surface of nanoparticles allowing to adsorb a large quantity of pollutant, and 2) high efficiency of magnetic particle separation.
Under an external magnetic field, the nanoparticles are first attracted to magnetized microspheres, which are easily removed from the polluted water by low induction external magnets. After washing, the nanoparticles can be re-used for another purification cycle.

We show in this work that, despite a strong Brownian motion, the nanoparticles can be effectively captured by a magnetic microsphere if the interparticle interactions are large enough to provoke a condensation phase transition in the vicinity of the microsphere. In experiments, under an external magnetic field we observe formation of thick anisotropic "clouds" of magnetite nanoparticles around a magnetic microsphere, and try to estimate the shape and the concentration profile of these clouds from the fundamental thermodynamic relations.



Anisotropic « cloud » of nanoparticles around a magnetic microsphere under an external magnetic field

Structural and magnetic properties of Ni-Fe and Ni-Ag nanoparticles embedded in polymer

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Bimetallic magnetic nanoparticles have generated considerable research interests in recent years for their unique physical properties and potential application in sensors and carrying pharmaceutical particles for drug delivery applications. These bimetallic nanostructures are composed of either magnetic-nonmagnetic or magnetic-magnetic elements. Bimetallic nanoalloys are found to exhibit several anomalies in terms of lattice constant, thermal expansion coefficient and average magnetic moments and chemical reactivity. Here we report on the chemical synthesis of such bimetallic nanopartcles of Ni-Ag (Ni-7.2%, Ag-92.8%) and Ni-Fe (Ni-45%, Fe-55%) in polymer (polypyrrole) and obtain their structural and magnetic properties.

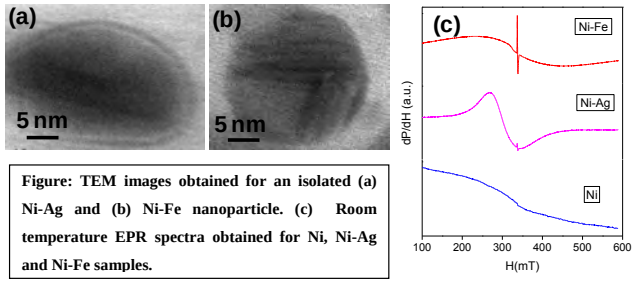

Figure: TEM images obtained for an isolated (a) Ni-Ag and (b) Ni-Fe nanoparticle. (c) Room temperature EPR spectra obtained for Ni, Ni-Ag and Ni-Fe samples.

Structural characterizations reveal the coexistence of both the nanoalloy phase and mixed phase of the bimetallic clusters of size 20 ±2 nm. The nanoparticles stabilize either in core-shell (Ni-Ag) or mixed pattern (Ni-Fe) in conformity with theoretical calculations. The interfacial alloying behavior of such bimetallic nanoparticles is further analyzed with electron paramagnetic resonance (EPR) spectra. The temperature dependent EPR spectra clearly outline the influence of paramagnetic silver or ferromagnetic iron on the spin resonance behavior of Ni^{2+} in such bimetallic nanoparticles.

Optimization of 4 different SPION as potential diagnostic carriers in magnetizable stent assisted drug targeting applications.

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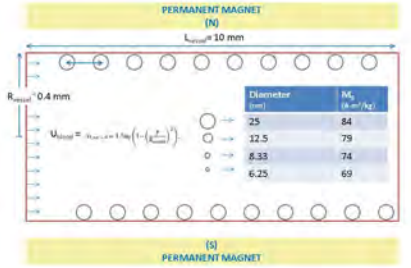
Superparamagnetic iron oxide nanoparticles (SPIONs) continue to offer promise for clinical applications since they are employed as magnetic resonance imaging (MRI) contrast agents and their features could be easily tailored by including targeting moieties, fluorescence dyes, or therapeutic agents. The magnetizable stent assisted magnetic targeted drug delivery system, which uses high gradient magnetic separation (HGMS) in a physiologically stretched vessel was previously studied by the authors in a full 2D mathematical model. Ferromagnetic, coiled wire stent is implanted to aid collection of magnetic drug carrier particles in an elastic tube that has similar mechanical properties to the blood vessel and the changes in the mechanical behaviors were analyzed under the influence of mechanical forces generated. Here, we focus on the theoretical modeling of the interaction between 4 different sizes SPIONs derived from polyol methods coated with oleic acid in a magnetizable stent assisted magnetic targeted drug delivery system. The objective of this study is to understand if SPION size can influence the payload delivery at the stent site as potential application in pharmaceutical applications. Key point of this study is the amount of SPIONs included in the drug carrier particles which is inversely proportional to the diameter changes within the equally exposed area in each simulation. This allow for the optimization of the total amount of carrier particles targeted at the desired site under the influence of different magnetic field strength and blood velocity. The unique combination between physico-chemical properties, the multiparametric theoretical model and its analysis is therefore allowing for the optimal identification of the "functionally-optimal" nanoparticle requirements prior to moieties, fluorescence dyes, or therapeutic agent functionalisation for targeted diagnostic or pharmaceutical applications.



Schematic of the control volume used for studying the behavior of functionalized SPIONs as pharmaceutical carriers in magnetizable stent assisted drug targeting applications.

ACKNOWLEDGEMENTS
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Magnetic nanoparticles coated by gold

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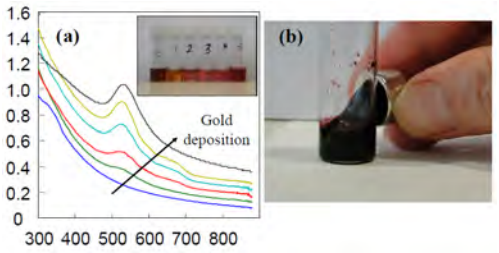
The gold coated magnetic nanoparticles are very interesting for scientific investigation and also can be used for optical and biomedical applications. Au@Co nanoparticles have a great interest as catalysts.
We have synthesized Au @ magnetic particles by different ways, with magnetite and Co magnetic particles, in water and in toluene.
Gold coated cobalt nanoparticles (Au@Co) are synthesized via redox reaction by reducing Au precursor solution in toluene in the presence of Co-Magnetic fluid which was prepared via thermal decomposition of Co carbonyl in presence of $Al(octyl)_3$ under ambient conditions.
Using the magnet the gold coated cobalt particles were separated from pure gold nanoparticles. After washing a few times with toluene and ethanol the Au@Co particles were easily redispersed in toluene by just adding some amount of toluene and disperse 1-2 min in US- bath.

In result, the long time (longer then 1 year now) stable (chemically and colloidal) Au@Co particles and Au@Co – Magnetic fluid were developed.

The obtained Au@Co particles can be transfer into water by tiols.



(a) – UV-Vis-Spectra; (b) – Au@Co- magnetic fluid

Dual-purpose Cancer Therapy Using Functionalized Iron Oxide Nanoparticles

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In recent years, iron oxide nanoparticles have been adapted for use as drug delivery platforms because of their ease of surface functionalization, biocompatibility, and low cytotoxicity. Additionally, their intrinsic magnetic properties enable magnetic localization to target sites, magnetic resonance imaging, and inductive heating through alternating magnetic field (AMF) exposure. Their heating potential, in particular, has been utilized for hyperthermia therapy, wherein cancer tissues are targeted and heated to induce apoptosis. We have recently synthesized and surface-functionalized iron oxide nanoparticles and demonstrated their use for *in vitro* drug delivery. Specifically, we attached an analog of the anticancer drug Doxorubicin to iron oxide nanoparticles using a cationic linker. After incubation with MCF-7 cells, AMF induction resulted in the release of the drug analog and consequent cell death. Further modifications of the linker are providing insight into the nature of the binding interactions between the drug conjugate and the surface of the nanoparticles. We present here our optimization of this nanoparticle based drug delivery system for use in concert with hyperthermia treatment as a dual-purpose cancer therapy.

The Stability of Poly(ethylene glycol) Stabilized Iron Oxide Nanoparticles: A study on ligand displacement under biological conditions.

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There have been recent significant advances in the use of magnetic nanoparticles for various biomedical applications, including MRI contrast enhancement[1], magnetic hyperthermia[2], and drug delivery vessels.[3] All of these potential applications depend on the stability of the systems inside the human body. For example, particle clustering has been shown to have an impact on both the contrast enhancement[4] and heating properties of the systems. Furthermore, clustering may have an impact on uptake by the reticuloendothelial system. It is therefore crucial to understand the stability of these particles under biological conditions in order map the efficiency and performance of these systems.

The major factor that contributes to particle instability and clustering in biological media is the body's ability to interfere with particle stability mechanisms. This includes screening of particle surface charges with biological salts, protein conjugations, and displacement of particle ligands. One of the most studied factors in particle stability from a grafting to perspective is the strength of the ligand binding group, or anchor. For magnetite, there are several anchor groups that provide robust attachment to the surface of the particle. These include carboxylic acids, phosphates, silanes, and catechols. One of the more recently studied anchors is the catechol, more specifically dopamine and L-DOPA. These have shown significant adhesion to the surface of magnetite, but hierarchy of binding strengths between anchor has still not been well established. For the purpose of this study, three anchor groups have been chosen to study the relationship between particle stability and biological salt content. These anchor groups are L-DOPA, NitroDOPA, and Tri-NitroDOPA. The stability of these three anchor groups were tested against phosphate buffered silane (PBS) and Fetal Bovine Serum (FBS) concentration. Particle stability was measured using dynamic light scattering (DLS) and indicated particle protien interactions are sigificantly dependant on the bind ligand use.

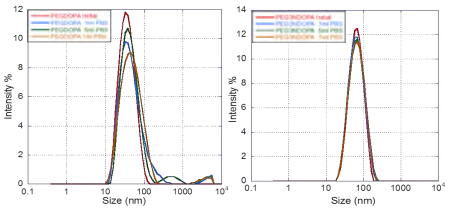

Figure 1: DLS intensity average of PEG DOPA coated particles and PEG TriNitroDOPA stabiles particles with increasing concentration of PBS

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Nanospheres loaded with ferrimagnetic carbon nanotube and chlorin$_{e6}$ for application in hyperthermia and photodynamic cancer therapy

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Carbon nanotubes (CNT), filled with magnetic nanoparticles (MNP), are presented as a promising material for biomedical application. Biocompatibility combined with good dispersion in aqueous solution allow their use as a multifunctional drug carrier in cancer therapies. Hyperthermia, a new and noninvasive therapy, aimed to destroy tumor cells by local heating. Photodynamic therapy, which is based on the administration of a photosensitive drug followed by exposure to visible light at a specific wavelength and in the presence of oxygen, generates cytotoxic species and promotes cell death. In this study we report on the production and test of polymeric nanospheres loaded with ferrimagnetic multi-wall CNT and chlorin$_{e6}$ as the selected photosensitizer (FMCNT/C$_{e6}$-NS). The material system was successfully prepared by double-emulsion technique. Maghemite (γ-Fe_2O_3) nanoparticles were incorporated into CNT using a highly-stable ionic magnetic fluid sample. The obtained nanospheres present no agglomeration whereas revealing a narrow monomodal size distribution, with average diameter in the range of 252-354 nm and polydispersity index around 0.230. The obtained nanospheres presented negative surface charge, corresponding to a zeta-potential of –25.6 mV. The *in vitro* antitumor activity was assessed using murine melanoma cell line (B16-F10). The influence of nanospheres on cell viability was investigated by classical MTT assay. The current study showed that FMCNT was more biocompatibility than CNT pure. Additionally, the FMCNT/C$_{e6}$-NS (3.7×10^{14} particle.mL^{-1} of maghemite, 12.5 µg.mL^{-1} of CNT, and 2.5 µmol. L^{-1} of Chlorin$_{e6}$) showed no toxicity in the absence of both light and applied AC magnetic field. However, under AC magnetic field (40 Oe amplitude), the FMCNT/C$_{e6}$-NS reduced the cell viability down to 55%. Furthermore, the cell viability reduction was more pronounced while combining AC magnetic field with light stimulation; the B16-F10 cell viability reduced to 11%. The present study indicates that the as-produced FMCNT/C$_{e6}$-NS material system represents a promising multifunctional drug nanocarrier with synergic effect while employed in photodynamic and hyperthermia therapies.

Magnetic relaxation switching biosensor: sensing mechanism and applications

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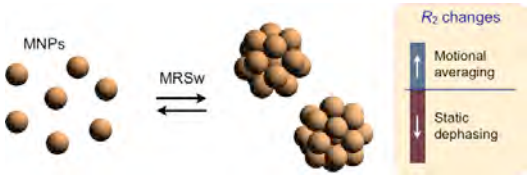
Magnetic relaxation switching (MRSw) assays that employ target-induced aggregation (or disaggregation) of magnetic nanoparticles (MNPs) have beed used to detect a wide range of biomolecules. When MNPs cross-link upon the recognition and binding of target biomolecules, these clustered particles change the transverse (R_2) relaxation of water protons, which can be detected by nuclear magnetic resonance (NMR; **Scheme 1**). The assay procedure is simple and fast, as it does not require the separation of bound and free MNPs. Moreover, due to the intrinsically low magnetic susceptibility of biological media, the assay can be performed in complex, native specimens with little interference from biological background. The precise working mechanisms of the assay, however, remain poorly understood, often leading to confounding and conflicting results. We herein present a systematic investigation of the MRSw phenomena. A panel of MNPs with different physical properties (size and magnetization) were synthesized and utilized for MRSw assays. We specifically focused on 1) characterizing the nature of MNP clustering; 2) elucidating the different transverse relaxation modes with such clustered MNPs; and 3) establishing the relationship between MNP's material properties and its MRSw detection sensitivities. The study found that clustered MNPs are universally in a quasi-solid, fractal state (dimension of ~2.4). Accordingly, a new model for transverse relaxation was constructed, that describes the observed MRSw phenomena. Importantly, the study led to an analytical MRSw model that could predict the detection sensitivities and dynamic ranges for a given type of MNPs. These findings will aid in not only interpreting existing experiment data but also designing new MNPs and assay protocols to further improve MRSw sensitivities.



Scheme 1. Magnetic relaxation switching (MRSw) assay. Dispersed magnetic nanoparticles (MNPs; left) form clusters upon binding with target molecules (right). Depending on the cluster size, the transverse relaxation of samples can assume two separate modes, motional averaging and static dephasing, resulting in opposite changes in its relaxation rate (R_2).

Open gradient magnetic red blood cell sorter evaluation on model cell mixtures

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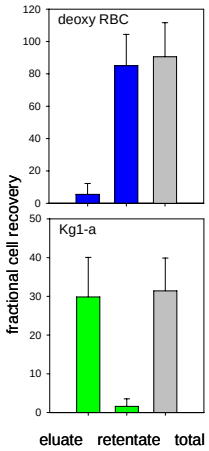
Magnetic properties of red blood cells are a function of the heme oxygen binding: the bonds are purely ionic in the oxygenated hemoglobin and purely covalent in deoxygenated hemoglobin. There are four heme groups in the hemoglobin molecule and the protein bulk of the hemoglobin molecule is diamagnetic resulting in a large paramagnetic contribution from the unpaired electrons in the heme iron of the deoxyhemoglobin (four Bohr magnetons per heme). This leads to a marked change in the difference in magnetic susceptibility of the hemoglobin relative to that of water, from negative for weakly diamagnetic oxy-hemoglobin to positive for the deoxy-hemoglobin. Consequently, the oxygenated erythrocytes in physiologic aqueous solutions are pushed away from the magnet (behave as the diamagnetic particles) while the deoxygenated erythrocytes are attracted to the magnet (behave as the paramagnetic particles). We have tested if the increase in the paramagnetic contribution to deoxygenated RBCs is sufficiently large to provide basis of a practical sorting system for enrichment of mature RBCs from hematopoietic cell cultures in an open-gradient system that avoids potentially cell damaging ferromagnetic inserts typical of the high-gradient magnetic separator designs.

The pilot scale magnetic separation system based on a quadrupole magnet with 2 mm aperture, 76 mm length, a maximum field of 1.6 T, a nearly constant, axially-symmetric field gradient of 1,600 T/m incorporating an annular flow channel of approx. 2.3 mm^2 cross-section area was set up in various configurations on a test bed allowing for capture-and-release or continuous modes of operation. The tests consisted of mixtures of normal donor RBC and KG1-a (blood progenitor cell line from ATCC, Manassas, MD) as models of hematopoietic cell culture. The results of three experiments on 50:50 mixtures at the total cell number concentration of 1×10^8/mL and the total cell number throughput of 3×10^5/min resulted in nearly 90% recovery of RBC in the "retentate" magnetic fraction with only a small contamination (approx. 1%) of KG-1 cells (Figure). The ongoing tests show feasibility of throughput scale-up to 7×10^7/min that approaches practical laboratory applications requirements.



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Biomineralization process of Fe_3O_4 nanoparticles by the magnetotactic bacterium *Magnetospirillum gryphiswaldense*

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Magnetite Fe_3O_4 nanoparticles present outstanding properties for biomedical applications due to their high magnetic susceptibility and biocompatibility. A relatively unexplored method for the production of magnetite nanoparticles is the culture of magnetotactic bacteria. Magnetotactic bacteria are microorganisms that have the ability to align in and navigate along the geomagnetic field lines due to the presence of a chain of magnetosomes. These magnetosomes are magnetic nanoparticles covered by a lipid bilayer membrane. The type of magnetic nanoparticle, the shape and size of the magnetosomes depend on the species of magnetotactic bacteria. In particular, the bacterium *Magnetospirillum gryphiswaldense* MSR-1 produces magnetite, Fe_3O_4, cubo-octahedral shaped nanoparticles with an average size diameter of ≈ 50 nm and covered by a membrane of around 2-4 nm. These nanoparticles are single domain, present high crystallinity and narrow size distribution so they are specially interesting for biomedical applications. In this work we plan to study this biomineralization process, which is complex and still poorly understood, through a time-resolved study. For that purpose, the bacterium *Magnetospirillum gryphiswaldense* MSR-1 was grown microaerobically at 28^{o}C in low iron medium. For induction of magnetite biomineralization, iron starved cells were harvested by centrifugation during logarithmic growth phase, and transferred to fresh medium supplemented 100 μM Fe(III)-citrate. At given time intervals, after measuring the bacterial growth, images of the cells have been obtained by Transmission Electron Microscopy (TEM) and the magnetic properties have been measured. In order to maximize the magnetic signal the cultures have been centrifuged to concentrate the cells in a volume of approximately 50 μl.

As shown in figure 1a, TEM reveals the presence of nanoparticles in some cells after 2h of Fe addition but without a clear formation of the chain. Coincidentally, the appearance of a hysteresis loop (figure 1c) that according to the butterfly shape of the low-field signal (inset), points out to the presence of two different magnetic phases, that could be explained by the coexistence of both small and bigger particles. After 6h, most of the bacteria present nanoparticles organized in chains (figure 1b) and the hysteresis loop shows only one magnetic phase with increased saturation magnetization, a clear indication of a progressive increase of the amount of magnetic nanoparticles. Finally, upcoming X-ray absorption spectroscopy measurements will allow us to clarify the Fe oxidation along the magnetosome growth process.

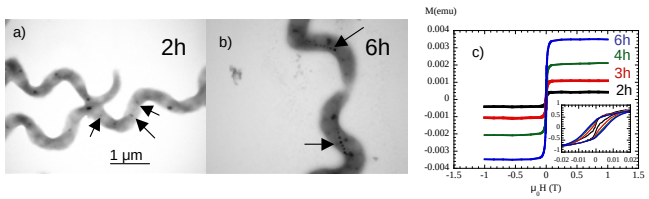

Figure 1: TEM images of *Magnetospirillum gryphiswaldense* after a) 2h and b) 6h of incubation with Fe(III)-citrate. c) Room-temperature hysteresis loops obtained after 2, 3, 4 and 6 hours of Fe(III)-citrate addition. Inset: normalized hysteresis loops at low applied magnetic fields.

Drug impregnated magnetic nanospheres

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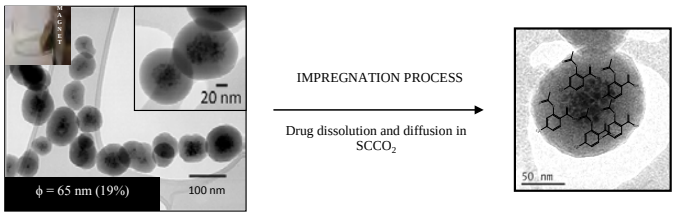
The use of supercritical carbon dioxide ($scCO_2$) as a synthesis medium as well as a solvent to perform adsorption and impregnation processes and materials functionalization has received considerable attention as a viable and sustainable alternative to conventional liquid solvents. We will present the use of supercritical fluid assisted sol-gel method for the production of a multi-core magnetic silica carriers as well as the use of supercritical carbon dioxide to impregnate a therapeutic agent (triflusal) in the nanospheres. Trifusal is an antithrombotic therapeutic agent used here as a model of a hydrophobic and moisture sensitive active agent with poor solubility in water.

Fabrication of the magnetic silica nanospheres was done in a straight forward one-pot method combining sol-gel chemistry and supercritical fluids technology [1]. Resulting nanoparticles present a narrow particle size distribution with sizes of the order of 100 nm. Each nanosphere consists of a magnetic multi-core of non-contacting Fe_3O_4 nanoparticles surrounded by a microporous silica shell. Nanospheres are superparamagnetic at room temperature. Some advantages of the method are short reaction times, purity of the product and potentiality of the process to be scaled up. Cytotoxicity studies of the composites will be presented.

We have previously reported on the potential use of the nanospheres as enhanced T_2 contrast agent for MRI [1,2]. In addition, the designed material may find applications as a target drug delivery system having the greatest therapeutic potential in those clinical scenarios that require the delivery of active agents at a specific point of the body while avoiding systemic effects of toxicity. The silica–based matrix is found to prevent the hydrolization of the active ingredient more efficiently than a polymeric matrix PMMA used for comparison, the drug vehicle serving in this way as a moisture protection barrier. Moreover, the trifusal is dispersed in a molecular form inside the material and fast release kinetics has been assessed, both features being of great interest to enhance the bioavailability of low solubility drugs.



[1] Taboada et al., *Advanced Functional Material*, **19**, 14 (2009) 2319.
[2] Taboada et al., *Mater. Res. Soc. Symp. Proc.* **1257**, (2010), 1257-O05-06

Self-heating temperature and ac hysteresis of magnetite nanoparticles and their dependence on secondary particle size

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Magnetic nanoparticles are expected to be used as hyperthermia agents. The mechanism of self-heating of the magnetic nanoparticles under ac magnetic field is different according to their size. In this study, the temperature rise and both of ac/dc hysteresis loops of magnetic nanoparticles were evaluated in order to clarify the contribution of Néel and Brownian relaxations to heat dissipation. The samples were dextran-coated Fe_3O_4 nanoparticles of different hydrodynamic diameter (40, 54, and 86 nm). Their primary diameter was 5 nm for all these three samples.

The dc hysteresis loop was measured using a VSM at room temperature. The hydrodynamic particle size was measured by dynamic light scattering. The self-heating temperature and ac magnetic properties were measured by applying an ac magnetic field of 50 Oe at 50-500 kHz.

Dc hysteresis loops indicated that all three samples were superparamagnetic particles. This means that heat dissipation of these samples is attributed to magnetic relaxation loss. The average hydrodynamic diameters of the samples were 40, 54, and 86 nm. From these hydrodynamic diameters, the peak frequencies for Brownian relaxation are calculated to 7.59, 2.93, and 0.74 kHz, respectively. The peak frequency for Néel relaxation at 3.6 MHz is also calculated for a primary particle size of 5 nm [1]. Self-heating temperature rise of the sample of 86 nm was higher than those of 40 and 54 nm. This agrees with the measured area of ac hysteresis loops which are normalized by their saturated magnetization (Fig. 1). The sample of 86 nm exhibited a higher value in imaginary component of susceptibility than the samples of 40 and 54 nm as shown in Fig. 2. The Néel relaxation time is much shorter than the Brownian relaxation time for these samples. Although Néel relaxation, determined by a primary particle size, is dominant, the results suggest the effect of magnetic interaction between the nanoparticles depending on the hydrodynamic diameter.

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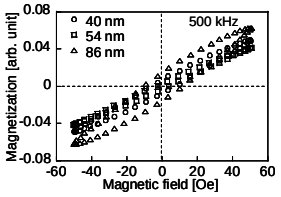

Fig. 1. Ac hysteresis loops measured at 500 kHz.

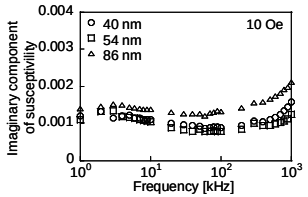

Fig. 2. Dependence of heat efficiency on magnetic field frequency.

Poster 93

Magnetically modulated single particle tracking as a tool to study cytotoxicity of airborne nanoparticles

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Macrophages are the primary defence cells in the lung responsible for uptake and clearance of foreign particles, debris, viruses and bacteria. These biophysical processes can be studied by tracking the trajectory of individual particles as they diffuse through the medium, bind to the cell surface, and are internalized. However, it is challenging to track translation in situ at low magnification. We have developed a novel technique to track adherence, release, and intracellular transport of phagocytosed magnetically modulated optical nanoprobes (MagMOONs). These MagMOONs are micron sized tracer particles with one hemisphere coated by gold creating an orientation-dependent scattering and fluorescence signal. The particles align with an external magnetic field and blink when they rotate in response to a rotating magnetic field. Tracking rotational transport via intensity changes allows analysis of many particles simultaneously even at low magnification. In this study, we track the MagMOONs blinking signal as they non-specifically bind onto J774A macrophage cell membrane, and then are released during membrane rupture (see Figure 1).

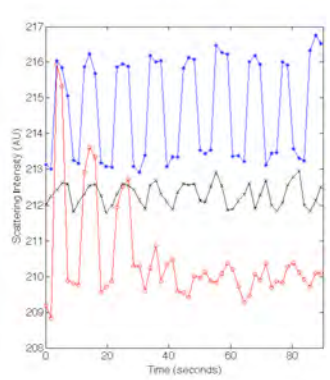

Figure 1. Scattering intensity of a magnetically modulated particle before and after it binds to J774A macrophage. Particle: 3.9 μm Fe_3O_4, hemi-spherically coated with 50 nm gold. The ~ 0.5 mT magnetic field is rotated 180^0 "on" and "off" every 5 seconds. Red line (○): before MagMOON attaches to macrophage surface there is intense modulation which decreases when the particle attaches to the surface. Black line (x): minimal modulation after 1 hour the particle attaches on macrophage surface. Blue line (*): restoration of modulation after the cell membrane ruptures.

Poster 94

Quantitative Real-Time Study of Magnetic Particle Clearance from Blood and Biotransformation in Animal Organs *in vivo*

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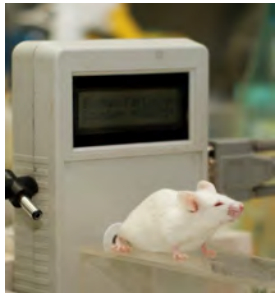
Biomedical applications of magnetic nanoparticles (MP) continue to expand due to unique properties of these nanoagents: possibility to be guided and heated by external magnetic field. Besides, MP can be quantified with high sensitivity by external magnetic field even in opaque medium or living body [1-4]. However, their biocompatibility and non-toxicity suggest their suitability for *in vivo* theragnostic purposes. For example, magnetic particles are considered to be potentially successful agents for diagnosis of different disorders such as atherosclerosis and cancer, and therapy of cancer (via hyperthermia), anemia, etc.

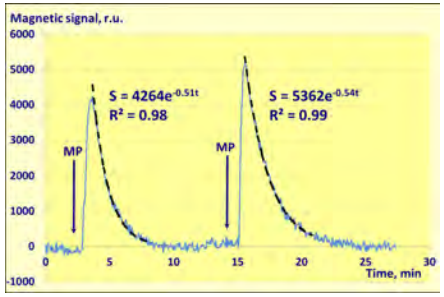
The choice of the proper nanoparticles for each particular application significantly depends on their pharmacokinetic behavior in living body. In terms of pharmacokinetics the most crucial characteristics for nanoagents are their blood clearance and their biodegradation and/or clearance from the organism. To study such parameters of different MP, we have improved our method of non-invasive MP detection in blood and organs of small animals by non-linear magnetization [1-4]. We investigated influence of the following parameters on the dynamics *in vivo*: nanoparticle size, coating, concentration and injected amount, anesthesia, number of injections, etc.

The first part of the work was dedicated to the study of MP dynamics in blood flow of mice. For the experiments the tail of a mouse was placed in the induction coil of the device (left figure). After retroorbital MP injection (near the eye of the mouse), the MP blood concentration dynamics in tail arteries and veins were recorded in real-time with 3 sec resolution. Interestingly, for the most cases the MP blood concentration was strictly exponential (right figure). However, in some variations of the experiment (large MP doses, certain MP coatings, etc.) the concentration curves were complex and could not be fitted with a single exponential curve. The developed method allows non-invasive recording of the MP dynamics with high time resolution and sensitivity.

The second part of the work was dedicated to the non-invasive study of MP degradation in organs of mice. After intravenous injection of MP the mouse was scanned by external induction coils which detected magnetic nanoparticles also on combinatorial frequencies. During two months the signal in the liver region was recorded for every mouse and MP degradation curve was plotted for every mouse. Non-invasiveness of the measurement procedure allowed carrying out more humane experiments using and sacrificing much less mice than it would be necessary for invasive studies. Influence of coating, size and dosage of MP on the degradation dynamics was investigated.

The developed methods and acquired data can be useful for understanding kinetics of processes underlying nanoparticle clearance and degradation by organs of reticulo-endothelial systems. The knowledge about these processes can be of great importance for the development of biomedical agents based on magnetic nanoparticles as well as any other nanoparticles.





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Magnetic epidermal growth factor conjugate for targeted delivery to tumor in xenograft mouse model

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It is known that epidermal growth factor (EGF) receptor is over-expressed in cancer cells. Therefore the malignant tumors can be monitored by magnetic resonance imaging (MRI) with enhanced contrast using magnetic nanoparticles (MNP) conjugated with EGF. The feasibility of using MNP-EGF conjugates in MRI of xenograft tumors in mouse model was evaluated. MNPs were prepared by co-precipitation of Fe salts under N_2 purging and minor modification of electrolyte composition. The MNPs were coated by cross-linked dextran. EGF samples were provided by Protein Contour (Russia). MNP-EGF conjugates were synthesized by covalently linking EGF to dextran shells of MNPs via carbodiimide linker. Conjugates were characterized by relaxometry measurements, ELISA, quasi-elastic light scattering (QELS) and Doppler laser magnetophoresis in static and dynamic (antigen induced agglutination) conditions. Iron concentrations were determined spectrophotometrically by absorbance of iron–thiocyanate complex at λ_{ex}=480 nm.

The MNP-EGF hydrodynamic diameter values as obtained by QELS were within the range of 100-300 nm. The NMR measurements of magnetic relaxation rates and relaxivity coefficients of MNP-EGF in suspensions were carried out using Bruker CXP-300 spectrometer at 7.1T. The high relaxivity R_2 value of conjugates corresponds to negative contrast agents for MRI, producing thus the immune active magnetic delivery system. The sample of murine melanoma cell line Clone-M3 was obtained from Institute of Cytology, Russian Academy of Sciences (St. Petersburg, Russia). The cells of murine melanoma were cultured in Dulbecco's modified Eagle's medium (DMEM) containing 10% v/v of fetal calf serum (FCS), in a damped atmosphere of 5% CO_2, at t=37°C. Syngenic DBA strains of mice were used for xenograft melanoma model. Tumors were induced in the subcutaneous connective tissue by injection of 10^6 melanoma cells. The mouse and gel phantom images were registered with the help of Bruker Avance II NMR spectrometer at 11 T. The measurements were carried out using gradient-echo sequence (GEFI ORTO) and multiscan-multiecho (MSME). The *T1, T2* weighted images were obtained under RARE-T1 and Turbo-RARE-T2 scanning regimes. Series of transverse and coronal sections of tumor were acquired after intravenous injection of MNP-EGF conjugate at different terms and regimes of acquisition. The MRIs of tumor-bearing mice before and after injection of MNP-EGF are shown in Figure 1, where the tumor xenografts (dark areas) are negatively contrasted by the conjugate binding.

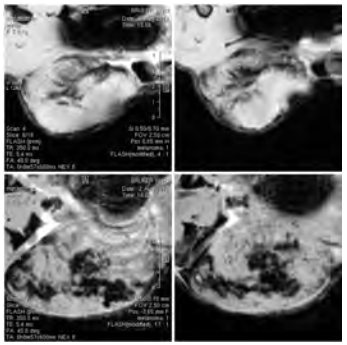

Figure 1. MRI axial image of xenograft melanoma (Clone M-3, 10 days) obtained by gradient echo. Upper row: control scans before contrast agent MNP-EGF administration; lower row: in 60 min. after intravenous administration of MNP-EGF contrast agent.

The study demonstrated that MNP-EGF conjugate intravenously injected in tumor xenograft mouse model accumulates in the tumors and enhance the contrast of their MRI images. MNP-EGF conjugates show high relaxivity and can be used as negative contrast agents for MRI diagnostics of malignant tissues with over-expressed EGF receptors.

Highly Sensitive Magnetic Immunoassay Compatible with High Volume Samples

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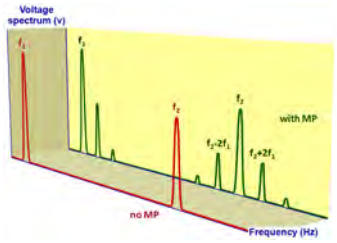
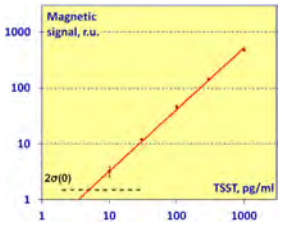
Novel highly sensitive Magnetic ImmunoAssay vertical-flow (MIAflo®) format have been developed, which allows direct analysis of complex liquids such as neat milk, whole blood, drinks, water used for washing vegetables or meat, etc. For many complex liquids the format does not require preliminary sample preparation and is compatible with large sample volume, which leads to sensitivity increase due to direct immunoconcentration of the antigen on solid phase.

The MIAflo® format is based on 3D porous filters employed as solid phase for sandwich assay and magnetic nanoparticles (MP) used as labels, which are detected at combinatorial frequencies by non-linear magnetization [1] (left figure). The related readers can detect as little as few ng of MP [2,3] and direct comparison with radioactive technique for MP based on ^{59}Fe isotope demonstrated similar sensitivities of both methods [4]. Thus, the MIAflo® allows realizing main advantages of old radioimmunoassays, but in much more safe and affordable ways. The combination of 3D filters and magnetic nanolabels removes many kinetic limitations intrinsic to antigen interaction with antibody layer immobilized on 2D flat surface of traditional biochips or ELISA plates. The MIAflo® format permits fast immunofiltration of antigens from complex samples, reduces the assay time and allows analyzing practically unlimited sample volumes.

In our experiments, several toxins produced by *Staphylococcus aureus* such as Staphylococcal Enterotoxin A (SEA), Toxic Shock Syndrome Toxin (TSST) have been detected directly in neat milk with no sample preparation, which is obligatory in standard methods. A detection limit for TSST in 30 ml of net milk was as low as 4 pg/ml (right figure), which is ca. 50 times better, than for ELISA. The assay time of such sample (2 hours) is less, than that of standard methods (3-14 hours). For express MIAflo®, 25 min-long, using standard sample volume of 0.15 ml the limit of detection was 0.1 ng/ml, which is in ca. 2-5 times better, than for standard, much longer methods. The developed assay has wide dynamic range (>3 orders of magnitude of both concentration and signal) as well as large angle of the signal dependence on concentration, which is close to its theoretical maximum of 1.0 in log-log scale. This allows obtaining reliable results for both low and high concentrations. Analysis of high-volume samples is of special interest not only for testing food extracts to detect pathogens and toxic chemicals, but for a wide variety of applications such as detection of Legionella in air-conditioning systems, inspection of transport visiting infected regions by testing washing liquids, highly sensitive identification of comorbidity during plasmapheresis, when large volume of blood plasma is available, etc.





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Poster 97

Magnetofection using DNA/Polyethylenimine coated on magnetic nanoparticles

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Recently magnetic nanoparticles (MNPs) attract rising attention for using as transfection vectors. Nonviral transfection vectors, for example, cationic polymer and cationic liposome are higher biocompatible than viral vectors, but their transfection efficiency is lower. Transfection vectors using MNPs are enable high transfection efficiency and biocompatibility. In this study, γ-Fe_2O_3 nanoparticles coated with polyethylenimine(PEI) were prepared. Dependences of the weight of MNPs and the number of DNA/PEI/MNP complexes on transfection efficiency were evaluated.

PEI coated γ-Fe_2O_3 nanoparticles conjugated with plasmid DNA were prepared. The primary size of the γ-Fe_2O_3 nanoparticles was 29 nm. In this transfection experiment, HeLa cells from human cervical carcinoma line were used, which were incubated in 35 mm culture dishes. After 24 hours, DNA/PEI/MNP complexes were exposed to incubated cells, and magnetic field was applied by using a Nd-Fe-B magnet for an hour. After 48 hours post-transfection, transfection efficiency was evaluated by a fluorescent microscopy. Transfection efficiency was also evaluated with using PEI coated Fe_3O_4 nanoparticles (primary particle size of 20-30 nm). In order to estimate the number of complexes from the weight of MNPs, the hydrodynamic diameter of the complexes was measured by dynamic light scattering (DLS).

Figure1 shows dependence of the weight of γ-Fe_2O_3 and Fe_3O_4 nanoparticles on transfection efficiency. The transfection efficiency increases with the weight, but higher weight leads to a drop in transfection efficiency. It has been reported that this drop is attributed to toxicity [1]. The DLS measurement indicated that DNA/PEI/MNP complexes aggregated. Figure2 shows dependences of the transfection efficiency and the number of complexes for the case of using PEI coated γ-Fe_2O_3 nanoparticles. It was indicated that the decrease in transfection efficiency was related to the decrease in the number of complexes.

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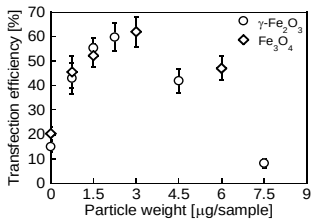

Fig.1 Transfection efficiency depending on particle weight.

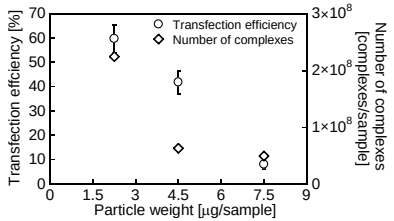

Fig.2 Transfection efficiency and the number of complexes depending on particle weight.

Poster 98

A Minimally Invasive Approach to Remove Circulating Tumor Cells from the Body

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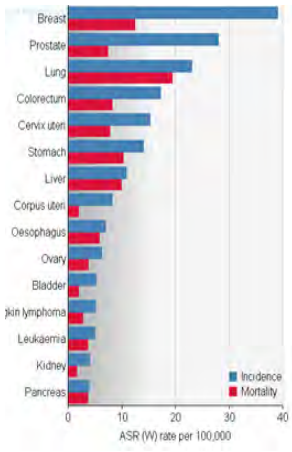

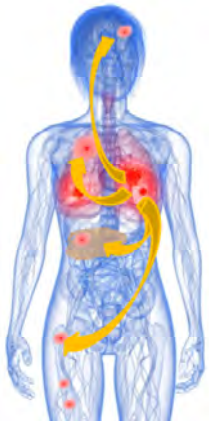

Each year, almost 1.6 million new cases of cancer are diagnosed and over 570,000 people will die of cancer. Metastases are responsible for about 90% of these deaths.

Fatal metastases affect patients with all major cancers, such as lung, breast, prostate, colon. Patients are left suffering under the threat of relapse after successful primary therapy.

Metastases form when aggressive circulating tumor cells (CTCs) from a primary site enter distal tissues to grow into secondary tumors. These CTCs seed new and maintain existing tumors.

Blood vessels and the lymphatic system act as a conduit to transport the malignant circulating tumor cells of many aggressive solid tumors.

We are developing a novel approach by which tumor cells are magnetically removed from circulation through a minimally invasive procedure. We demonstrated that we can successfully capture functionalized magnetic nanoparticles from circulation *in vivo* without obvious side effects to the mice. Microparticles were injected intravenously and recovered after three hours of circulation by applying a small magnet to the tail vein.

Our active magnetic mixing technology can significantly enhance the kinetics of binding. We envision using a flexible arm patch for magnetic mixing and a stronger bracelet for magnetic collection and removal.

Our goal is to reduce the risk of metastases formation or cure the patient without the need for invasive and expensive therapy and hospitalization. Treatment results will translate into effective personalized treatment plans that augment limited existing treatment options for many cancers after primary therapy.

Injection of nanoparticles | Incubation and mixing | Collection of labeled CTCs | Removal by venipuncture

We gratefully acknowledge research funding for this project by the State of Hessen, Germany, and corporate support by Merck KGaA, Darmstadt, Germany.

Poster 99

Multifunctional Magnetic Hydrogels as Cancer Nanotheranostic Agents

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A growing number of stimuli-responsive nanoscale drug-delivery vesicles have recently attracted great attention as combined diagnostic and therapeutic (theranostic) agents. In this work, we show our progress towards developing a multifunctional thermoresponsive magnetic nanostructure for non-invasive tumor destruction through hyperthermia and photodynamic therapy (PDT) as adjuvant cancer treatments. Here, we present a platform technology for a theranostic hydrogel system comprised of a biodegradable thermoresponsive polymer, magnetic nanoparticles and a photodynamic therapy (PDT) drug. The synergies between the magnetic, thermoresponsive and optical properties of the hydrogel nanosystem can result into a versatile platform for drug delivery, imaging and effective non-invasive cancer treatment.

Over the past years, iron oxide (Fe_3O_4) nanoparticles have been regarded as a promising nanoplatform for hyperthermia treatment because of their capability for magnetic field guided transport and efficient thermal energy transfer, which can result into sustainable heat release. By exploiting the great competence of this nanoparticle system for biomedical applications, we devote our efforts to develop a nanotheranostic agent in the form of multifunctional magnetic polymeric hydrogels. Here, we systemically explore methods to effectively incorporate ~30 nm Fe_3O_4 nanoparticles into a thermoresponsive polymeric matrix in order to facilitate biocompatibility, and efficient drug delivery and therapeutics. As a model system for our study, we explore nanohydrogels based on the extensively studied thermoresponsive polymer, poly(N-isopropylacrylamide) (PNIPAM). In prior reports, chitosan-grafted PNIPAM was found to have a lower critical solution temperature (LCST) value of ~42°C, in which the polymer transforms from a swollen state to a more compact shrunken state.[1] This behavior allows it to be a smart polymer capable of an on-off heat-triggered drug release mechanism. By covalently attaching a cancer therapy drug to the PNIPAM-encapsulated nanoparticles, a magnetically-controlled release behavior can be facilitated. For our initial studies, we use the photosensitizer, methylene blue (MB), which has been approved as a potent PDT drug for tumor treatment.[2] By subjecting the drug-loaded magnetic nanohydrogel to an AC magnetic field, the resulting hyperthermia effect allows the PDT drug to be expelled from the polymeric coating upon reaching the LCST, thereby promoting a triggered drug-release process. Combined with selective light excitation, our novel system can exhibit photo-induced cytoxicity towards cancer cell lines. In our study, we use real-time fiber optic fluorescence spectroscopy monitoring to investigate the drug release *in situ*. The resulting multifunctional magnetic hydrogels is characterized by a variety of electron microscopy techniques (i.e.TEM, SEM) and analytical methods such as AAS, DLS, TGA and DSC. This nanoplatform serves as a versatile drug delivery system and provides a modular platform for the incorporation of other chemotherapeutic drugs (i.e. doxorubicin) and near-IR fluorescent PDT agents (i.e. IR700) to enable simultaneous optical and magnetic resonance imaging of cancers.

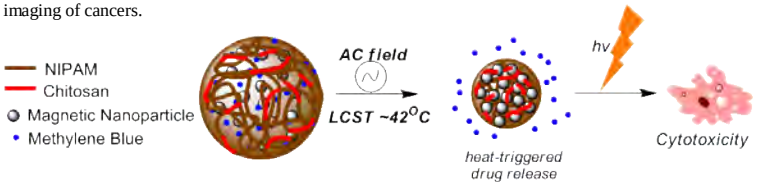

Figure 1. *Schematic representation of the mechanism of action of drug-loaded magnetic thermoresponsive hydrogel.*

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Poster 100

DNA Interaction of Pt-Decorated Iron Oxide Nanoparticles

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Small platinum (Pt) nanoparticles (< 5 nm) have recently shown great potential in therapeutic applications, such as double strand DNA dissociation, free radical scavenging and enhanced radiotherapy. However, such small sized Pt nanoparticles tend to aggreagate and difficult to target. Here, we report the synthsis, characterization, and DNA interaction of small Pt nanoparticle decorated iron oxide nanoparticles. These multifunctional nanoparticles could potentially provide silmultaneous imaging and therapy, where the small Pt nanoparticles sever as therapeutic agents and iron oxide nanoparticles can be used as contrast agents in magnetic resonance imaging.

First, multiple Pt-decorated iron oxide nanoparticles were synthesized using a previously reported procedure.[1] Second, the DNA (plasmid) interaction of these nanoparticles was studied by agarose gel electrophoresis (Figure 1). Figure 1 a and b shows transmission electron microscopy (TEM) images of the multiple Pt-attached iron oxide nanoparticles. The gel electrophoresis suggest two types of interactions betwee DNA and Pt-iron oxide integrated nanoparticles by comparing with control DNA(Figure 1c). One band that run slower than the control DNA was likely related to the plasmid DNA wraped aroud the whole integrated nanoparticles; the other band run slightly faster than the control DNA can be assigned to DNA attached with small Pt nanoparticles. The small Pt interacted DNA migrated faster likely because the DNA coiled around the small Pt nanoparticles.

This nanodrug system could potentially open up new possibilities in the design of antitumor agents using multifunctional nanoparticless. Continuous efforts are directed to investigate the *in vivo* characteristics of this nanodrug.

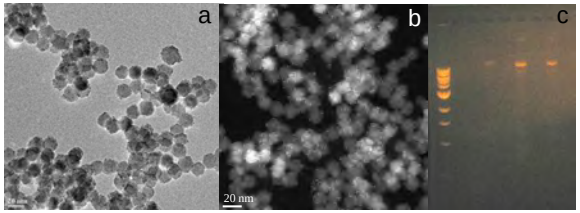

Figure 1. (a) Bright field TEM image of Pt-iron oxide nanoparticles, (b) dark field image of Pt-iron-oxide nanoparticles, and (c) DNA interaction agarose gel.

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Hetero-coated magnetic microcarriers for point-of-care diagnostics

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We report on the latest advances in the development of our magnetic encoded microcarriers [1]. Thin magnetic strips ('bits') are encapsulated in a biocompatible polymer backbone to form `tags'. The tags can be used to generate a large library of magnetically labelled bio-chemical analytes. Since the magnetic encoding can be applied post fabrication, all microcarriers are nominally identical, which makes them a cost effective micro-tagging strategy [2]. The number of unique codes doubles with every extra bit added, which also makes magnetic encoding extremely scalable. For instance a 7-bit tag offers 2^7=128 codes, but a 32-bit tag would offer over 4 billion unique codes. Applications range from DNA/protein analysis for genotyping and point-of-care diagnostics to drug development and combinatorial chemistry.

At the last meeting, we focussed on some novel aspects of SU8 surface chemistry and the effects of various linker molecules on binding efficiency [3]. Since then we have introduced a thin layer of gold on to one side of the microcarriers to provide a second functional coating. With this, we can now pursue two different chemical routes (carbodiimide chemistry and thiol containing self assembled monolayers) to add particular probe molecules to each side. While one probe remains specific to the analyte of interest, the other acts as a hybridisation control to interrogate the assay's binding conditions.

The complimentary target (pre-labelled with TAMRA) is added to the sample serum as a positive control. This eliminates the possibility of seeing no fluorescence and not being confident of whether the analyte was absent (true negative) or whether the binding conditions were insufficient (false negative). The target strand can be labelled with a different colour, e.g. with PicoGreen in an additional step, so that now a positive result requires the microcarrier to fluoresce red on one side and green on the other. As can be seen from the figure, there is a clear signature (peaks vs troughs) in the intensity profile corresponding to the microcarrier's orientation.

The microcarriers are read in-flow through a 50µm wide channel, which includes a TMR sensor able to detect the stray field (magnetic signature) of the passing microcarrier [4]. Thus, by combining the fluorescence data and the TMR data it is possible to conduct a multiplexed assay very quickly and cost-effectively on a small footprint device.

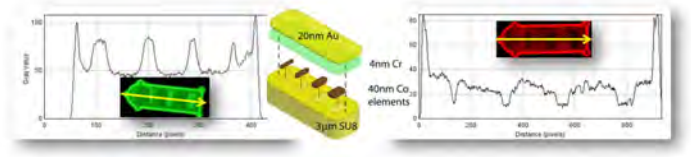

Figure: Exploded schematic of a hetero-functional magnetic microcarrier (centre) and line-intensity profiles showing microcarriers dual-labelled with fluorescein and TAMRA. The peaks and troughs are indicative of whether the fluorescence is on the top or bottom side respectively.

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Towards an optimized preparation of negatively-charged SPION

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A typical way of transferring SPION from organic solvents to aqueous solutions is by using a ligand-exchange strategy (1,2), where the hydrophobic molecules coating the surface of the SPION are replaced by hydrophilic molecules that also have affinity for the surface. The new ligand may also be the basis for subsequent functionalization steps of the SPION and therefore, different ligands with different reactivity are chosen depending on the desired application. In this work, we have explored two routes to produce hydrophilic SPION. Nanoparticle syntheses were performed both in aqueous solution and organic solvent, leading to hydrophilic and hydrophobic SPION, respectively. The hydrophilic SPION were synthesized by a one-pot hydrothermal route and stabilized by ascorbic acid (3), while the hydrophobic were synthesized by thermal decomposition of $Fe(acac)_3$ in benzyl ether in the presence of oleic acid and oleylamine, for particle stabilization(4). To transfer the latter into aqueous solution, the oleic moieties were exchanged by citric acid through a ligand-exchange reaction (1) which was performed at different temperatures (100°C, 60°C and room temperature). Samples were characterized by DLS, TEM and VSM. We have obtained hydrophilic SPION with aggregate sizes between 15 nm and 170 nm, where the larger aggregates resulted from the one-pot synthesis in aqueous media in the presence of ascorbic acid. Although presenting larger hydrodynamic diameters and higher zeta potential value then hydrophilic SPION obtained through ligand exchange, these monodisperse particles had the same polydispersity index as the hydrophobic SPION prior to phase transfer. For the hydrophilic SPION obtained through phase transfer from organic solvent to aqueous media, we showed that temperature plays a critical role, being the reaction favored at higher temperatures (Figure 1). The developed protocols are useful for the development of multifunctional SPION for clinical applications.

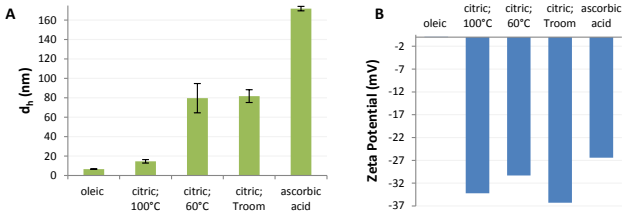

Figure 1. Size and charge characteristics of the prepared SPION. (A) - Mean hydrodynamic diameter, taken from the size distribution by intensity measured by Dynamic Light Scattering; (B) - Zeta Potential.

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Poster 103

Magnetic Silica Composite with Satellite Ag Nanoparticles: Magnetically Recyclable Nanocatalyst and Antibacterial Material

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Magnetic silica composite with satellite silver nanoparticles (nAg@MSC) was fabricated and applied for a recyclable nanocatalyst and antibacterial material. The superparamagnetic cluster (~300 nm in diameter) of Fe_3O_4 nanoparticles incorporated in the center of the composites provided strong magnetic response to external fields at room temperature, allowing efficient magnetic separation of nAg@MSC. Silver nanoparticle satellites (~5 nm in diameter) were firmly immobilized on the outer layer of the MSC using aminopropyl moieties.

To illustrate the potential catalytic efficacy, the nAg@MSC was tested in a model reaction, which reduces 4-nitrophenol by $NaBH_4$ in aqueous solution. The reaction was monitored by UV-Vis spectroscopy. The 4-nitrophenol was completely reduced to 4-aminophenol within 20 minutes in the presence of nAg@MSC, while the reaction did not proceed without nAg@MSC even with a large excess amount of $NaBH_4$. Moreover, nAg@MSC was successfully reused for five cycles and all the reaction cycles were completed with 100 % conversion yield within 20 minutes. Even after 5 cycles of catalytic reaction, silver nanoparticles did not aggregate or change the shape on the TEM images.

Along with the catalytic activity of nAg@MSC, their antibacterial activity was also evaluated using *Escherichia coli* (gram-negative bacteria). The composite material exhibited excellent antibacterial properties against *Escherichia coli*, furthermore, nAg@MSC could be easily removed from the reaction mixture by using an external magnetic field to avoid contamination of environment. Therefore, our novel composite nAg@MSC is believed as the promising reusable nanocatalyst for industrially important catalytic reactions, and antibacterial material.

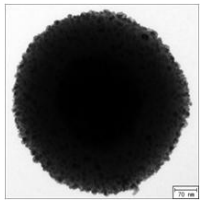

Figure 1. TEM image of nAg@MSC.

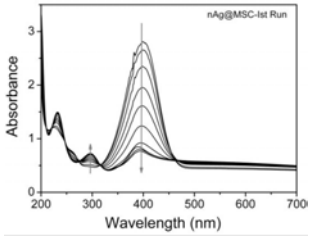

Figure 2. Temporal evolution of catalytic reaction.

Poster 104

Chitosan-coated Magnetic Nanoparticles as a Carrier for Delivery of Urokinase in Targeted Thrombolysis

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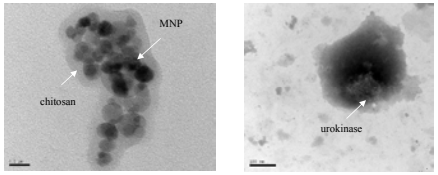
Fibrinolysis induced by tissue plasminogen activator is currently the only approved therapy for treatment of acute ischemic stroke. Delivery of urokinase-type tissue plasminogen activator by binding the thrombolytic drug to a magnetic nanoparticle (MNP) as a drug carrier will ensure the drug to be delivered under magnetic guidance and retained in a local area in circulation, which is potentially useful for targeting fibrin clot in vivo. MNP as a drug carrier is usually composed of a magnetite core with superparamagnetic characteristics, and a polymer coating layer providing functional groups for drug binding, inhibiting aggregation, and increasing colloidal stability. Chitosan is a highly cytocompatible polysaccharide coating material for MNP. It stabilizes the nanoparticle colloid by its positively charged amine groups, thus creating a low-fouling surface-coating layer to limit nonspecific protein adsorption, and providing abundant anchor functional groups on particle surface for covalent binding with drug molecules.

In this study, we examine the feasibility to use chitosan-MNP as a magnetic nanocarrier for delivery of urokinase. Chitosan coating on the particle surface provides abundant $-NH_2$ functional groups for conjugating with urokinase through glutaraldehyde-mediated covalent bond formation. *In vitro* amidolytic activity of urokinase was determined by chromogenic substrate assay. Thrombolysis effect of free *vs.* immobilized urokinase (U) on clot induced by $CaCl_2$ was measured with thromboelastography. Urokinase at concentration of 30 to 300 U/ml induced concentration-dependent thrombolysis. An overall lysis induced by free *vs*. immobilized urokinase (300 U/ml; with MNP 58 g/ml) was 77 ± 8% *vs.* 65 ± 11%, respectively. Pre-incubation of free urokinase with whole blood for 5 min attenuated the thrombolysis activity by 58%, whereas the activity of immobilized urokinase was not attenuated. The results suggest that the activity of immobilized urokinase may be protected from the inhibitors in the blood. Similar enhanced storage stability in buffer at 4 °C was also observed for immobilized urokinase *vs*. free urokinase (98% *vs.* 60% after 40 days). Effective thrombolysis with immobilized urokinase under magnetic guidance is demonstrated in an *ex vivo* intravascular thrombolysis model where 40% reduction in blood clot lysis time was observed compared with runs without magnetic targeting or with free urokinase using the same drug dosage.



TEM images of chitosan-MNP (left, bar = 200 nm) and urokinase bound to chitosan-MNP (right, bar = 100 nm) after staining with phosphotungstic acid.

Poster 105

Integrating technologies for the development of peanut allergen detection bio-assays

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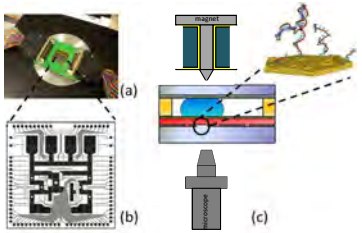
Peanut allergy is a common and often severe condition with no medical treatment available so far and the only existing therapy being avoidance of allergen-containing food. Because rigorous labeling of products is required, it is essential to improve the performance of current bio-assays for detecting potential allergens in food samples. In order to challenge this problem, we are employing magnetic detection technology combined with bioreceptors against one of the most important peanut allergens, Arah1 protein. Magnetic fields are used to increase the speed of the assay by transporting the on bead captured Arah1 proteins to the detection surface while magnetic forces discriminate specific from non-specific bonds between the particles and the surface.

In this assay we used both antibodies against Arah1 protein as well as aptamers selected and characterized by our group[1]. An aptamer with high affinity (Kd 92.3 nM) and specificity for Arah1 was selected using capillary electrophoresis (CE)-SELEX approach. The performance of this bioreceptor is compared to already established biosensing technology with antibody coated superparamagnetic particles that capture Arah1 protein. In these model experiments we apply magnetic forces to the Arah1 protein-antibody bond and investigate dissociation properties of such a formed complex. For this purpose one of the biomolecules is immobilized on the surface of superparamagnetic particles while the complementary molecule is on a solid phase.

The assay is performed using an instrumental set-up consisting of: (1) a sample holder that supports the fluid cell in which the beads are incubated, (2) a microscope-camera system for imaging the particles and (3) an electromagnet to apply a constant mechanical pulling force in picoNewton regime. When applying a force, a measurement of the time dependence dissociation reveals the rate constant (k_{off}) and binding characteristics. In addition, the necessary applied force needed for the removal of unbound and non-specifically bound particles can be measured. We consider this a very promising technology as understanding the interaction between Arah1 protein and either of its bioreceptors will help in further optimization of novel assay technologies for achieving higher sensitivity and specificity.

In a later phase, we will integrate the magnetic detection technology with a digital microfluidic chip. Digital microfluidics involves the individual droplet (300 nL) movement on a planar surface by means of the 'electrowetting-on-dielectric' principle. The integration allows the execution of this force-discrimination assay in a highly automated and miniaturized way. Schematic images of the complete setup and images of chip design and holder are drawn in the figure below. The digital lab-on-a-chip will be biofunctionalized by the creation of spatially controlled micropatches[2], allowing the covalent attachment of aptamers and antibodies against Arah1 protein on the hydrophobic chip surface.



(a) Chip holder, placed on inverted microscope; (b) chip design; (c) scheme of integrating magnetic detection system with biofunctionalized digital chip.

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Poster 106

Towards a multifunctional nano-object for optical and magnetic detection of the sentinel node in early breast cancers

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Magnetic markers are already used for magnetic resonance imaging (MRI). The generally labelled SPIO (Super Paramagnetic Iron Oxide) are made of nanoparticles of less than 10 nm in size, coated with polymers. These biocompatible coatings are able to promote dispersion in aqueous media, but they are not robust and can easily be detached from particle surfaces under *in vivo* conditions. In order to increase the grafting efficiency and to ensure the possibility of tuning the characteristics (morphology, functionalities, physico-chemical properties …) of the organic coating, we have recently proposed an innovative strategy based on a combined use of dendritic molecules and phosphonic acid as coupling agent.[1] This strategy has been applied to design multimodal markers intended to be detected by magnetic and/or optical hand-held probes. With this aim in view, we synthesized batches of magnetic iron oxide (IO) of controlled sizes and narrow size dispersion, and pegylated dendrons bearing (**1**) or not bearing (**2**) the vital blue dye (Patent Blue VF) at the periphery of the central tetraethyleneglycol branch.[2,3]

We present here the markers obtained by grafting the dendrons **1** and **2** on IO nanoparticles (NPs) of 10 ± 3 nm (NP10) or 20 ± 5 nm (NP20) in size. They were shown to be non toxic on red blood cells (RBCs) and Caco-2 cells. Investigating two different sizes of NPs is quite interesting since the smallest are superparamagnetic at room temperature and can be used for magnetic resonance imaging, while the largest (NP20) present an easy magnetization direction, a small magnetic hysteresis and a higher magnetization favourable to detection by a magnetic probe. Animal experimentations were performed using a rat model of lymph node hyperplasia. After injection, the blue dye was detected by an optical probe. Chemical analyses of iron concentration in the nodes pointed out an accumulation of iron when nanoparticles of 20 nm were injected. Finally, comparing a mixture of dye and dendron **2**-grafted NP (NP10@**2** and NP20@**2**) to dye-derivatized dendronized NPs (NP10@**1** and NP20@**1**) highlighted a strong dye-nanoparticle binding which survives *in-vivo* conditions.

* This work was supported by the European community through the NANOMAGDYE, CP-FP 214032-2(7thPCRD2007-13) and the NANOMATRIX project (Interreg IV Oberrhein Rhin supérieur A21)

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Biodegradation of Magnetic Nanoparticles in Rat Brain Studied by Mössbauer Spectroscopy

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Drug delivery to specific locations in the brain is a challenge in the treatment of diseases related to central nervous system. The main difficulty in solving this problem is to pass through the blood-brain barrier. The blood-brain barrier is a barrier system of endothelial cells that separates the blood from the underlying brain cells, providing protection to brain cells. Colloidal drugs such as nanoparticle dispersions consisting of particles of 10 - 100 nm in diameter show a great promise as drug delivery systems for this task. The advantage of using magnetic nanoparticles is two-fold. First, an external magnetic field can be used to increase the concentration of the nanoparticles in the brain. Second, the localization of the magnetic particles inside the brain can be controlled by magnetic resonance imaging method. One of the key challenges for the nanomedical agent is its fast and full biodegradation and/or clearance from the brain.

Direct transcranial injection of ferrofluid with superparamagnetic Fe_3O_4 nanoparticles in the ventricle of the rat brain

The brain has four major fluid compartments: 1) the blood that flows through entire brain structures, 2) the interstitial fluid bathing neurons and neuroglia, 3) the cerebrospinal fluid that circulates around brain ventricles and spinal cord, 4) the intracellular fluid within brain cells. There is no barrier between the interstitial and the cerebrospinal fluid. Thus, nanoparticles staying in either of these two fluid compartments are free to exchange and reach entire brain structures. In this work we investigated the biotransformation of magnetic nanoparticles, injected in cerebrospinal fluid in the brain of rats. Transcranial procedure invasively bypasses the blood-brain barrier by drilling a hole in the skull and injecting the magnetic nanoparticles intracerebrally. At present, intracerebral injection is the "gold standard" for *in vivo* brain experiments.

In our study the superparamagnetic particles of Fe_3O_4 in ferrofluid were injected transcranially in the ventricle of the rat brain. At three months after the injection the rat was sacrificed and its brain was investigated. To obtain quantitative information about the biodegradation process in the brain we used our experimental method, based on Mössbauer spectroscopy, and presented at the previous Conference in Rostock and at the Conference "Frontiers in BioMagnetic Particles 2" in Charleston. The method is based on measuring the Mössbauer spectra of each sample of the brain at two different temperatures and in an external magnetic field and the subsequent simultaneous fit of these three Mössbauer spectra, corresponding to the same stage of the nanoparticles biodegradation, with one set of parameters. The method makes it possible to separate both the partial superparamagnetic component of the spectra, which characterizes changes of exogenous magnetic nanoparticles in the process of their biodegradation, and the partial paramagnetic component of the spectra, which characterizes changes of endogenous ferritin-like proteins.

Synthesis and Characterization of Hybrid Magnetic-Plasmonic Nanoparticles

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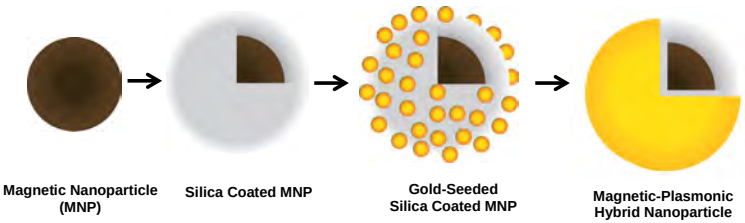
Magnetic-plasmonic nanoparticles continue to attract research interest due to their great potential in biosensing and drug delivery applications. The integration of noble metals and their associated localized surface plasmons into nanostructured magnetic materials provides a unique system for investigating the interaction of magnetic and plasmonic properties,[1,2] and can give rise to a useful strategy towards designing smart nanosensors.

In this work, we describe the fabrication of tunable magnetic-plasmonic hybrid nanoparticles that are comprised of plasmonic gold nanoshells deposited on top of silica coated iron oxide magnetic nanoparticles. By tuning the thickness of the intermediate silica layer, the interaction between the gold nanoshell and the magnetic nanostructure is systematically investigated. Moreover, by using thermal decomposition synthesis methods, we are able to prepare monodisperse iron oxide nanoparticles of different compositions, which facilitate the tuning of the magnetic properties of the iron oxide core nanomaterial. The as-prepared magnetic nanoparticles are then coated with a silica layer using a microemulsion synthesis method, after which the surface is functionalized with (3-aminopropyl)triethoxysilane (APTES) to introduce an amine-terminated silane coating that promotes the addition of gold seeds on the surface. The resulting gold-seeded nanoparticles are then added to a gold plating solution, which by reduction with formaldehyde forms a continuous plasmonic nanoshell.

We can follow the formation of the magnetic-plasmonic hybrid nanoparticles using transmission electron microscopy (TEM), which allows the investigation of the shape, composition, and size distribution of the nanoparticles. To monitor the optical properties of the hybrid magnetic-plasmonic nanoparticles we use visible to near-IR spectrophotometry. Moreover, using an inductive heating apparatus we evaluate the hyperthermia properties of the hybrid nanoparticles.



Schematic representation of the formation of the hybrid magnetic-plasmonic nanoparticles.

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FORMULATION DEVELOPMENT AND PHARMACEUTICAL EVALUATION OF LORNOXICAM MAGNETIC NANOEMULSION

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Lornoxicam is a potential NSAID belonging to the oxicam class widely used as analgesic, anti-inflammatory and anti-arthritic agent, with plasma half-life of 3 – 5 hours. In this study, lornoxicam magnetic nanoemulsion (LMNE) was developed and the possible use in targeted and sustained release drug therapy in animal model of rheumatoid arthritis was evaluated. Lornoxicam was dissolved in chloroform and diluted with ethyl alcohol; Tween 80 and Span 80 were added to the oil phase of oleic acid. Aqueous ferrofluid was synthesized by co-precipitation method by mixing ferric and ferrous salts in the ratio of (2:1) under alkaline conditions. Lornoxicam magnetic nanoemulsion was formulated by spontaneous emulsification method and achieved by slow addition of synthesized aqueous ferrofluid to oil phase, controlled evaporation of organic solvent under stirring followed by exposure to ultrasonication. FT-IR spectra revealed no chemical interaction exists between the drug and other excipients used in the formulation. Physical and related pharmaceutical parameters such as type of emulsion, droplet size, viscosity, refractive index, zeta potential and encapsulation efficiency, saturation of magnetization curve, percentage drug release, pattern of drug release and stability of LMNE were evaluated and shown in Table 1 and Fig. 1. Transmission electron microscopy revealed spherical emulsion droplets with magnetic material in the core Fig. 2. Magnetic targeting and therapeutic efficacy of filter sterilized lornoxicam magnetic nanoemulsion at various i.v. doses upon filtration sterilization were analyzed in adjuvant induced arthritis model Fig. 3. The developed lornoxicam magnetic nanoemulsion might be useful to provide sustained drug release in the targeted site.

Table 1. Pharmaceutical parameters of LMNE

Parameters	Results
Type of emulsion	Oil in water
Mean droplet size	120 nm
Viscosity	0.8872 cP
Refractive index	1.3300 ± 0.002
Zeta potential	−17 mV
Encapsulation efficiency	85.2%
Saturation of Magnetization	17.6 emu/g @ 9000G
% Drug release	91.5% in 36 hours
Type of drug release	Zero order release
Stability	> 3 months

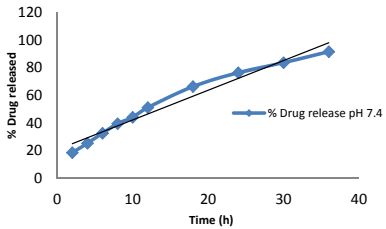

Fig 1. Drug release pattern of LMNE

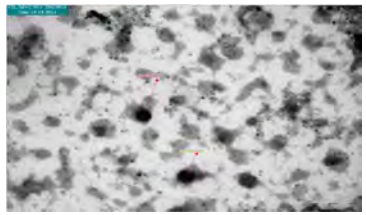

Fig 2. TEM image of LMNE

Fig 3. In-vivo drug targeting of LMNE

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Protein-Templated Magnetic Nanoparticle

Tanya Prozorov, Carmen Valverde-Tercedor, Concepcion Jimenez-Lopez,
Dennis A. Bazylinski, Surya K. Mallapragada, Marit Nilsen-Hamilton, Ruslan Prozorov

The biomineralization of magnetotactic bacterial magnetite nanoparticles is a topic of intense research due to the particles' narrow size distribution, and magnetic properties. Magnetic nanoparticles with narrow size distribution, large magnetic moment and controlled magnetic anisotropy have important technological applications in a wide variety of areas, from high density data storage and ferrofluidic devices, to quantum computing and targeted drug delivery. For many applications, the particles must have well-defined shape, exhibit good crystallinity, belong to one of the highly magnetic compounds, such as magnetite or cobalt ferrite, and be just below the superparamagnetic limit as to remain in a monodomain state at the operation temperature.

Uniform magnetic nanocrystals are synthesized via a template growth in the presence of the recombinant iron-binding protein, Mms6. Use of low-temperature synthetic approach permits control over size, shape, and orientation of the magnetic nanostructured crystals. The protein-templating mechanism is probed via numerous analytical techniques, including transmission electron microscopy, magnetization measurements, magneto-optical imaging, and X-ray photoelectron spectroscopy. Uniform magnetic nanocrystals can be further functionalized to address specific applications. Effect of the synthetic route and incorporation of impurities on the magnetic properties of synthesized magnetic nanostructured materials is investigated.

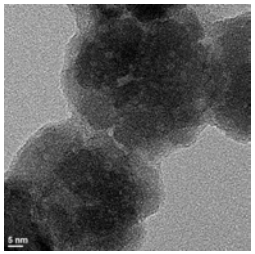

Figure 1. Image of magnetite nanocrystals formed in the presence of Mms6 taken at 360,000 magnification.

Specialized technique of tissue Doppler imaging for locating magnetic nanoparticles.

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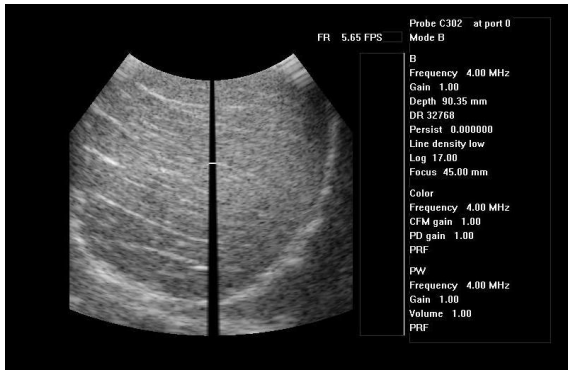
We continue to improve our new method of ultrasonic synchronous tissue Doppler imaging with magnetic modulation for *in vitro* and *in vivo* detection and visualization of magnetic ultradisperse particles in soft tissues. Use of higher ultrasound frequency in the new version of the prototype hardware with custom software increased its sensitivity to the presence of magnetic particles and modulating magnetic field intensity by one order of magnitude compared to the previous version of the device. The higher sensitivity combined with an increase of the modulating magnetic field frequency improved the spatial resolution of the method and reduced artifacts in the images.

A new improved version of the device (UDS-12) was created by the Medical Acoustical Imaging Center, Ltd in February 2012. The testing of the unit is underway, and the preliminary results are encouraging (see Figure).



The Effect of Ligand Molecule Length on the Size of Synthesized Iron Oxide Nanoparticles

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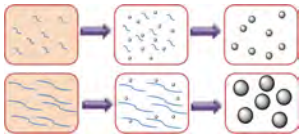
Iron oxide nanoparticles (IONPs) with controlled size and shape are preferred as magnetic carrier in theragnostics area. e.g magnetic resonance imaging (MRI) contrast agent, drug delivery carrier and magnetic hyperthermia. Saturated aliphatic amines, which work as a capping ligand in the reaction, has much higher purity comparing to commonly used oleylamine in thermodecomposition procedure and favors synthesizing nanoparticles with narrow size distribution as well as controlling size increment precisely. In previous results, it shows that the particles size may increase with increasing ligand concentration in some cases.[1,2] However a systematic study focused on ligand length effects on particles size have been are rarely reported.

To measure this effect, monodisperse IONPs were synthesized by decomposing iron(III) acetylacetonate ($Fe(acac)_3$) as precursor and selected 1-hexylamine, 1-dodecylamine and 1-octadecylamine capping ligands. Benzyl ether was used as the high boiling point solvent (300^oC) to keep the consistency of the reaction. Size evolution of IONPs during heating process was characterized. Thermostability of precursor in the presence of capping ligand had been measured by thermo gravimetric analysis (TGA). Particles size was characterized by transmission electron microscopy (TEM) images and dynamic light scattering (DLS) instrument.

The results indicate that the particles size would decrease with increasing ligand concentration, which is opposite to the published reports.[1,3] Crystal growth process was traced by UV-Vis spectrophotometry and which indicated that it could be restrained by increasing ligand molecule length as well. Magnetic properties were determined by vibrating sample magnetometer (VSM) and specific absorption rate (SAE) measurements. By investigating the kinetic process in nanoparticles synthesis, it is possible to prepare monodisperse nanoparticles with finely tuned size and shape, thereby obtain magnetic fluid with desired heating efficiency.



Schematic illustration of ligand length effect on nanoparticle size.

Reference

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Evaluation of gastrointestinal tract absorption and distribution patterns using ACB technique associated to magnetic nanoparticles

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The alternating current biosusceptometry (ACB) system is a biomagnetic technique quite successfully for studies regarding the gastrointestinal tract (GIT). Now, the association of an ACB system to magnetic nanostructured particles provides new approaches able to evaluate a whole other class of biological properties. By this new method it became possible to analyze the biological absorption pattern of a magnetic tracer from the stomach and gut through liver and its arrival on kidneys. It is also possible to obtain information about function and clearance of those organs, by absorption through the GIT. These magnetic tracers can still be monitored and related to the capability of each organ to absorb, retain and release a specific material. The main objective of this paper is to associate the gastrointestinal transit of magnetic marked solutions and its absorption, with the arrival of these particles in the liver and kidneys of rats to insure the relation between the ingestion of a determined particle, its absorption, arrival and clearance at those organs. The working principle of Alternating Current Biosusceptometry is based on a double magnetic flux transformer with air core. A single ACB sensor consists of two pairs of coils separated by a fixed distance (baseline). Each pair is composed of an excitation coil and a detection coil in a first-order gradiometric configuration. One pair works as the reference and the other as the detector probe, gathering information regarding the magnetic flux variation around the sensor. This variation is related to the magnetic tracer concentration in the analyzed region. This study was developed using a nanostructured non absorbable magnetic tracer (Manganese Ferrite – $MnFe_2O_4$) with 13.5 nm diameter, coated with citrate molecules. A marked solution was injected by gavage in a group of 6 male Wistar rats. After monitoring the variation of the magnetic material concentration in 5 pre-determined regions of each animal, related to stomach, cecum, liver, right and left kidneys, was plotted a magnetic tracer concentration through time for each specific region, shown in figure 1. Then, by monitoring the magnetic tracer arrival and clearance of each organ, it was possible to observe the relation between gastrointestinal transit, absorption and distribution patterns, and also liver and kidneys function. From the obtained data, it can be anticipated that it will be possible to constitute a normality parameter for each function and its relations, and analyze the normal behavior of each organ and each process, and also the influence of specific drugs, not only on one function or region, but also on the relation between two or more physiological process. Partial financial support: FAPESP, CAPES and CNPq.

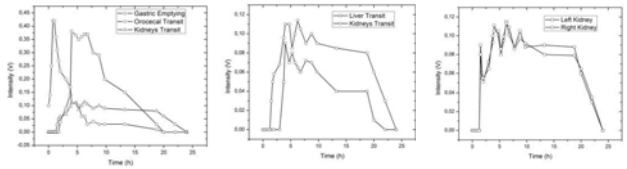

Figure 1: Illustration of the relation between each analyzed process.

Simulating magnetic particle interaction for targeted drug delivery

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Introduction

Accurate and efficient techniques for the simulation of magnetic drug and cell delivery are crucial in improving experimental design and allow insight into the expected retention *in vivo*. Previous numerical models have characterised particle dynamics in patient derived geometries subject to physiological flow rates [1]. However, these studies do not address the issue of particle interaction, which has so far only been addressed for small particle numbers [2]. Particle dynamics, particularly in the presence of strong magnetic fields, are dominated by magnetic dipole interactions resulting in chain-like aggregates [3]. Here this issue has been addressed for multiple particle interactions of fully and partially magnetised particles.

Methods

Modelled as particles suspended in a fluid, nanoparticles are represented as rigid particles which are subjected to fluid, magnetic, gravitational, electrostatic, inertial and contact forces. Using a discrete particle approach [4] the resulting dynamics of these particles are determined from the momentum balance that is integrated in time using an explicit centre difference method. In order to model the nonlinear response of magnetic particles to an applied magnetic field, a Langevin approximation was used. The magnetization of particles and their local magnetic field were calculated interactively for each time step. An adaptive integration time was used which limited the maximum displacement of particles to 2.5% of their diameter to avoid numerical instabilities and account for magnetization changes for particles at close proximity in weak fields. In the case of particle overlap where dji<0 (**Figure A**), contact forces are added to the force and torque balance.

Results

When particles are separated by a few particle diameters, drag and hydrodynamic interaction forces are dominant. However in close proximity magnetic forces are dominant. Simulations of particles in homogenous fields led to formation of chains aligned with the main field (**Figure B**). Inhomogeneous fields also leads to chain formation, however particle movement is dominated by the field gradient. The coefficient for the rolling friction had a strong influence on the stability of chains during movement and the impact of other particles.
Figure C illustrates some of the forces acting between two particles. One particle has a fixed position while the other one is attracted to the first particle due to the magnetic forces. The magnetic force increases rapidly when the particles are coming into contact and during the overlap. Van der Waals forces become strongly repulsive when particle overlap (the force has been limited to a minimum separation of 10 nm). A strong elastic response in the linear contact force can be observed as this model used hard spheres. Since the two particles do not collide along with their two centres perfectly aligned, a small tangential interaction force resumes. Simulations with a large variation in particle diameter lead to faster chain formation compared to a uniform particle diameter.

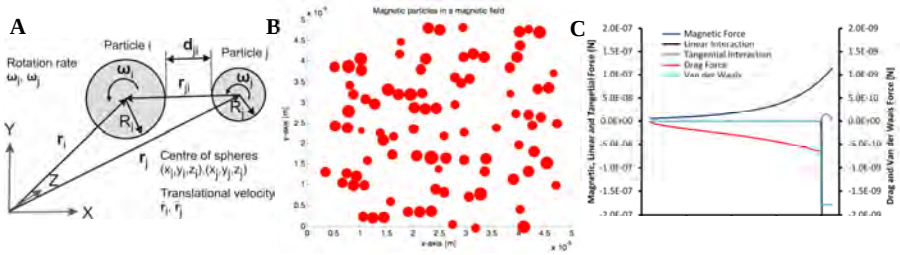

Figure A: Particle-particle kinematic variables; Figure B: Many particle simulation showing chain formation 100us after starting with particles distributed at grid points. Figure C: Some of the forces acting between two particles. The reference particle is fixed in place while the particle under investigation is attracted by the magnetic force.

Conclusions

Discrete particle models can be employed to model the behaviour of magnetic particle suspensions and can potentially be integrated into a macroscopic model of fluid flow for more realistic simulations of magnetic drug delivery. Such simulations can be used to optimize particle diameter distribution for different magnetic field geometries employed during magnetic targeting applications.

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High Saturation Magnetization FeCo Nanostructures

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High-saturation FeCo nanostructures have been considered to have great potentials for many technological applications including biomedical sectors [1], [2]. However, there are two major problems with the FeCo nanostructure developed so far: one is that the grain sizes are larger than the sizes of the biomolecule cells and the other is that the maximum room temperature saturation magnetization, M_s, is still low, primarily due to the oxide formation during growth. These issues have significantly limited their use in biomagnetic sensing, for example. Furthermore, the relationship between the internal state of FeCo nanostructures and M_s has not yet been fully understood, and the questions as to how M_s of these nanostructures can be enhanced, remains unanswered.

The physical properties of the FeCo nanostructures are governed by the methods in which they are developed [2], [3]. In this study, FeCo nanostructures have been produced by alternately stacking Fe and FeCo layers in various ratios using pulsed-current deposition, similar to the one given in [4]. The relationship between the pulsed-current and the nanostructure is shown in Fig. 1. The pulsed-current deposition favors the initiation of grain nuclei and results in fine-grained deposits with better physical properties over the nanostructures prepared using other methods [4].

The effect of stacking number on M_s at various compositions was studied using a vibrating sample magnetometer. The result is presented in Fig. 2. Only the results of nanostructures grown at Co at % 25 are presented here. The nanostructure exhibited dramatically high M_s of up to 240 emu/g at room temperature as the stacking number increased. We will explain the relationship between the enhanced M_s and the lattice constant both using microstructural studies and 3-d band models. The increase in M_s with n is considered to be due to the intermixing of atoms in the atomic level.

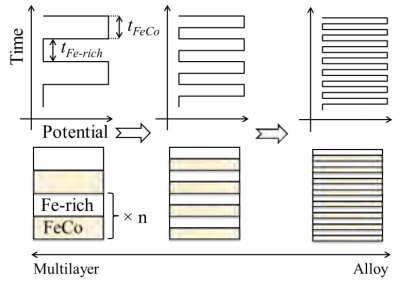

Fig. 1 Schematic of the pulsed-current and the corresponding nanostructure. The notation n outside the parenthesis denotes the stacking number.

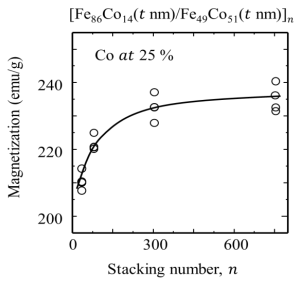

Fig. 2 Saturation magnetization versus stacking number.

If the biocompatible magnetic nanostructures presented in this work are labelled with the cancer biomarkers and used in conjunction with the giant magnetoresistance sensors [5], these nanostructures have been considered to have great potential for use in biomagnetic sensing.

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Controlled Targeting and Biodistribution of Magnetic Nanoparticles Across the Blood-Brain Barrier

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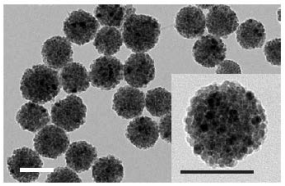
Blood-brain barrier (BBB) is a major challenge for an effective delivery of therapeutic or diagnostic agents to the brain parenchyma. Here we demonstrate in an *in vivo* mouse model, the selective accumulation of magnetic nanoparticles (MNPs) in the brain under an external magnetic field. Following their systemic administration, MNPs permeate across the BBB and localize within a narrow perivascular zone under an applied external magnetic field. Fluorescent labeling of MNPs enabled their direct tracking and cellular localization inside endothelial cells and in the perivascular extracellular matrix *in vivo*. To address the important concern of nanoparticle toxicity, we assessed the effects of MNPs on the brain parenchyma using a non-invasive reporter for astrogliosis, biochemical and histological studies. None of these readouts demonstrated any acute or long-term toxicity indicating that these engineered MNPs were inert and safe. Further analysis using *in vitro* cell culture models and atomic force microscopy demonstrated that MNPs were internalized by endothelial cells, suggesting that trans-cellular trafficking may be a mechanism for the BBB crossing observed *in vivo*. Together, these results establish an effective strategy for regulating the biodistribution of MNPs in the brain through the application of an external magnetic field.

(a)



(b)

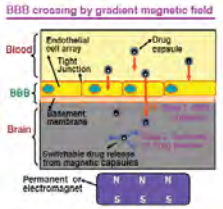


Figure. (a) TEM micrograph showing trapped magnetic nanoparticles in MNPs. Scale bar, 100 nm. (b) Schematic illustration of the extravasation of MNPs. Translocation of MNPs via DC gradient magnetic field is processed first, followed by switchable drug release inside the brain parenchyma.

Title: Enhancing Magnetic Properties of Cyanide Based Molecular Magnetic Materials: The role of Single Ion Anisotropy

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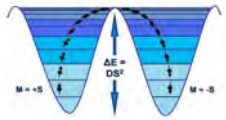

Figure 1 Energy Level Diagram for Single Molecule Magnet

Single Molecule Magnets (SMMs) are a remarkable class of molecules that display magnetic bistability of a molecular origin which arises from the combination of a high ground state electron spin (S) and a large negative zero-field splitting $|D|$. This magnetic bistability provides an excellent potential for molecular spintronics. A major focus in this field is to increase the barrier to the reversal of the magnetization by tuning the anisotropy parameters and ground state spin value in order to increase the blocking temperature T_b to a range suitable for applications. Much attention has been recently directed at complexes containing heavier transition metal ions for their high spin orbit coupling terms as well as diffuse d orbitals which provide better overlap with cyanide orbitals, leading to large exchange constants |J|. In this vein, our group has previously reported a highly anisotropic building block $[Re^{II}(triphos)(CN)_3]^-$ (triphos = 1,1,1-tris(diphenylphosphino-methyl)ethane)) that was incorporated in a family of molecular cubes $[Re^{II}(triphos)(CN)_3]_4[M^{II}Cl]_4$ (M = Mn, Fe, Co, Ni and Cu). The $[Re^{II}_4Mn^{II}_4]$ molecular cube **1** was found to behave as a SMM. Current efforts focus on enhancing the magnetic properties of these clusters by introducing more single ion anisotropy using anisotropic manganese salen-type complexes. The reaction with $[(Mn(5\text{-}Br\text{-}Salen))_3]ClO_4$ resulted in cluster **3** which exhibits very weak antiferromagnetic interactions. The $[Re^{II}(triphos)(CN)_3]^-$ building block was also incorporated in a family of trigonal bipyramidal clusters (TBP) to test the hypothesis that lanthanide assemblies with axial crystal field could give rise to SMM behavior. The Re/Sm TBP **2** shows very large temperature independent paramagnetism.

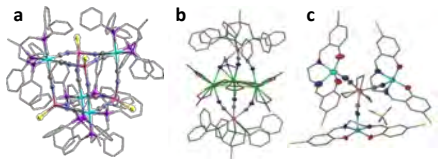

Figure 2 Structure of: a) $[Re^{II}(triphos)(CN)_3]_4[Mn^{II}Cl]_4$ (**1**) b) $[Re^{II}(triphos)(CN)_3(Sm(NO_3)_3)_3]^{2-}$ (**2**) c) $[Re^{II}(triphos)(CN)_3(Mn(5Br\text{-}Salen))_3]^+$(**3**)

Biography

M.R.Saber has completed his MSc from Fayoum University, Egypt. He joined Gabbai's research group at the chemistry department, Texas A&M University, as a Fulbright visiting scholar for 10 months then he started his PhD in the Dunbar research group. He has published 4 papers in reputed journals and participated in several ACS meetings as well as the Austrian Physical Society Meeting 58 [OPG 58].

Acute Necrosis and Reactive Oxygen Species Generation by Magnetic Hyperthermia Leads to Effective Elimination of Cancer Stem Cells

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Cancer stem cells (CSCs) are a sub-population of stem-like cells that are thought to be responsible for tumor drug resistance and relapse. Therapies that can effectively eliminate CSCs will, therefore, likely decrease tumor recurrence. The objective of our study was to determine the susceptibility of CSCs to magnetic hyperthermia, a treatment that utilizes superparamagnetic iron oxide nanoparticles (SPIO NPs) placed in an alternating magnetic field to generate localized heat and selective tumor cell kill.

SPIO NPs synthesized in this study had a magnetite core of 12 ± 3 nm and a saturation magnetization of 58 emu/g of magnetite. The heating rates were dependent on the concentration of SPIO NPs; a 2.5 mg/ml concentration of SPIO NP was found to increase the temperature of the dispersion from 37°C to 43°C in less than 5 minutes when placed in an AMF of 6 kA/m and frequency of 386 kHz. This heating rate induced effective cell death in both A549 (non-small cell lung cancer) and MDA-MB-231 (breast adenocarcinoma) cells. Multiple assays for CSCs, including side population phenotype, aldehyde dehydrogenase expression, mammosphere formation and in vivo xenotransplantation, indicated that magnetic hyperthermia reduced or, in some cases, eliminated the CSC sub-population in treated cells. Interestingly, conventional hyperthermia, induced by subjecting cells to elevated temperature (46°C) in a water bath, was not effective in eliminating CSCs.

Our studies show that magnetic hyperthermia has pleiotropic effects, inducing acute necrosis in some cells while stimulating reactive oxygen species generation and slower cell kill in others. These results suggest the potential for lower rates of tumor recurrence after magnetic hyperthermia compared to conventional cancer therapies.

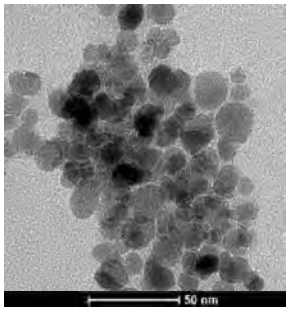

Figure – TEM image of SPIO NPs

Magnetically responsive biocomposite materials for water technology applications

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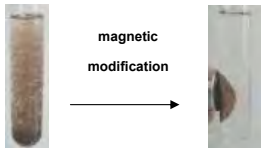
Magnetically responsive nano- and microparticles used in various areas of biosciences, medicine, biotechnology, environmental technology etc. can be usually described as composite materials, where the magnetic properties are caused by the presence of magnetic iron oxides nano- or microparticles. In addition to magnetic composites based on synthetic or inorganic materials, a large amount of magnetic biocomposites has been developed and used. In many cases magnetic fluids can be used to convert diamagnetic materials of biological origin (e.g., microbial and algae cells, plant-derived materials, activated carbon etc.) into their magnetic derivatives. Alternatively biological materials can be magnetically modified by binding of maghemite or magnetite nano- or microparticles on their surface during alkaline precipitation of ferrous and ferric ions, by heating of modified materials impregnated with iron or nickel salts at high temperatures or by encapsulation of the modified materials together with magnetic particles in an appropriate biopolymer gel.

Magnetic biocomposites have been successfully used as efficient adsorbents for the removal of both organic and inorganic xenobiotics, such as dyes, pesticides, heavy metal ions and radionuclides from water, exhibiting high maximum adsorption capacities. Magnetic biocomposites with immobilized enzymes and cells, as well as ferrofluid modified or gel entrapped living microbial cells can serve as efficient biocatalysts useful for the detection of important xenobiotics or degradation of signal molecules responsible for the formation of biofilms in water systems. Magnetic modification of biological materials enables to prepare stimuli responsive materials with a great potential for water technology applications.



Simple modification of biological materials leads to the formation of smart, magnetically responsive biocomposites

Universal Scaling Law to Predict the Efficiency of Magnetic Nanoparticles as MRI T2-Contrast Agents

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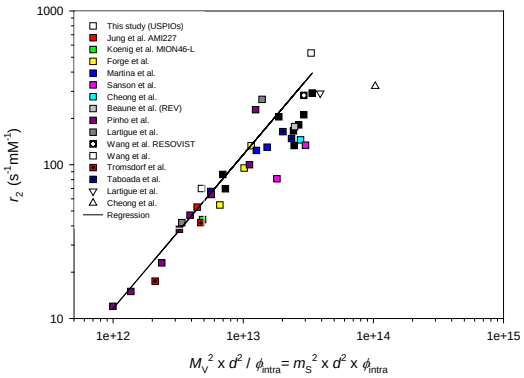
Magnetic particles are very efficient Magnetic Resonance Imaging (MRI) contrast agents. In the recent years, chemists have unleashed their imagination to design multi-functional nanoprobes for biomedical applications including MRI contrast enhancement. In this study, we focus on the direct relationship between the size and magnetization of the particles and their nuclear magnetic resonance relaxation properties, which condition their efficiency. Experimental relaxation results on maghemite particles exhibiting a wide range of sizes and magnetizations are compared to previously published data and to well-established relaxation theories with a good agreement. This allows us to derive the experimental master curve of the transverse relaxivity *versus* particle size and to predict the MRI contrast efficiency of any type of magnetic nanoparticles. This prediction only requires the knowledge of the size of the particles impermeable to water protons and the saturation magnetization of the corresponding volume. To predict the T_2 relaxation efficiency of magnetic single crystals, the crystal size and magnetization – obtained through a single Langevin fit of a magnetization curve – is the only information needed. For contrast agents made of several magnetic cores assembled into various geometries (dilute fractal aggregates, dense spherical clusters, core-shell micelles, hollow vesicles…), one needs to know a third parameter, namely the intra-aggregate volume fraction occupied by the magnetic materials relatively to the whole (hydrodynamic) sphere. Finally a calculation of the maximum achievable relaxation effect – and the size needed to reach this maximum – is performed for different cases: maghemite single crystals and dense clusters, core-shell particles (oxide layer around a metallic core) and zinc-manganese ferrite.



Data of transverse relaxivity r_2 at high field (≥1T) for samples of individual USPIOs and clusters (either of low size or dilute) satisfying the MAR condition, *i.e.* $\Delta\omega\tau_D<1$. The MAR is characterized by r_2 linear with the squares of magnetization M_v and of diameter d divided by the intra-aggregate volume fraction ϕ_{intra}. Introducing the specific magnetization m_S of magnetic cores in the cluster and relating it to the whole body magnetization $M_v=\phi_{intra}\times m_S$, r_2 becomes also linear with $m_S^2\times d^2\times\phi_{intra}$.

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Nanocomposite Biocompatible Magnetite-Encapsulated Hemoglobin

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The development of biocompatible nanocomposites has generated many studies in recent years by their applications in various fields such as biomedicine, electronics, microelectronics and others [1]. That work consists in the syntesis and caracterization of biocompatible magnetic nanocomposite (*BMNC*) from magnetite nanoparticle (*MNP*), synthesized using a co-precipitation technique, complexed in arrays of human hemoglobin (*Hb*) (Fig. 01). *Hb* for being a biocompatible material is very promising in the complexation of magnetic probes and biomedical applications [2,3].

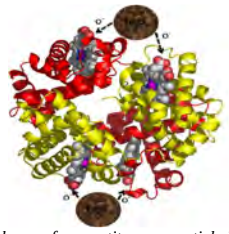

Figure 01. *Nanocomposite Scheme of magnetite nanoparticle in alfa-beta hemoglobin.*

The *Hb* been collected of six (06) donors and blood type: *AB+*, *A+* e *O-*. After a process of purification, the samples been doped with MNP in five (05) different concentrations at a temperature of 309 K. In the samples of *BMNC*, the structural characterizations were performed using optical spectroscopy covering the region from 1100 to 2500 nm with a spectrophotometer (*NIR900 PLUS FEMTO*) and a X-ray diffractometer (*Shimadzu XRD 6000*). The morphological characteristics of *MNP* have been raised using the technique of transmission electron microscopy (*TEM*) with the instrument (*JEOL JEM 1010*).

X-ray diffractometry of *BMNC* showed a region with variation in the magnetic particles crystalline and another region with amorphicity represented by *Hb*. The micrography of the *MNP* showed that the morphology is approximately spherical, the average diameter of 4,5 nm with polydispersity of 0,02 nm. The infrared spectra showed the fluctuation of the *HEME* group in function of the concentration of *MNP* trapped in the *Hb* polymer.

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Permeation Magnetic Colloid in Stratum Corneum as Model in the Permeation of Human Tissue

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The strength of some solid tumors through chemotherapy is partly due to low penetration of drugs or doses in cancerous cells. Thus, treatments that achieve better orientation and penetration of tumor cells are urgently needed. Another challenge is to achieve high concentrations of non-aggregated particles, in the specific area within the polymer matrix. Therefore penetration studies of membrane barriers are important for understanding the processes of interaction with plasma proteins, and macrophages action of the reticuloendothelial system [1]. The magnetic colloid (*MC*) system of maghemite nanoparticle, polymer of essential oil of *pepper hispidum* (*PH*) widespread in the stratum corneum (*SC*) is the purpose of this work (Figure 01).

This work represents the synthesis of magnetic nanoparticle of maghemite, the preparation of the stratum corneum of Wistar rats, the preparation of essential oil of pH, incubation of SC and diffusion was measured in six different concentrations of maghemite samples (*5, 6, 7, 8, 9* and *10 ml/g*) in the stratum corneum. The characterization was performed by transmission electron microscopy, optical microscopy, X-ray diffraction, *UV-VIS-NIR* and *DC* Susceptometry at room temperature. The magnetic colloid is stable in the presence of magnetic field. The samples were incubated and read at times of *1, 2, 3, 6, 12, 24, 36, 72* hours and six days. The dynamic permeation *MCPH-SC* were made in the region *190-2500 nm* (*NIR900S FEMTO PLUS*). The structural measures were performed using X-ray diffractometer (*XRD SHIMADZU 6000*). The morphological characteristics of *MCPH* been raised using the technique of transmission electron microscopy (*TEM*) with the instrument (*JEOL JEM 1010*).

(a) (b)

Figure 01. Optic Micography SCPH (a) and MCPH (b).

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Magnetically responsive alginate scaffolds for tissue engineering

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Externally-controlled drug delivery and remote control of cellular behavior for therapeutic and tissue engineering purposes are important clinical areas in which polymeric composite materials have the potential to make significant advances. It is known that apart from molecular signals (e.g. growth factors), additional physical cues, such as electrical signaling, mechanical stimulation, and medium perfusion are required in tissue engineering in order to obtain functional tissue transplants. Hydrogel nanocomposites impregnated with magnetic particles are attractive materials for achieving remote actuation with adjustable direction, force and possibility of *in vivo* applicability. An applied magnetic field can be coupled to the particle to actuate a process within a target cell regardless of whether there are intervening structures, such as tissue.

In this work, we explored whether impregnation of alginate scaffolds with magnetically responsive nanoparticles (MNP) and further exposure to an alternating magnetic field would exert a direct effect on endothelial cell activity and organization. The inclusion of magnetic particles had no significant effect on the porosity, stability and wetting properties of the composite scaffolds making them appropriate for cellular support and cultivation. The added magnetic properties of composite scaffolds in combination with an externally applied alternating field led to an induced capillary-like organization of endothelial cells. This strategy combined with chemical signals can potentially lead to the creation of an efficient pre-vascularized tissue construct *in vitro* which can be transplanted and efficiently integrated *in vivo* with the host tissue.

Optimal Permanent Magnet Halbach Designs for Deeper Tissue Targeting

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We present optimization methods to design permanent magnet Halbach arrays [1] to maximize the forces applied on magnetic particles at deep tissue locations. In magnetic drug targeting, where magnets are used to focus therapeutic nanoparticles to disease locations, the sharp fall off of magnetic forces with distances from magnets has limited the reach of magnetic drug targeting. Generating stronger forces at depth by optimally designing and constructing Halbach arrays would allow treatment of a wider class of patients, e.g. patients with deeper tumors.

The goal is to find the magnetization direction of each sub-magnet in the array so that the magnetic force, pull (for accumulating particles) or push (for injecting them), is maximized at a desired location. We know that the magnetic field around an array of magnets is the sum of the magnetic fields generated by each sub-magnet [1][2], and that the magnetic force on particles is proportional to the gradient of the magnetic field squared [3]. Thus there is a quadratic map between the magnetization of each sub-magnet and the resulting force on particles.

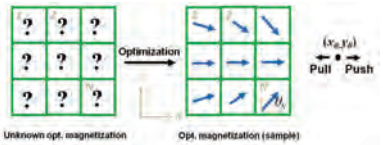

Figure 1: The optimization task: find the angle θ (blue arrow) for each sub-magnet (green box) in order to maximize force (push/ pull) at the deep location (x_0,y_0).

We maximize this quadratic map using semi-definite quadratic programming, and we rigorously prove that we achieve *globally optimal* Halbach designs in 2 and 3-dimensions. A sample optimal magnetic push array is shown on the left of Figure 2. We can also use our method to select optimal magnet shapes. Our optimal Halbach designs significantly outperform benchmark magnets of the same size and strength. For example, a 3-dimensional 36 element 2000 cm^3 array with optimal shape and magnetization directions (Figure 2, right) yields a ×5 greater force at a 10 cm depth compared to a uniformly magnetized magnet of the same size and strength. We have already constructed an optimal Halbach array with four sub-magnets and its performance matches predicted behavior. Larger Halbach arrays are currently under design and will be tested and validated in future experiments.

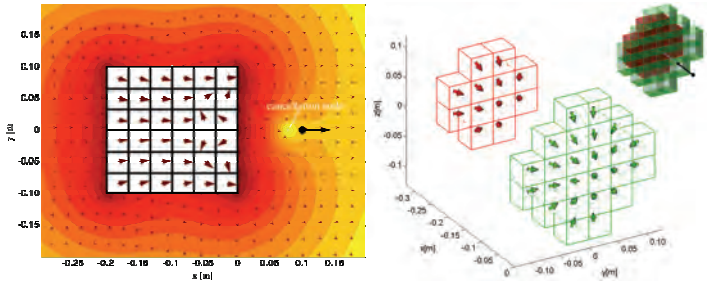

Figure 2. (Left) Optimal 36 element 2D push array. Brown arrows show optimal magnetization directions, colors indicate the resulting magnetic field strengths, and the black dot and arrow shows the push force at depth. (Right) Optimal geometry and magnetization directions for a 36 element 3D Halbach pull array. The optimal geometry is shown at top right, magnetization of the two layers is shown by red and green arrows for the back and front magnets.

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Fractionation of Particles and Cells by Continuous Flow Magnetophoresis: Effects of Sample Concentration

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Low throughput is a major limiting factor in continuous flow fractionation systems. Sample concentration has to be maximized in order to overcome this deficit. Concentration dependent phenomena such as magnetically induced aggregation and hydrodynamic coupling are effects that have been shown to influence particles and magnetically labeled cells. The present study was aimed at enhancing our understanding about these phenomena and their effect on continuous flow magnetic fractionation processes.

We investigated the influence of sample concentration on the fractionation in an open gradient, continuous flow magnetic fractionation system (Q_{Sample} : Q_{total} = 0.1, Q_{total} = 30-60 ml/hr), employing a glass flow channel (*width* × *thickness* × *length* = 8.85 mm × 0.75 mm × 152.4 mm) placed in a customized dipole magnet (B_{max} = 2.2 T, dB/dy = 0.154 T/mm). We studied a wide range of sample concentrations spanning two orders of magnitude using monodisperse magnetic microparticles (d = 6.3 µm; micromod Partikeltechnologie GmbH, GER) and immunomagnetically labeled T-lymphocytes (d = 10 µm). The results were compared to a mathematical model based on the magnetophoretic mobility distribution measured by cell tracking velocimetry (CTV). This model ignores potential interaction of particles and cells.

We observed a strong effect of particle and cell concentration on continuous flow magnetic fractionations even at low volume fractions. In contrast, static systems such as the one employed for (CTV) are less affected by concentration dependent phenomena. This resulted in considerable discrepancies between experimental results and model predictions at moderate sample volume fractions.

Our results show that concentration dependent particle and cell interaction can severely affect the outcome of open gradient, continuous flow magnetic fractionation, resulting in a shift of the fractionated sample towards higher outlet numbers with increasing sample concentration. The underlying principles are still poorly understood but are likely to be related to hydrodynamic coupling and magnetically induced formation of aggregates. Mathematical models used to predict fractionation outcome have to be augmented to account for these effects.

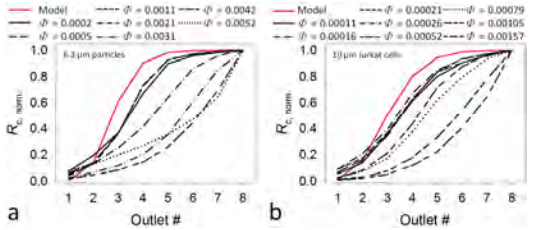

Figure. Normalized cumulative recovery of DMF fractionations with 6.3 µm magnetic microparticles (a) and 10 µm immunomagnetically labeled Jurkat cells (b). Data shown as cumulative mean recovery (n = 2).

Tissue model for the study of heat transition during magnetic heating treatment

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A promising therapy for cancer diseases without any chemotherapeutic agent and also without any side effects is the so-called magnetic heating treatment. Depending on concentration and specific heating power of the magnetic material, as well as on parameters of the magnetic field temperatures between 43-55°C can be reached. This paper deals with the evaluation of the heat distribution around such a heat source. Especially, the heat transfer from tissue enriched with magnetic nanoparticles to a region of no or little enrichment of nanoparticles in which in a clinical treatment temperature measurement could be performed is studied..

The evaluation of the temperature distribution took place with the help of a tissue phantom. The phantom is composed of two concentric cylinders. The inner cylinder consists of a defined mixture of polyurethane gel and magnetic fluid. This cylinder represents tissue enriched with nanoparticles. The outer cylinder, which stands for pure tissue consists of polyurethane only.

This tissue phantom has been exposed to an alternating magnetic field according to the protocol of magnetic heating treatment. The temperature measurements were performed by thermocouples which are placed on defined positions as shown in Figure 1a).

The experimentally obtained temperature data presented in Figure 1b) is the basis for a finite element method (FEM) simulation model. The FEM model allows the determination of heat transition from regions enriched with magnetic nanoparticles to regions with no or minor nanoparticle accumulation.

a) b)

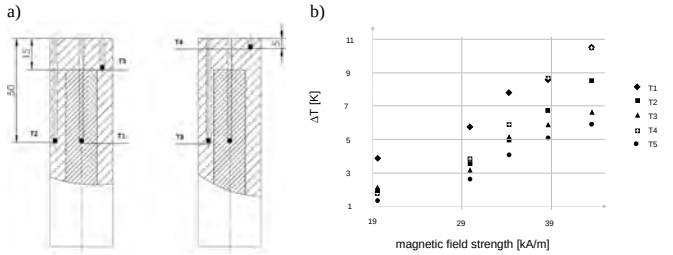

Figure 1a) Phantom with well-defined positions of the thermocouples named T1 to T5. b) Magnetic field dependence of the maximal temperatures for the different thermocouples.

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Control of Thermoresponsive Protein Functionality by the Combination of Magnetic Nanoparticle and AC magnetic field

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Magnetic nanoparticle subjected to an AC magnetic field show heating effect related to losses during the magnetization reversal process of the particles. Applications of this heating effect to the biomedical fields are intensively studied. In the conventional studies such as magnetic hyperthermia, heat generated by the magnetic nanoparticles is dissipated by the medium to raise the target temperature. The target of this study is to use this heating effect for controlling the functionality of the thermoresponsive protein, while keeping the temperature of the solution unchanged. As a magnetic nanoparticle material, Therma-Max™ was used. It shows a reversible transition between flocculation and dispersion at around the critical temperature of 32ºC by the thermoresponsive polymer on its surface. We have confirmed that the application of AC magnetic to the Therma-Max™ solution keeping the temperature at 20ºC resulted in the formation aggregates, indicationg that the heat generated by the magnetic nanoparticle has significant effect to the surface molecules. Tk-subtilisin, which is a subtilisin homologue from *Thermococcus kodakarensis*, was used as a model thermoresponsive protein. It is a highly thermostable subtilisin and shows higher enzyme activity by increasing the solution temperature. The nanoparticle-protein hybrid was formed by coupling reaction between carboxyl group on Therma-Max and amino group in Tk-subtilisin. The AC magnetic field was applied to the solution of the nanoparticle-protein hybrid keeping the solution temperature constant. The activities of the Tk-subtilisin in the hybrid were significantly enhanced by the application of AC magnetic field. The result indicates that the thermoresponsive protein was directly activated by the magnetic nanoparticles under AC magnetic field, and not by the medium heat. This finding would contribute to the development of new research fields for the application of magnetic nanoparticles.

Iron Oxide Nanoparticle-Mediated Hyperthermia of Hepatocellular Carcinoma

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Exposing cancer cells to temperatures exceeding 39 ^{o}C promotes protein misfolding and alters the activity of proteins involved in DNA repair and apoptosis. Because these effects can sensitize cancer cells to damage by radiation or chemotherapy there is interest in hyperthermia as an adjunctive treatment for cancer. One method of selectively heating cancer cells is with iron oxide nanoparticles, which deposit heat when exposed to alternating magnetic fields (AMF). Here we present early results in an ongoing study of the efficacy of bionized nanoferrite (BNF) particle-mediated hyperthermia in the treatment of hepatocellular carcinoma (HCC) using *in vitro* and animal models.

Initial *in vitro* studies were conducted to measure toxicity of hyperthermia in the human HCC cell line HepG2. First, HepG2 cultures were heated to between 37 ^{o}C and 45 ^{o}C for 30 minutes in water bath. Clonogenic assay showed reduced survival of heated cells compared to 37 ^{o}C controls (survival fraction per cent ± SEM at 42 ^{o}C: 71.7 ± 2.3; at 43 ^{o}C: 11.3 ± 0.7; at 45 ^{o}C: 5.4 ± 0.7). Next, HepG2 cells in media with BNF particles (0.4 mg iron ml^{-1}) were heated to approximately 42 ^{o}C for 30 minutes by AMF (AMF strength varied from 55.7 to 63.7 kA m^{-1}, frequency 154-155 kHz to maintain media temperature at 42 ± 1 ^{o}C). Cells in media with PBS underwent simultaneous AMF treatment, but media temperature did not exceed 37 ^{o}C. Clonogenic assay showed reduced survival in heated cells compared to controls (cells with BNF survival fraction per cent ± SEM: 28.45 ± 11.7).

Further experiments to evaluate BNF particle-mediated hyperthermia in male athymic nude mice with subcutaneous HepG2 tumors are ongoing. In these experiments we also examine the addition of lipiodol to the BNF particle formulation. Lipiodol is used in transarterial chemoembolization of HCC in humans due to its uptake and retention by tumors. We hypothesize that using lipiodol with BNF particles would promote uniform distribution of particles in the tumor, leading to more widespread heating and greater therapeutic effect. Briefly, tumors of approximately 0.1 cm^3 are injected with vehicle (PBS + 1% Tween 20), BNF (1:1 BNF:PBS by volume + 1% Tween 20, 6.9 mg iron cm^{-3} tumor volume), or a mixture of BNF and lipiodol (1:1 BNF:lipiodol by volume + 1% Tween 20, 6.9 mg iron cm^{-3} tumor volume). 18 hours after injection mice undergo AMF treatment at 39.8 kA m^{-1}, 155 kHz for 15 minutes with constant monitoring of tumor surface, contralateral surface, and rectal temperatures using fiber optic probes. Following AMF treatment tumors are either taken for histology to assess apoptosis, necrosis, and iron content, or are monitored for a tumor growth delay study.

Initial data show that tumors injected with BNF with or without lipiodol heated to greater than 39^{o}C while rectal, contralateral surface, and control tumor surface temperatures did not exceed 39^{o}C. Tumors injected with BNF and lipiodol appear to heat more consistently than those injected with BNF alone. However, at this early point in the study no significant differences in growth rates between tumors in each treatment group have been observed. While these early results are promising, further study of tumor growth rates and examination of tumors by histology are necessary to evaluate the efficacy of lipiodol and BNF particle-mediated hyperthermia as a therapy for HCC.

Preparation and properties of magnetic $ZnFe_2O_4$-chitosan core-shell nanoparticles

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Abstract

Superparamagnetic single phase zinc ferrite nanoparticles have been prepared by wet chemical co-precipitation methodusing NaOH solution as precipitating agent without any subsequent calcination. X-ray diffraction patterns indicated that the magnetic nanoparticles were pure $ZnFe_2O_4$ with a cubic inverse spinel structure. Then $ZnFe_2O_4$ - chitosan nanoparticles were obtained through crosslinking the amino groups on the chitosan using glutaraldehyde. Transmission electron microscopy results showed that the $ZnFe_2O_4$ - chitosan nanoparticles were quasi-spherical with a mean diameter of 5 nm. The binding of chitosan to the $ZnFe_2O_4$ nanoparticles was also demonstrated by measuring the Fourier transform infrared spectra and thermogravimetric analysis. Magnetic measurement revealed that the nanoparticles were superparamagnetic at room temperatureand magnetization of the composite nanoparticles was5.21emu/g at a maximum external field of 10kOe.

Fluorescence and Reflectance Co-Registered 3D Tissue Imaging System based on a Cryostat

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An automated system was developed to image the 3-dimensional distribution of fluorescent magnetic nanoparticles in tissue samples. The system enables easy measurement of magnetic nanoparticle distributions, for small and large tissue samples (currently up to a maximum size of 2 cm × 2 cm × 2.5 cm), in 3-dimensions, with a 5-20 µm resolution in plane and 1-30 µm in the axial direction. A cryostat is used to continuously cut off thin slices from the top of a frozen tissue block. Before each new slice is removed, two cameras simultaneously take both a reflectance and a fluorescence image of the top of the sample thus imaging both the histology and the spatial distribution of fluorescent magnetic particles in that slice. Successive slices are used to reconstruct the entire 3-dimensional distribution of the particles in the tissue sample.

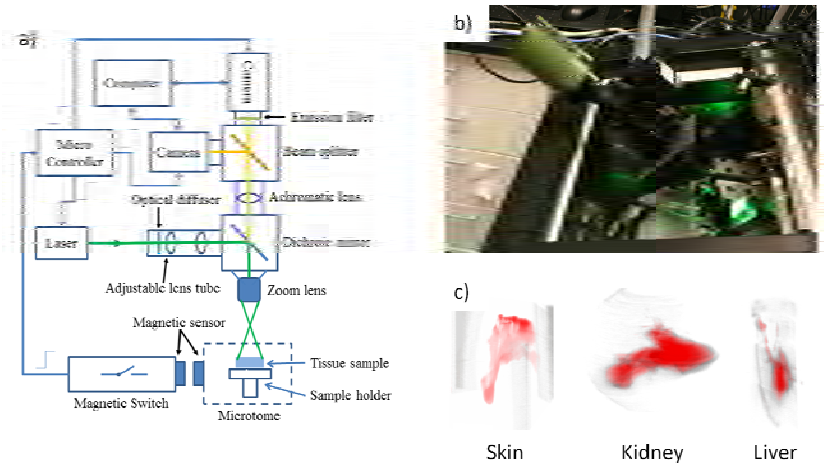

Figure 1: a) Schematic and b) photograph of the cryostat based co-registration 3D tissue imaging system. c) Sample measured magnetic nanoparticle distributions in skin, kidney and liver (fluorescent image only).

After a magnetic drug targeting experiment, an excised tissue sample is either first frozen or formalin fixed and then encased in OCT for increased mechanical stability during slicing. A cryostat (5030 microtome, Bright) was modified to achieve the measurements. Each slice can be as thin as 1 µm, and this slice thickness sets the vertical spatial resolution of the system. A homogenized laser beam (532nm, 150mW with optical diffuser ED1-S20-MD, Thorlabs) illuminates the top of the sample after each slice. Two cameras (Manta G-145, Allied Vision and QIClick, qimaging) are synchronized to take both reflectance and fluorescence images and imaging software is used to assemble these planar images into a 3D stack. The two cameras share the same imaging optics and their sensor sizes are chosen to be identical, allowing for the images taken by the two cameras to be co-registered.

The system has been successfully applied to investigate magnetic-fluorescent nanoparticle distributions in mouse and rat cochleas, kidneys, skin, brain, spleen, heart and livers. Figure 1c shows sample data of only the fluorescent images taken from the skin, kidney and liver. The nanoparticle distribution through the various tissues can easily be distinguished (red) as they travel through various tissues within vessels and interstitial spaces.

Fluorescent, superparamagnetic nano carriers for drug storage, targeting, and imaging

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This presentation reviews advances in the design and development of multi-functional magnetic nano carrier systems for early cancer diagnosis and spatially and temporally controlled therapy. The critical issues in cancer diagnosis and treatment are addressed based on novel nano technologies such as real time in-vivo imaging, drug storage and release, and specific cancer cell targeting. For early cancer diagnosis and treatment, a nano carrier system is designed and developed with key components uniquely structured at nano scale according to medical requirements. For imaging, quantum dots with emissions near infrared range (~800 nm) are conjugated onto the surface of a nano composite consisting of a spherical polystyrene matrix (~150 nm) and the internally embedded, high fraction of superparamagnetic Fe_3O_4 nanoparticles (~10 nm). For drug storage, the chemotherapeutic agent paclitaxel (PTX) is loaded onto the surfaces of these composite multifunctional nano-carriers by using a layer of biodegradable poly(lactic-*co*-glycolic acid) (PLGA). A cell-based cytotoxicity assay is employed to verify successful loading of pharmacologically active drug. Cell viability of human, metastatic PC3mm2 prostate cancer cells is assessed in the presence and absence of various multifunctional nano-carrier populations using the MTT assay. PTX loaded composite nano-carriers are synthesized by conjugating anti-Prostate Specific Membrane Antigen (anti-PSMA) for targeting. Specific detection studies of anti-PSMA-conjugated nano carrier binding activity in LNCaP prostate cancer cells are carried out. LNCaP cells are targeted successfully *in vitro* by the conjugation of anti-PSMA on the nano carrier surfaces. To further explore targeting, the nano carriers conjugated with anti-PSMA are intravenously injected into tumor-bearing nude mice. Substantial differences in fluorescent signals are observed *ex vivo* between tumor regions treated with the targeted nano-carrier system and the non-targeted nano-carrier system, indicating considerable targeting effects due to anti-PSMA functionalization of the nano carriers.

Biocompativel Magnetic Colloid Based–Triterpene Polymer

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Magnetite (Fe_3O_4) nanoparticle is interesting for many areas of science and technology. Magnetic fluid containing magnetite nanoparticles can be used for biomedical applications, magnetic recording, magnetic refrigeration, sensing, catalysis, among others [1–3]. Therefore, the control of the magnetic properties of nanosized systems is of great importance to reach the goals imposed by the specific application.

This work Triterpene Polymer (*TP*) was obtained of essential *Tucuman oil* (*TO*) be used for to coat the magnetite nanoparticle. *TO* was prepared using pop of the fruit seed tree. Magnetite nanoparticles were chemically synthesized and peptized as a surfacted magnetic fluid (*MF*) sample, following the standard two-step procedure described in the literature [4]. In the first step, magnetite nanoparticles were synthesized by precipitating Fe^{+2}/Fe^{+3} aqueous ion in alkaline medium. In the second step oleic acid was used to peptize the as precipitated magnetic nanoparticles in triterpene polymer at a final particle concentration of about 1.6×10^{16} *particle*/cm^3 of the magnetic fluid *TP* magnetite-based (*MFTP*).

After *MFTP* was characterized using *UV-VIS-NIR* (*190–2500 nm*). Morphology was made through of the optic microscopy (*OM*) showed alters form through image. Morphology and nanoparticle diameter was obtained to transmission electronic microscopy (*TEM-JEOL JEM 1010*) (see Figure 01). The crystalline nanostructure was fined through of the X-rays diffraction technique (*Shimadzu XRD 6000*).

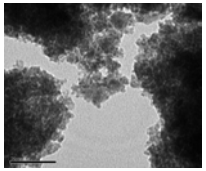

Figure 01. *TEM micrograph of the nanoparticles of magnetite*.

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Development of Fe_3O_4 dendritic nanoadsorbents for water remediation

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Dendritic Fe_3O_4 magnetic nanoadsorbents (DMNA) were fabricated by a facile soft-chemical approach based on Michael addition/amidation reaction. XRD and TEM analyses revealed the formation of highly crystalline single-phase Fe_3O_4 inverse spinel nanostructures. The growth of dendritic nanostructures having terminal amino acid (i.e., arginine) is evident from FTIR spectroscopy, dynamic light scattering, and zeta-potential measurements, elemental and thermal analyses. These nanoadsorbents are of average size about 10 nm and exhibit superparamagnetic behavior at room temperature with strong field dependent magnetic responsivity. The terminal amino acid on the shell of nanoadsorbents allows us to create functionalized exteriors with high densities of organic moieties (both amine and carboxyl) for adsorption of toxic heavy metal ions. It has been observed that these nanoadsorbents have strong affinity for simultaneous removal of toxic metal ions (Ni^{2+}, Cu^{2+}, Cd^{2+}, Co^{2+}, Pb^{2+} and As^{3+}) and bacterial pathogens (*E. coli, S. aureus*) from water (Fig. 1). Further, the removal of metal ions and killing/capturing of bacteria are strongly dependent on pH of the medium and initial concentration of nanoadsorbents. Depending upon the surface functionality, nanoadsorbents capture metal ions either by forming chelate complexes or ion exchange process or electrostatic attraction. Furthermore, these nanoadsorbents can be used as highly efficient separable and reusable materials for removal of toxic metal ions and bacterial pathogens.

Keywords: *Nanoparticles, Magnetic, Surface functionalization, Heavy metal ions, Bacterial pathogens*

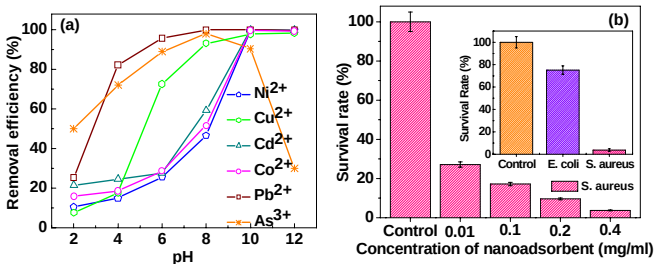

Fig. 1. (a) Removal efficiency of toxic metal ions after incubation with DMNA for 24 h at different pH and (b) Survival rate of *S. aureus* after incubation with different concentrations of DMNA for 6 h (Inset shows the survival rate of *E. coli* and *S. aureus* after incubation with 0.4 mg/ml of DMNA for 6 h).

Magnetic fluid reverses inhibition of cell growing caused by protein amyloid fibrils

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Protein amyloid deposits formed by a variety of proteins are associated with incurable amyloidosis and have toxic consequence to various cell types leading to their dysfunction or death by deregulation of cell homeostasis, disruption of the intracellular transport and membrane integrity. These facts correlate with the findings suggesting that reduction of amyloid aggregates is beneficial for cells and animals.

In our work we investigated effect of insulin/lysozyme amyloid fibrils (IF/LF) and magnetic fluid (MF) on the growth curves of V79 and LLC-PK1 cells. Protein amyloid aggregation was achieved by incubation of protein in presence of NaCl at 65°C and constant stirring. Formation of amyloid fibrils was verified by spectroscopic and microscopic techniques. Magnetic fluid was prepared by co-precipitation method consisting of Fe_3O_4 stabilized by sodium oleate and modified by bovine serum albumin.

To investigate effect of amyloid fibrils on the cell growing, the cells were plated in 24-well plate and amyloid fibrils at different concentration were added to the cell culture. As it shown in Fig. 1 the protein amyloid fibrils inhibit cell proliferation and affect the cell grown curves. The results reveal that fibrils are toxic to the cells and inhibit proliferation in a dose-dependent and time-dependent manner.

Our previous results demonstrated *in vitro* anti-aggregation effects of magnetic fluid in the case of protein lysozyme [1]. We were interested if protective effect of MF can be observed also in cells affected by amyloid fibrils. Interestingly, the cell viability was significantly improved when the MF was added into cell culture media. It has been noted that MF alone caused no significant changes in viability at studied concentrations.

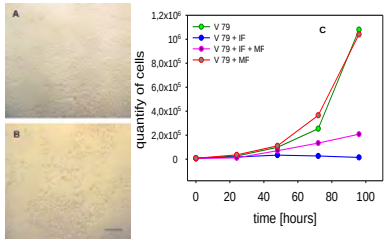

Fig. 1: Proliferation of LLC-PK1 (A) and inhibition of proliferation by LF (B). Viability of cells determined by MTT test in presence of IF, IF+MF and MF alone (C).

The obtained results indicate that prepared fibrils are toxic to the both studied cell lines and MF is able to alleviate the negative effect of amyloid fibrils on the cells. We assume that MF has ability to reduce the amount of fibrils leading to decreasing of the cytotoxic effect of amyloid aggregates.

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Novel magnetic targeting models with superparamagnetic iron oxides

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Magnetic drug targeting describes the selective targeting of therapeutics bound to magnetic nanoparticles (so called magnetic drug carriers) in a tissue or a region in the body, e. g. in a tumor, by an external magnetic field in order to allow a controlled drug release. The field exerts an attractive force on the magnetic nanoparticles and concentrates them in vivo at a target site where the strongest magnetic force and the highest magnetic field gradient dominate. However, magnetic drug carriers should not only be able to carry a wide variety and a high quantity of chemical agents. It is also most important that they can deliver significant amounts of the bounded drugs to the tumor site. Considering this, a new concept of placing an array of permanent magnets and coils inside hollow organs of the body was developed. This gives the possibility to target endoluminal tumors, e. g. prostate carcinoma, esophagus adenocarcinoma or bile duct Klatskin tumors, which allow the minimally invasive endoscopic insertion of permanent magnets and coils very close to the target site. Hence, a stronger magnetic field and a higher magnetic field gradient in the tumor are achieved.

Accordingly, a novel targeting model using FEM simulations of magnetic nanoparticles in blood flow under the influence of a magnetic field was designed considering the physical and chemical properties of differently synthesized SPIOs. The targeting model describes the interaction of an external magnetic field with blood flow containing homogeneously suspended SPIOs with a certain mass fraction (SPIOs' concentration). The simulations show that for the same concentration of nanoparticles, different settings of a single magnet lead to different velocity changes in the range of 2 % to 10 % of the maximum velocity and that below a SPIOs concentration of 3.3 % no targeting effect can be achieved. Furthermore, biophysical models of the prostate gland, esophagus and bile duct were built and different magnetic field configurations of permanent magnets as well as coils were simulated in order to find the best setup for a high targeting efficiency. In the simulations the parameters: current, size, orientation, number, and position of the coils or permanent magnets were varied. With optimized geometries an improvement of the targeting efficiency up to a factor of 40 could be achieved. The targeting efficiency is defined as the weighted sum over the magnetic fields strengths at each point of the simulated area, giving a representative entity for SPIO accumulation.

First simulation results were validated in animal trials with pigs. After application of the optimized targeting setups, the magnetic behaviors of the pig tissues were analyzed using a superconducting quantum interference device. The results confirmed the accumulation of SPIOs at the target site, where the magnetic behavior of tissues changed from diamagnetic to paramagnetic.

This novel magnetic drug targeting model shows a way of prediction and improvement of the delivery rate for drug targeting systems and is a suitable tool for enhancing the efficacy of current treatment by reducing the dose of the administrated drug and, in this way, minimizing its side effects.

Investigation of mesh implants with incorporated magnetic nanoparticles for MR visualization

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Textile implants usually consist of polymers which cannot be distinguished from the ambient tissues with conventional radiological imaging methods such as X-ray, ultrasound, computed tomography, and magnetic resonance imaging (MRI). The concept of a visible mesh implant in MRI is realized by compounding superparamagnetic iron oxides (SPIOs) into the polymer of the mesh. This new mesh material can be visualized as signal voids in MRI due to the magnetic field distortion caused by the SPIOs. Therefore, signal loss in MRI is associated with the presence of these particles and, as a result, the mesh is depicted. Consequently, the visual investigation of the implant supports the surgeon to decide whether a surgery is necessary and may reduce the exposure of the patient to redundant surgical interventions or to plan the surgical procedure more accurately.

The base material of a hernia implant is PVDF which is incorporated with SPIOs during the spinning process. Subsequently, threads are extruded with diameters between 85 µm and 170 µm and knitted to meshes of different sizes and patterns for different applications such as hiatal hernia repair or stoma hernia repair. For a high quality product, the nanoparticles must be homogeneously distributed within the material of the threads and have optimal magnetic properties for MRI. Therefore, fundamental investigations on SPIOs and threads with integrated SPIOs were performed. For this purpose, different SPIO nanoparticles were synthesized by co-precipitation and thermal decomposition methods with systematic variation of the reaction parameters and used for manufacturing the threads. The determination of the key parameters that influence the physical and chemical properties of both SPIOs and the threads was accomplished using different techniques: The magnetic behavior was investigated using a superconducting quantum interference device. The size of the nanoparticles was determined by dynamic laser scattering, transmission and scanning electron microscopy as well as atomic force microscopy. The measurement of cluster formation, cluster size and distribution of the nanoparticles within the threads was performed using combined atomic and magnetic force microscopy. The relaxation times in order to determine the imaging characteristics were measured in phantoms by means of magnetic resonance imaging.

Referring to the determined properties of the nanoparticles with the methods mentioned above, co-precipitation particles were chosen to be incorporated into the mesh material due to a high magnetization, high relaxivity, small size and high iron concentration values. The best parameter configuration was achieved for uncoated iron oxide nanoparticles with a grain size of 1 µm incorporated in threads with an iron concentration of 10 mg/g and of 20 mg/g and for different sizes and knit fabrics. The investigation of the influence of SPIOs' integration in polymers on the relaxation of adjacent protons of an agarose phantom showed the best results for R2* susceptible sequences creating signal voids due to the strong local static magnetic field gradients generated by the SPIOs.

In conclusion, the applied methods are a feasible approach to control the quality of surgical implants for MR-visualization and to support the optimization process in the development of various mesh types for different applications.

Carbon nanotube-based magnetic nanohybrids

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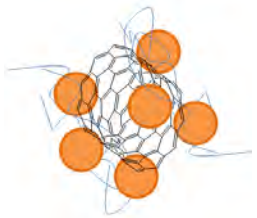
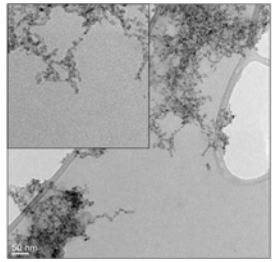
Carbon nanotubes (CNTs) with tailored functionalities are multifunctional carriers that can potentially provide new approaches for diagnosis and treatment of diseases [1]. Synthesis of magnetic CNTs with required physical properties (size distribution, magnetic saturation, etc…) for biomedical applications still remains a challenge to accomplish.

We developed a simple multi-step chemical route for synthesis of carbon nanotube-based magnetic nanohybrids. On one hand, tip sonication and chemical oxidation [2] of CNTs has been used to control the length distribution of CNTs and provide them carboxylic groups for further functionalisation. On the other hand, superparamagnetic iron oxide (SPIO) nanoparticles have been synthesized [3] as a tool to render the CNTs magnetic. Finaly, covalent functionalisation was used to decorate the oxidised CNTs with the SPIO nanoparticles. Such carbon nanotube-based nanohybrids can be used for various biomedical applications as imaging (MRI contrast agent) or sensoring (nanoparticles-based diagnostics).



Concept of magnetic nanohybrids



TEM image of magnetic nanohybrids

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Simulating of biogenic magnetite nanoparticles behaviour in external magnetic fields

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Discovery of biogenic ferrimagnetic particles in human tissue emerges new questions to human-magnetic field interaction [1]. Previous studies take into account only thermal effects of Faraday currents. To simulate the interaction mechanism we use our 'Cube' model approach [2]. It allows more realistic calculation of nanoparticle magnetic moment in comparison with generally used 'Sphere' model (**Fig 1**). In "Cell Unit" (CU) approach a magnetic moment of particle is derived from magnetite cell unit shape and size: $\bar{\mu}_{magCU} = 8\bar{\mu}_{FU}V_{mag}/V_{CU}$, where V is the volume of the magnetite and CU respectively, and μ_{FU} is the magnetic moment of magnetite "Function Unit". In "Bulk" approach, the magnetic moment is derived from bulk saturation magnetization.

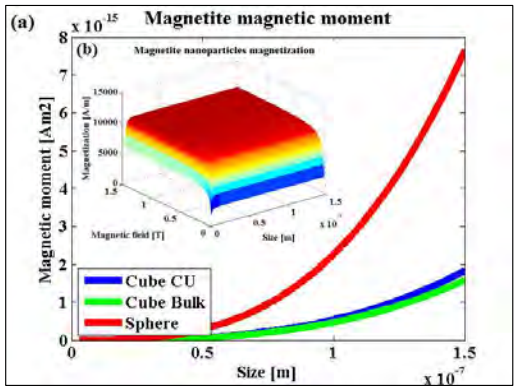

Fig 1: (a) Magnetic moment of magnetite particles for "Cube" and "Sphere" model. (b) Magnetization of biogenic nanoparticles with respect to particle size and magnetic field strength.

Single domain particles interact with external magnetic fields in two ways: translation in gradient fields and rotation in uniform field. Based on above mentioned model rotation interaction energies of biogenic magnetite nanoparticles in single domain range with **geomagnetic field are ≈ 10^{-25} - 10^{-20} J**. In **MRI tomographs the energies are ≈ 10^{-20} - 10^{-15} J**, which exceed the typical **biochemical bond energy (≈ 10^{-19} J)**. **Translation interaction energies** can be neglected **(≈ 10^{-29}-10^{-24} J)**. These findings may be crucial in understanding of animal magnetoreception mechanism as well as human - magnetic field interaction (magnetic hazard, magnetotherapy).

Acknowledgments
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Cyclical Magnetic Field Flow Fractionation for the Separation of Magnetic Nanoparticles

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Introduction: In this study, a novel magnetic field flow fractionation (FFF) system was designed and modeled by using finite element simulations. Other than current magnetic FFF systems [1, 2], which use static magnetic fields, our system uses cyclical magnetic fields for the separation of magnetic nanoparticles. In the cyclical magnetic FFF system, in addition to the magnetic field strength, frequency of the magnetic field can also be adjusted to achieve the optimum separation parameters. Simulation results show that cyclical magnetic FFF system can effectively be used for the separation of magnetic nanoparticles.

Methods: Cyclical Magnetic FFF system is composed of a microfluidic channel and 2 electromagnets, shown in Figure1. A pressure driven flow is generated, resulting in a parabolic flow profile in the channel. Square wave currents with 90 degrees of phase difference are applied to the top and bottom electromagnets, so that the particles are driven away from the channel walls. The particles with higher magnetophoretic mobilities will move longer distances away from the channel walls. As a result, they stay in the faster fluid regions and elute earlier than the lower mobility particles.

A microfluidic channel (length=5cm, height=30μm) was modeled in Comsol Multiphysics. Square wave magnetic fields were generated by the electromagnets (B=0.8T, f=1Hz). An inlet velocity of 0.4mL/h was defined, resulting flow profile and magnetic field can be seen in Figure2.

By using the simulated magnetic field profile, magnetic force acting on the magnetite (Fe_3O_4) nanoparticles was calculated according to eqn1 (next page). Where $\mathbf{V_p}$ is the volume of the particle, $\boldsymbol{\chi_p}$ is the particle susceptibility, **B** is the magnetic flux density and $\boldsymbol{\mu_0}$ is the magnetic permeability of the free space. To obtain the volume magnetic susceptibility for different size magnetite particles at given magnetic fields, equations supplied by Rosensweig [3] was used.

By using the magnetic force acting on the particles, resulting particle velocity $(\boldsymbol{v_p})$ was obtained according to eqn 2. Where, $\boldsymbol{\eta}$ is the fluid viscosity, $\mathbf{r_p}$ is the particle radius, and $\boldsymbol{v_f}$ is the velocity of the fluid resulting from the pressure driven flow.

To find the particle trajectories in the channel, a Matlab code was generated which solves the particle velocity equation eqn2.

Results: Particle trajectories of 30 and 50nm magnetite particles were obtained for the first 3.6 seconds of fractionation (Figure 3). As shown in the figure, 50nm particle moves much faster throughout the channel. The elution times of the particles ranging from 10nm to 50nm were calculated and plotted (Figure 4). As can be seen, there is a significant difference between the elution times of the particles.

Discussion & Conclusion: In this work, a cyclical magnetic FFF system was modeled and it was shown that by the application of cyclical magnetic fields, the separation of magnetic nanoparticles can be done efficiently. Compared to the current magnetic FFF systems, this system can be easily adjusted for different types of particle samples, and it is done by just modifying the strength and frequency of the magnetic field.

As an improvement to this work, we added diffusion equation in our simulation code. Diffusion has a significant detrimental effect on the separation quality. Our recent results show that to eliminate the diffusion problem, one of the elctromagnets should apply higher magnetic fields compared to the other electromagnet. Preferably the top electromagnet should be the more powerful one (since the outlet is at the top). According to the latest modeling results, to separate magnetic nanoparticles in the range of 10nm, the top electromagnet should be at least 40% more powerful than the below electromagnet. Furthermore, our recent code has the advantage of visualizing the separation process by outputting the movie file showing the motion of individual nanoparticles in the channel. Currently, we are working on the fabrication of the system. But instead of using bulky electromagnets we are using strong permanent magnets and cyclical magnetic fields are obtained by robotic motion of the permanent magnets via the help of electric motors.

Cyclical Electrical Field Flow Fractionation for the Separation of Magnetic Nanoparticles

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Introduction: In this study, potential of Cyclical Electrical Field Flow Fractionation (CyEFFF) for the separation of magnetic nanoparticles is investigated. Recently, we demonstrated for the first time that by the application of appropriate voltage waveforms, one can separate nanoparticles with sizes less than 100 nm. By using proper voltage waveforms, detrimental effect of the particle diffusion is eliminated and particles in the range of 10nms can be fractionated. We believe that CyEFFF has also a great potential for the separation of magnetic nanoparticles.

Background: Field Flow Fractionation (FFF) is a powerful method for the separation and characterization of macromolecular, colloidal and micron-sized particles [1]. Cyclical Electrical Field Flow Fractionation (CyEFFF) is one of the subtechniques of FFF which separates the particles according to their size and electrical mobilities [2]. In CyEFFF, separation channel is composed of bottom and top electrodes which are separated by a thin spacer. A typical schematic of the CyEFFF system can be seen in Figure 1. In this system, oscillating voltages are applied to the electrodes which result in a cyclical electric field inside the channel. As a result of the cyclical electric field, particles move back and forth between the electrodes. Particles with high electrophoretic mobilities will move longer distances away from the channel walls and they spend more time at the faster fluid regions. As a consequence, they elute earlier than the lower mobility particles.
Earlier studies showed that diffusion of the nanoparticles is a limiting factor in CyEFFF. It gives rise to band broadening in the UV fractogram and prevents to achieve good separations. We address and solve this problem by changing the shape of the applied voltage waveform. In the earlier works, researchers used square wave voltages with DC offset voltages. In this work, we don't apply any DC offset voltages but we use square wave voltages with higher duty cycles.

Methods:
Separation experiments were done with a mixture of 15 and 40 nm mean diameter gold nanoparticles (NanoComposix, CA, USA). Square wave voltages (10Hz, 10Vpp) with duty cycles ranging from 50% to 80% were applied. De-ionized water (18.2 MΩ cm^{-1}) was used as the carrier which was pumped at a flow rate of 1ml/min.
Another set of experiments were done at the same experimental conditions with a magnetic particle sample. MACS anti-mouse IgG1 microbeads were used as the injected sample. Those are superparamagnetic particles which conjugated to epitope tag specific antibodies. Finally, we made another experiment with MACS particles at a 75% duty cycle voltage and a 0.5ml/min carrier flow speed. In this experiment, in addition to the UV detector DAWN® HELEOS™ II light scattering detector was used to measure the rms radius of the particles.

Results:
Figure 2a shows the UV fractograms of the CyEFFF experiments done with gold nanoparticles. As can be seen, as we increase the duty cycles of the applied voltages, we are getting 2 separate peaks, which are corresponding to 15 and 40 nm particles. The highest resolution was achieved at a duty cycle of 75%.
Figure 2b is the experimental result obtained for MACS superparamagnetic nanoparticles. It is clear that as the duty cycle of the applied voltage increased, magnetic particles retained more in the channel. Figure 3 shows the UV fractogram and light scattering data for magnetic particles. Mean rms radius of the magnetic nanoparticles is measured as 140nm and the particles eluted later have slightly less rms radius compared to the ones eluted earlier. This result shows that MACS nanoparticles have an average rms radius of 140 nm with a narrow range +-10nm. But the particles have a broad range of elecrophoretic mobiltities (as determined from the wide range of retention times). We predict that the difference in the electrophoretic mobilities can be resulted from the difference in the number of attached antibodies to the naoparticles.

Conclusion: It has been shown for the first time that Cyclical Electrical Field Flow Fractionation can be used for the size and electrophoretic mobility analysis of the magnetic nanoparticles. As we increase the

Biomagnetic methods in clinical practice: evaluation of influence of immunosuppressants on gastrointestinal transit

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The range of the possibilities covered by magnetism applied to medicine and biology continues to expand with improvements in instrumental sensitivity and easiness of use as well as the growing interest from diverse areas of study. Renal transplantation is the preferred renal replacement therapy. It restores quality of life and increases longevity for individuals at end stage of renal failure. However, the incidence of gastrointestinal (GI) complications after renal transplantation is relatively high and it may be regarded as side effects of immunosuppressive therapy. Despite this, motility disorders in these patients are not well studied. The aim of this study was to employ the Alternating Current Biosusceptometry (ACB) to evaluate the influence of immunosuppressants on GI transit after renal transplantation.

ACB employs a setup of detection and excitation coils for noninvasive monitoring of magnetic signals from the response of ferromagnetic materials to an externally applied magnetic field. These sensors are robust, don't need to operate in magnetically shielded rooms and the electronic instrumentation can be even more simplified than other biomagnetic technologies. Additionally, the ferromagnetic particles are not previously magnetized. The study protocol has been approved by local Ethics Committee. Eighteen renal allograft recipients and 12 healthy volunteers were enrolled in the study. All the patients were receiving triple immunosuppressive therapy: 12 were taking prednisone (PRED), azathioprine (AZA) and tacrolimus (FK) and 6 were taking PRED, AZA and Cyclosporine A (CsA). After an overnight fast, subjects consumed a standard 500 Kcal breakfast and 4 gelatin capsules filled with approximately 1000 mg of ferrite powder ($75 \leq \phi \leq 90\mu m$; Imag, Brazil) with 200 ml of water. ACB sensor was used to monitor gastric and colonic region at 10 min intervals for at least 8 h. Magnetic images were obtained to quantifying GI transit parameters. From the gastric emptying (GE) and colonic arrival (CA) time-intensity curves, the mean GE time (MGET), mean CA time (CAT) and mean small intestinal transit time (MSITT) were calculated. The results were expressed as mean±standard deviation. Differences were evaluated by ANOVA. P value <0.05 was considered statistically significant.

MGET obtained for FK, CsA and controls was 44±33 min, 147±88 min and 166±52 min, respectively. MGET was significantly faster (P<0.001) in FK group than CsA and healthy controls. MSITT for FK, CsA and controls was 183±93 min, 137±65 min and 196±66 min, respectively (P=0.52). MCAT was 229±101 min, 283±59 min and 363±55 min for FK, CsA and controls, respectively (P=0.002). Since these patients were taking concomitant drugs, the differences may be due to the use of FK. It was recognized that the macrolide structure of FK has a stimulatory effect on gastric emptying.

ACB sensors are versatile technologies that can be used for a wide range of applications. In clinical practice, their non-invasive and radiation free features provide an excellent approach for better and more reproducible monitoring of GI transit. New magnetic carriers can improve the sensitivity of ACB allowing other clinical applications.
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Hollow CoPt MNPs as a contrast agent for tracking transplanted neural stem cells in spinal cord slices

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Neural stem cells (NSCs) exhibit features that make them suitable candidates for stem cell replacement therapy and spinal cord reconstruction. Magnetic resonance imaging (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 magnetic CoPt hollow nanoparticles (CoPt NPs) into neural stem cells and to define appropriate MRI parameters. Both cell viability and multipotency analysis showed that CoPt NPs at a concentration of 16 mg ml_1 reduced T2 relaxation times in labeled rat NSCs, producing greater contrast on spin echo acquisitions at 4.7 T, yet did not affect cell viability and in vitro differentiation potential compared to controls. After optimizing nanoparticle loading concentrations and labeled cell numbers for MRI detection, CoPt-loaded NSCs were transplanted into organotypic spinal cord slices. The results showed that MRI could efficiently detect low numbers of CoPt-labeled NSCs with the enhanced image contrast. Our study demonstrated that MRI of grafted NSCs labeled with CoPt NPs is a useful tool to evaluate organotypic spinal cord slice models and has potential applications in other biological systems.

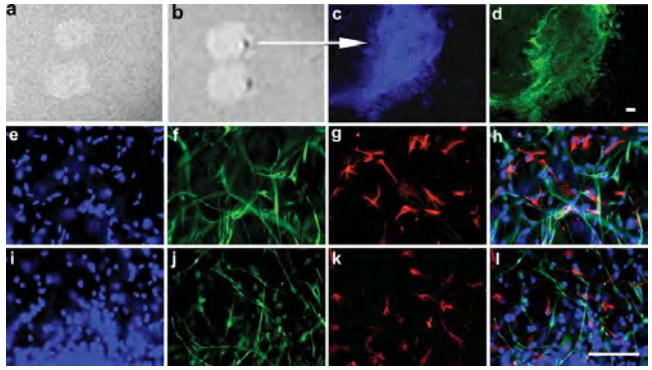

Fig 1. CoPt-labeled NSCs detected by MRI after transplantation into rat spinal cord slices

Core-shell gold coated magnetic nanoparticles and their interaction with thiolated DNA

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Core-shell magnetic nanoparticles have received significant attention recently and are actively investigated owing to their large potential for a variety of applications. Here, the synthesis and characterization of bimetallic nanoparticles containing a magnetic core and a gold shell are discussed. The gold shell facilitates, for example, the conjugation of thiolated biological molecules to the surface of the nanoparticles. The composite nanoparticles were produced by the reduction of a gold salt on the surface of pre-formed cobalt or magnetite nanoparticles. The synthesized nanoparticles were characterized using ultraviolet-visible absorption spectroscopy, transmission electron microscopy, energy dispersion X-ray spectroscopy, X-ray diffraction and super-conducting quantum interference device magnetometry. The spectrographic data revealed the simultaneous presence of cobalt and gold in 5.6 ± 0.8 nm alloy nanoparticles, and demonstrated the presence of distinct magnetite and gold phases in 9.2 ± 1.3 nm core-shell magnetic nanoparticles. The cobalt-gold nanoparticles were of similar size to the cobalt seed, while the magnetite-gold nanoparticles were significantly larger than the magnetic seeds, indicating that different processes are responsible for the addition of the gold shell. The effect on the magnetic properties by adding a layer of gold to the cobalt and magnetite nanoparticles was studied. The functionalization of the magnetic nanoparticles is demonstrated through the conjugation of thiolated DNA to the gold shell.

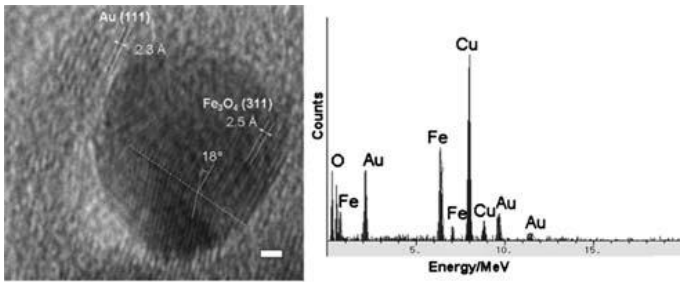

Fig 1. HRTEM image and EDX spectrum of a Fe3O4-Au NP

Hyperthermic effect in suspension of magnetosomes prepared by various methods

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The existence of biocompatible phospholipid membranes around magnetosomes and the obtained specific absorption rate (*SAR)* values predetermined the bacterial magnetic nanoparticles-magnetosomes to be as good materials for the biomedical hyperthermia applications [1,2]. The magnetozomes were prepared by biomineralization process of magnetotactic bacteria *Magnetospirillum sp.AMB-1*. The isolated chains of magnetosomes (sample M) were centrifuged at speed of 100 000 rpm (sample UM) and sonificated at 400 W for 3 hours (sample SM). The prepared suspensions were investigated with respect to structural, magnetic and hyperthermia properties. The results from scanning electron microscopy showed that isolated chains of magnetosomes were partially broken to smaller ones after ultracentrifugation. On the other hand the application of the sonification process the individual magnetosomes appeared only. These results were confirmed by coercivity and magnetic saturation measurements. Similarly, as it can be seen from figure 1, the contribution of hysteresis processes to the total release of thermal energy for the three samples as a function of magnetic field strength is the smallest one for sample obtained after sonification process. The found values for the specific rate absorption (SAR) of 1083 W/g for M, 934 W/g for UM and 463 W/g for SM at 10 kA/m are comparable to values found for similar samples [2].

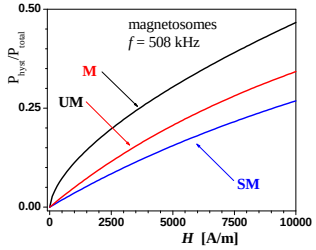

Fig. 1: The contribution of hysteresis processes to the total release of thermal energy for the three samples (M-normally isolated, UM-after ultracentrifugation, SM-after sonification) as a function of magnetic field strength.

Acknowledgements
This research was supported from the Projects No 26220120033, 26110230061 and 26220220005 in the frame of Structural Funds of European Union, Centre of Excellence of SAS Nanofluid, VEGA 0077 and APVV 0171-10.

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Radiation Stability of the BSA Stabilized Biocompatible Magnetic Fluid

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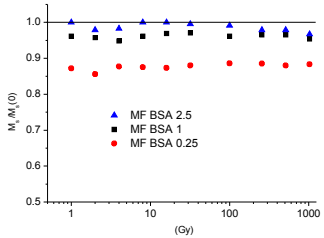
Superparamagnetic iron oxide nanoparticles have been widely used for medical applications, for example in drug targeting delivery, magnetic hyperthermia, arrangement of biological assemblies, contrast agents in Magnetic Resonance Imaging, biomagnetic separation, etc. Especially, *in vivo* applications of nanoparticles require highly biocompatible particle surfaces. Coatings can improve oxidation resistance, mechanical stability, and biocompatibility. Magnetic fluid hyperthermia is gaining increasing attention as a potential new cancer treatment. In this technique biocompatible superparamagnetic nanoparticles are injected into targeted region and heated by an external applied AC magnetic field. In work [1] the combined thermotherapy and radiation with 20 Gy was shown to be significantly more effective than radiation alone with 60 Gy.
The aim of the presented work was to investigate the stability of biocompatible magnetic fluid, i.e. water-based magnetic fluid containing magnetite nanoparticles stabilized by surfactant sodium oleate and modified by Bovine Serum Albumin (BSA) after electron irradiation. Samples with the same initial concentration of Fe_3O_4 but different mass ratio BSA/Fe_3O_4 (0.25, 1.0 and 2.5) were studied. The irradiation of samples was conducted by electrons with energy 8.6 MeV. The electron irradiation up to 4 Gy caused about 10 % reduction of the saturation magnetization in the case of the sample with mass ratio BSA/Fe_3O_4 0.25 comparing with the samples with mass ratio BSA/Fe_3O_4 1 and 2.5. We can conclude, that increasing mass ratio of BSA/Fe_3O_4 stabilises magnetic fluid against irradiation.



Dependence of reduced saturation magnetization on irradiation doses of water-based magnetic fluid containing magnetic particles coated with sodium oleate and modified with BSA. (The black line corresponds to value $M_s/M_s(0)$ of non irradiated sample).

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This work was supported by the Slovak Academy of Sciences grants VEGA 2/0077/09 and Centre of Excellence Nanofluid, Ministry of Education of Slovakia - Agency for Structural Fonds of EU in frame of project No. 26110230061 and by Ministry of Education and Science of Ukraine in the framework of project DB-715.

Influence of surface coating on magnetic and self-heating properties of Fe_3O_4 nanoparticles and *in vitro* experiment for hyperthermia

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Hyperthermia using magnetic nanoparticles is an attracting treatment for cancer. The magnetic properties, temperature rise under ac magnetic field and cytotoxicity of Fe_3O_4 nanoparticles coated with polyethylenimine (PEI), oleic acid, and Pluronic F-127 were evaluated in this study. *In vitro* hyperthermia effect of Fe_3O_4 nanoparticles coated with Pluronic F-127 was evaluated. The oleic-acid coated and Pluronic coated Fe_3O_4 nanoparticles immobilized with agar exhibited greater heat dissipation compared to the PEI coated nanoparticles, whereas the similar temperature rises of fluid Fe3O4 nanoparticles were observed. In cytotoxicity study, only oleic-acid coated nanoparticles exhibited cytotoxicity. These results indicate that the Pluronic-coated Fe_3O_4 nanoparticles, the heat dissipation of which is not related to surrounding viscosity and exhibited biocompatibility, will be suitable for hyperthermia. Appropriate temperature rise of the Pluronic-coated Fe_3O_4 nanoparticles significantly reduced the viability of HeLa cells and induced apoptosis.

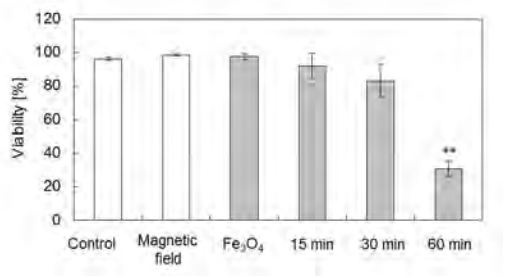

Fig. 1 Viability of HeLa cells treated with hyperthermia treatment using the Pluronic-coated Fe_3O_4 nanoparticles at the field strength of 200 Oe for 15, 30, and 60 min. *$P < 0.05$, **$P < 0.01$, n=3.

Highly efficient DNA extraction with droplet-based microfluidics and magnetic microcarriers

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Fast, cheap and reliable DNA analysis tools are getting increasingly important for all kinds of diagnostics, including the detection of gene mutations, cancer diagnosis, and archeological or criminal identification. From the technological side the miniaturization of large-scale laboratory techniques onto the size of a chip offers interesting opportunities to develop DNA based diagnostic assays at the micro- and nanoscale.

In this research we have investigated the DNA extraction from a heterogeneous sample mixture with high efficiency using bio-functionalized magnetic microparticles in a droplet-based microfluidic system.

Droplets were formed in a water in oil system where the magnetic particles were suspended in the water phase and mixed with the sample (Fig.1A), which contained a heterogeneous mixture of DNA stands. Complementary DNA strands were immobilized on the magnetic particles, in order to bind the target DNA when the droplets pass through a mixing zone. The target strands were then separated from the non-specifically bound DNA strands using a magnetic splitter (Fig.1B). Through a T-junction the droplets split into two daughter drops while a magnet inserted close to the lower arm ensured that the target DNA was isolated into one daughter drop [1]. With this method Dittrich [1] presented a complete separation of warfarin using equal droplet split. Nevertheless to increase the extraction efficiency we had to concentrate the major part of the magnetic particles into a tiny daughter drop. Therefore the symmetric T-junction was modified, the narrow part of one arm was elongated which resulted an unequal droplet split. The splitting ratio depended on the asymmetry of the T-junction [2]. The DNA extraction efficiency was studied by using different size of particles and by changing the position of the magnet.

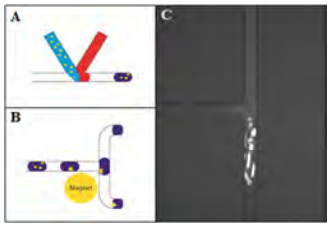

Fig.1. A Scheme of the droplet formation zone with magnetic particles and the sample mixture, **B** scheme of the magnetic splitter and the two daughter drops, **C** experimental image of the droplet containing magnetic particles inside the T-junction

[1] D. Lombardi, P.S. Dittrich, Anal. Bioanal. Chem. (2010), 399, 347-352
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Synthesis of iron oxide superparamagnetic nanoparticles with different sizes and their functionalization with a poly-ethylene-glycolated silane

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Tailoring the properties of magnetic nanoparticles (MNPs) is essential for various nano-based biological applications. Commercial MNPs are, however, difficult to tune as the properties and coatings are seldom clearly described, and are polydisperse in size and shape.[1] Having control over the properties of the MNPs from the bottom up, permits a maximum flexibility. We report the optimization and thorough characterization of a first (SMG[1]) and second seed mediated growth (SMG²) step by varying the surfactant amount and by optimizing the heating steps. The synthesized MNPs were thoroughly characterized with different techniques. We also show the functionalization of these MNPs with poly-ethylene-glycolated silanes, to render the MNPs dispersible in water. Hereto, different complementary characterization techniques were used to investigate the surface tailoring of the MNPs.

Transmission Electron Microscopy (TEM) revealed, starting from high quality MNPs produced by thermal decomposition[2] and by applying the seed mediated growth method, a growing monodisperse samples with sizes of 6.9 nm for the seed MNPs, 9.9 nm for SMG[1], and 12.9 nm for SMG² MNPs. In addition the relative size distribution dropped from 19% for the seed MNPs, to 13% for the SMG[1] and 10% for the SMG² MNPs (Figure A-B). Dynamic Light Scattering (DLS) was used to study the MNPs size in suspension and showed a similar size increase as TEM. X-ray diffraction (XRD) demonstrated the crystalline nature of all the samples. The magnetic properties of the MNPs were studied with Vibrating Sample Magnetometry (VSM) and were in concert with their size increase.

Functionalization of the MNPs was performed with poly-ethylene-glycolated silane. Back ground stained TEM showed a nice enwrapping of the silane around the MNPs, indicating a single core single shell functionalization of the MNPs (Figure C). Fourier Transform infrared Spectroscopy (FTIR) spectra clearly showed a peak at ~1100 cm^{-1}, specific for the asymmetric and symmetric stretches of the ethylene-oxide groups in the silane, which was absent in the FTIR spectrum of the non-functionalized MNPs, indicating the presence of the silane on the MNPs. (Figure D). XPS data disclosed uniquely a peak at 101.7 eV, representing oxidized silicium, on the functionalized MNP sample, confirming the successful engraftment of the silane on the MNPs' surface.

In conclusion, the proposed route of step-wise synthesis in combination with silane functionalization allows tuning the properties of iron oxide MNPs for applications in aqueous environment.

Funded by the SBO project iMAGiNe of the IWT (agentschap voor Innovatie door Wetenschap en Technologie, Belgium).

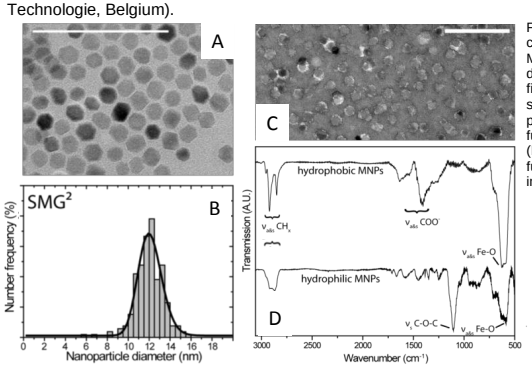

Figure. Bright field TEM-image (A) and corresponding histogram (B) of SMG² MNPs. The histogram, plotted as diameter *versus* number frequency, was fitted with a lognormal curve. Background stained bright field TEM-image (C) of poly-ethylene glycolated silane functionalized MNPs. The FTIR-spectra (D) of MNPs before and after silane functionalization. Scale bar of the TEM images is 100 nm.

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Iron-cobalt ferrite nanoparticles biocompatibility and distribution after intravenous administration to rat

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Magnetic nanoparticles (MNP) of iron-cobalt ferrite ($Co_xFe_{1-x}Fe_2O_4$, weight content of Co 1.5% or 25%), uncoated or coated with SiO_2, were synthesized for the purposes of magnetic hyperthermia and cell separation. Their biocompatibility *in vitro* and *in vivo* and distribution *in vivo* with respect to the effect of an external magnetic field were studied.

***In vitro* studies. Passage 1 (medium with MNP).** HEp-2 cells (human larynx carcinoma) or rat mesenchymal stem cells (MSC) were incubated for 3-4 days in a 24-well plate with 3 mg of MNP per a well. Morphology, viability and number of population doublings (PD) were examined. MSC and especially HEp-2 cells accumulated high amounts of magnetic material intracellularly. There were no decrease in viability of HEp-2 cells incubated with MNP containing 1.5% Co, but PD were decreased (78±6% and 85±3% of MNP-free reference for uncoated and SiO_2-coated MNP, respectively). MNP containing 25% Co were highly cytotoxic (PD 0±6% ref., viability 80±5%). The culture medium where MNP were incubated for 3 days was highly cytotoxic in case of MNP 25% Co, but not MNP 1.5% Co. The diffusion of Co ions from MNP into the medium was confirmed by atomic absorption spectrometry. **Passage 2 (after washing).** Cells were trypsinized, washed and incubated in MNP-free medium. At the end of the passage, cells still contained intracellular aggregates of MNP. The PD were comparable to the reference in case of MNP 1.5% Co (uncoated: 94±5% ref.; SiO_2-coated: 89±3% ref.) and were decreased in case of MNP 25% Co (39±8% ref.). Thus, iron-cobalt ferrite MNP with low cobalt content, as well as SiO_2-coated variant of these MNP, inhibit proliferation of HEp-2 cells moderately and produce virtually no effect on cells viability.

***In vivo* studies.** Single dose (1 ml of MNP suspension with Fe content 35 mg/ml) or fractioned dose (3 doses with the interval of 2h) were administered into tail vein of white rats (200±10g) . At 0.5, 1, 3 and 24 h after the last administration of MNP the rats were sacrificed and Fe content was determined in organs (liver, kidneys, spleen, lungs, muscles, brain) and model tumors (M-1 sarcoma and PC-1 alveolar hepatocarcinoma). The lethal doses (mg/kg) were LD16=84.2, LD50=314.1, LD84=541.0, LD100=659.0. The most MNP-accumulating organs were liver, kidneys and spleen. No accumulation was observed in brain. In the liver the accumulation maximum was at 3 h (105 µM Fe) when no external magnetic field was applied; at 24 h when NdFeB magnet was applied (gradual increase from 108 µM at 0.5h to 156 µM at 24h). In the kidneys without magnet the maximum was at 30 min (92 µM); with magnet – at 24 h (142 µM). In tumors, Fe content when no MNP were administered was 102 µM. With this reference value subtracted, MNP-attributed increase in Fe content was 58 µM without magnet and 139 µM (240%) with magnet.

Measurement of dynamic size distribution of magnetic nanoparticles

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Brownian relaxation of magnetic nanoparticle (MNP) is an interesting property that is used to monitor the hydrodynamic size, which could indicate the dynamic bio-chemical binding process. Previously it has been proposed that a mixing-frequency method can precisely measure the Brownian relaxation in real time [1]. MNPs are driven into the saturation region by a low frequency sinusoidal magnetic field, while a high frequency field of multiple tones is applied to generate mixing-frequency signals that are highly specific to the magnetization of MNPs. The mixing-frequency signals are picked up by a pair of balanced built-in detection coil as shown in the figure. The phase and amplitude of the mixing-frequencies signal will change due to the frequency dependence of Brownian relaxation. The phases and amplitudes at different frequencies will be used to estimate the hydrodynamic size distribution of MNPs by Least Mean Square method [2] to monitor binding events in real time.

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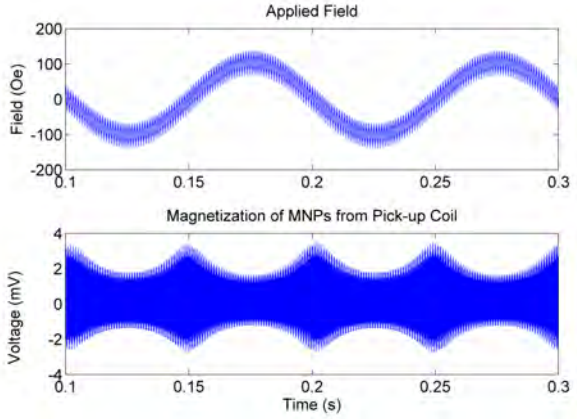

Signal from pick-up coil is digitized and recorded in time domain. The applied field (top) contains low frequency 10 Hz and multi-tone high frequencies (1 kHz, 5 kHz, 10 kHz, 15 kHz, and 20 kHz). The magnetization of MNPs (bottom) shows the periodic nonlinear response.

Co-Zn ferrite cores prepared by coprecipitation method for magnetic hyperthermia and magnetic resonance imaging

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In the search of a suitable mediator for magnetic fluid hyperthermia (MFH) and efficient contrast agents for magnetic resonance imaging (MRI) our group has focused on the nanoparticles of the cobalt-zinc ferrite. Namely the composition of $Co_{0.4}Zn_{0.6}Fe_2O_{4+\gamma}$ has been considered for MFH with respect to its Curie temperature and thus the possibility of the self-controlled heating mechanism in the range of 42 – 45 °C. For MRI the nanoparticles of $Co_{0.5}Zn_{0.5}Fe_2O_{4+\gamma}$ phase have been selected taking into account their high magnetization [1].

The ferrite cores were prepared by the coprecipitation method followed by annealing at 500 – 650 °C and mechanical treatment separating the individual particles. The XRD analysis evidenced single-phase composition of the products and their cubic spinel structure. The mean size of crystallites was ranging between 10 – 40 nm. Subsequently, selected samples were coated by silica in Stöber derived process. TEM studies revealed the silica shell with the thickness of 15 - 25 nm. The colloidal stability of aqueous suspensions was confirmed by DLS and the hydrodynamic size measurement indicated the mean values between 90 – 150 nm.

The silica coated $Co_{0.4}Zn_{0.6}Fe_2O_{4+\gamma}$ nanoparticles with the mean size of crystallites 12 and 24 nm were selected for the magnetic heating study. The experiments were performed on a home-made apparatus in an AC field of the amplitude H up to 13.8, 8.9 and 6.0 $kA{\cdot}m^{-1}$ for frequencies f = 107, 480 and 960 kHz, respectively, employing stable aqueous suspensions with concentration of 3 - 5 $mg_{(Co+Fe)}{\cdot}ml^{-1}$. The heating power of the samples was corrected on the thermal losses because of non-adiabatic conditions. The relaxometric studies were carried out with silica coated $Co_{0.5}Zn_{0.5}Fe_2O_{4+\gamma}$ nanoparticles. Particularly the field dependence of T_2 relaxivity was investigated (measurements at 0.5, 1.5, 3 and 4.7 T) as well as its temperature dependence. Namely, the aqueous suspension of the silica coated cores with the mean size of 12 nm and specific magnetization, $\sigma_{0.5T}(300K)$ = 42.8 $A.m^2.kg^{-1}$ exhibited transverse relaxivity r_2 = 185 $s^{-1}mM^{-1}_{0.2Co+0.8Fe}$ at 0.5 T. The sample containing 15 nm cores possessing specific magnetization, $\sigma_{0.5T}(300K)$ = 48.0 $A.m^2.kg^{-1}$, showed r_2 as high as 325 $s^{-1}mM^{-1}_{0.2Co+0.8Fe}$.

The support by the projects GACR P204/10/0035 and GACR P108/11/0807 is gratefully acknowledged.

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Thermal Plasma Synthesis of Superparamagnetic Iron Oxide Nanoparticles for Biomedical Applications

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There is currently intense interest in the use of superparamagnetic iron oxide nanoparticles for biomedical applications, including as contrast agents for magnetic resonance imaging and as targeted agents that can be heated by applying an alternating magnetic field, killing cancer cells by hyperthermia. Conventionally these nanoparticles are synthesized by wet chemical methods. However gas-phase synthesis methods have a number of potential advantages over wet chemistry, including higher production rates, the avoidance of impurity residues, the avoidance of the need to manage and dispose of hazardous solvents, and the avoidance of the need to remove surfactants before adding layers that impart additional functionality to the nanoparticle.

Here we report synthesis of superparamagnetic iron oxide nanoparticles using a DC thermal plasma. Ferrocene vapor and oxygen were injected into an argon/helium plasma that was then expanded through a subsonic nozzle. Particles were collected on glass fiber filters located in the reactor exhaust. In-situ measurements of particle size distributions were made using an aerosol sampling probe interfaced to a scanning mobility particle sizer (SMPS). Collected powder was characterized by transmission electron microscopy (TEM), X-ray diffraction, and vibrating sample magnetometry (VSM). Results showed that the synthesized powder consisted of a mixture of magnetic iron oxides depending on the oxygen flow rate. VSM measurements confirmed that the powder was superparamagnetic. Particles synthesized with optimum oxygen flow rate consisted primarily of magnetite (Fe_3O_4), and had a room-temperature saturation magnetization of 40.15 emu/g, with a coercivity and remanence of 26 Oe and 1.5 emu/g, respectively. TEM images show primary particle diameters of 5-8 nm, while SMPS measurements indicate that the aerosol at the reactor exhaust consisted of small agglomerates, with a modal mobility diameter of 8-9 nm. These results are the best yet reported for synthesis of superparamagnetic iron oxide nanoparticles by any type of plasma and are comparable to the best results reported to date for flame synthesis.

Primarily supported by the National Science Foundation (CBET-1066343), and by the Minnesota Futures Grant Program. Parts of the characterization work were conducted at the College of Science and Engineering Characterization Facility and the Institute for Rock Magnetism at the University of Minnesota.

Ex vivo quantification of magnetic nanoparticles in clinical samples at room temperature with high specificity and improved stability

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In the development of new clinical diagnostic and therapeutic applications of magnetic nanoparticles (MNP) in medicine, new equipment and detection strategies for suitable quantitative analysis of tissue samples are necessary. Therefore we developed a new magnetometer, based on copper wound coils for excitation and detection, that is able to detect very tiny amounts of MNP-content in biological samples at room temperature. To achieve this, stability and noise reduction are important properties of the apparatus to improve. Based on our experience, increased mechanical and thermal stability could be realized by introduction of liquid nitrogen cooling of the copper coils [1]. Meanwhile the samples, placed in a 11 mm diameter sample chamber, are kept intact at room temperature in an anti-cryostat, to eliminate effects on additional subsequent analysis.

Improved magnetic detection is achieved by a frequency mixing technique using combined excitation fields with different frequencies [2]. This mixed excitation makes the detection highly specific for non-linear properties of sample material. Parasitic linear contributions from the tissue itself are often interfering with several magnetometry techniques. Specific non-linear detection makes the measurement insensitive for linear magnetic components. Tissue samples containing MNPs can thus be detected and quantified with high accuracy. The sensitivity that could be achieved with the setup is in the sub-microgram range for iron-oxide nanoparticles.

Clinical application of this magnetometer is currently realized for magnetic Sentinel Lymph Node (SLN) detection in either breast or colorectal cancer. An iron-oxide magnetic nanoparticle tracer is injected near the tumor and is assumed to accumulate in the first lymph node draining the tumor area. For colorectal cancer the system provides an ideal protocol for magnetic selection of the SLN out of a series of harvested lymph nodes. Selected SLNs are subjected to additional microscopic analysis for ultra-staging of the disease.

[1]. Rabbers, J. J., B. ten Haken, et al. (2001). "Advanced ac loss measurement methods for high-temperature superconducting tapes." Review of Scientific Instruments **72**(5): 2365-2373.

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Ferromagnetic microdisks and polymer-encapsulated Fe_3O_4 nanoparticles for hyperthermia applications

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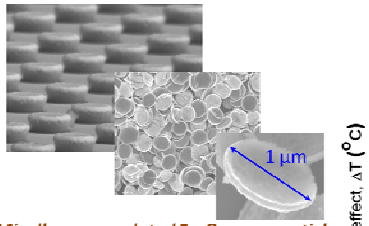
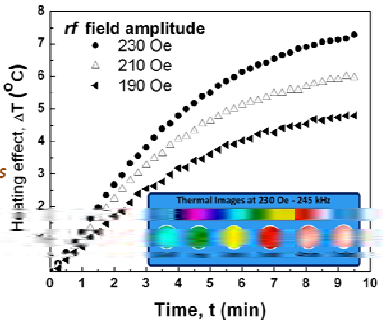
Superparamagnetic particles on the order of ten nanometers have been traditionally employed for magnetically-driven hyperthermia [1]. To achieve high efficiency in tumor heating, their applications typically call for large nanoparticle concentrations which could lead to cytotoxic effects. Anisotropically shaped ferromagnetic particles have a great potential for overcoming this limitation. In this work, we report on a different approach for magnetic hyperthermia using lithographically fabricated ferromagnetic microdisks (1 μm diameter, 60 nm thickness). These particles allow for multimodal treatment of cancer cells by utilizing different mechanisms controlling their dynamic behavior, depending on the frequency of the applied *a.c.* magnetic field. At the lower frequency range (~10-60 Hz, 90 Oe), these particles have proven to be instrumental for destruction of cancer cells by mechanically activating apoptotic pathways [2]. In the high frequency regime (hundreds of kHz), the microdisks can be employed for inductive heating applications due to the dynamic hysteresis losses, as we show here. For comparison, we also investigate the heating properties of 11 nm Fe_3O_4 nanoparticles encapsulated in polymer micelles [3] which serve as a superparamagnetic particle-based model system. The specific absorption rates of both particle types will be compared and the results of *in vitro* studies on cultured cancer cells will be presented.

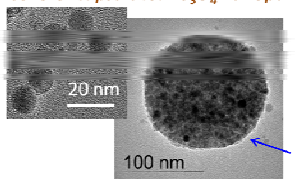
Lithographically defined ferromagnetic microdisks



Time-dependent heating of microdisks



Micelle-encapsulated Fe_3O_4 nanoparticles



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[3] D.-H. Kim, E. A. Rozhkova, T. Rajh, S. D. Bader, V. Novosad, *IEEE Trans. Magnetics* **45** (10), 4821-4824 (2009).

Magnetomechanical actuation of single cells by ferromagnetic disks induces intercellular calcium signaling

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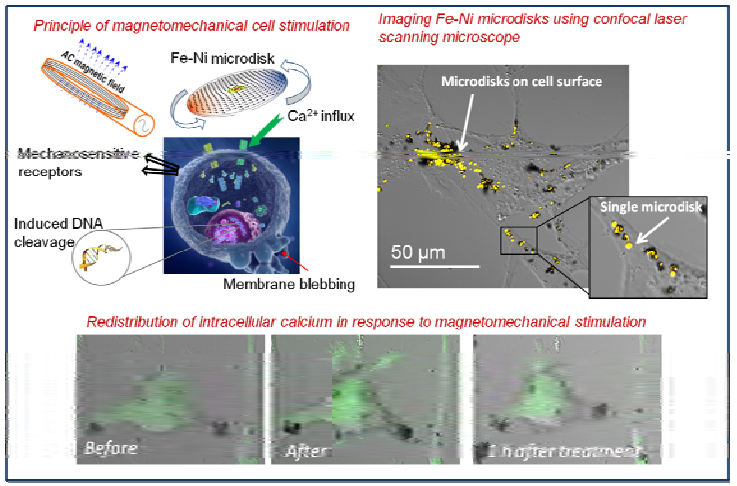
Magnetic materials allow for remote modulation of cell function by applying controlled forces [1]. Our group has recently demonstrated that ferromagnetic disks with spin-vortex ground state can be used for triggering programmed cancer cell death [2]. Although the results suggest that this phenomenon could be tied to cytoskeleton stress induced by magnitomechanical stimulation, the exact mechanism has not been fully understood. In this work, we demonstrate that magnetomechanical stimulation of a single cell results in signal transduction to the neighboring cells. The disks were fabricated by photolithography to define the shape of 1 μm diameter, followed by electron beam deposition of 60 nm permalloy layer and subsequent photoresist lift off. Upon application of a weak low frequency *a.c.* magnetic field we observe the calcium response in the neighboring cells. Therefore, the microdisks are serving as mediators of signal transduction which could be implicated in the mechanism of magnetomechanically induced apoptosis.



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[2] D.-H. Kim, E. A. Rozhkova, I. V. Ulasov, S. D. Bader, T. Rajh, M. S. Lesniak, V. Novosad, *Nature Materials* **9**, 165-171 (2010).

Quantification of *in vitro* and *in vivo* Magnetic Iron Oxide Nanoparticles (MIONs) using an Inductively Coupled Plasma Mass Spectrometer (ICP-MS)

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Many techniques are available to quantify the presence of magnetic iron oxide nanoparticles (MIONs) *in vitro* and *in vivo*. Popular, but less quantitative, are methods that make use of ferrous chelators and well-known chemical reactions, imaging-based methods including magnetic resonance imaging (MRI), x-ray microtomography, and histological examination of Perl's prussian blue reacted samples. Techniques based on atomic spectroscopy such as inductively coupled plasma atomic absorption spectroscopy (ICP-AAS) and inductively coupled plasma mass spectrometry (ICP-MS) have enabled more quantitative measurement of iron deposited by MIONs. ICP-MS in particular is a highly sensitive technique for iron quantification, able to reproducibly achieve resolution in the low part per billion (ppb) range. Due to such high sensitivity, ICP-MS is rapidly becoming the analytical method of choice for metallic and iron quantification in many types of samples. Our objective was to develop methods for quantitative digestion and analysis of aqueous MIONs, MION-loaded cells, and MION-containing murine blood and tissue to determine the presence and concentration of iron.

Samples were digested in nitric acid (HNO_3 optima grade) using a MARS5 Xpress microwave (CEM Corporation, Matthews, NC). The optimized digestion program was a ramp to temperature method achieving a final temperature of 175 °C. Post-digestion, samples were diluted to 1% HNO_3 in ultrapure water for analysis using an Agilent 7500ce ICP-MS (Agilent Technologies, Santa Clara, CA). In addition, an internal standard of scandium (Sc) was added to monitor instrument drift. Total iron content of each sample was calculated using an eight point calibration curve, blank correction, and the recovery of a standard reference material. In the case of aqueous MION solutions and MION-loaded cellular samples, the standard reference material utilized was SRM 2709a San Joaquin Soil (National Institute of Standards and Technology NIST, Gaithersburg, MD). For murine blood and tissue samples, SeroNorm Whole Blood (Sero AS, Billingstad, Norway) was used as the reference material.

Because of the innate sensitivity of ICP-MS and the ubiquitous nature of iron in the environment, development of precise and quantitative sample preparation free from environmental iron contamination is critical. This includes developing digestion and analysis techniques that match instrument precision and afford confidence in the accuracy of results. We developed and optimized digestion and analysis processes using multiple standard reference materials, internal standards, instrument standards, and replicate measurements to enable analysis of samples and estimation of confidence limits. We present iron content results obtained from cell culture and mouse studies containing MIONs. Using these optimized sample preparation and analysis methods, we are able to quantify iron content in samples well above baseline measurements.

Quantization of Spin Wave Levels and Ferromagnetic Resonance in Nanoparticles

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Ferromagnetic resonance is the absorption of energy from an rf electromagnetic field by spin waves in a magnetic material. The most commonly used process is the direct excitation of uniform mode spin waves whose wave vector is zero, thereby satisfying quasi momentum conservation. In bulk samples the uniform mode spin waves rapidly decay to other magnetostatic modes that dipole fields make degenerate with the uniform mode. The wavelength of these magnetostatic modes must be such that a whole number of half wavelengths is equal to the dimension of the sample which leads to large energy differences between spin wave energy levels in nanoparticles, thereby lifting the degenaracy of the magnetostatic modes, and removing the main relaxation channel. Heat flow from the grain to the matrix is restricted by a thermal contact resistance. As a result the number of spin wave modes, and the temperature of the grain increase very rapidly, until the decreasing frequency of the uniform mode is no longer in resonance. It is also possible to excite pairs of spin waves of equal and opposite wave-vector in the higher levels. This process has the advantage that the microwaves are only absorbed by grains in a narrow size range. With observed linewidths the calculated final temperature for 40 nm magnetite nanoparticles is about 90% of the magnetic transition temperature. Experimental results showed the time to reach this temperature was less than 0.01 secs, the shortest time experimentally available.

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Presentation preference: oral
Student presentation: no

Iron Oxide Nanoparticle for Cancer Therapy

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Multifunctional nanoparticles with the abilities to target tumors, carry therapeutic agents, and produce contrasts for tumor and stem cell imaging offer an exciting means for novel treatments of cancer patients. We have developed a theranostic magnetic iron oxide nanoparticle (IONP) platform that utilizes receptor-targeted IONPs to carry single or multiple therapeutic agents for drug delivery, optical imaging and magnetic resonance imaging (MRI). Our theranostic nanoparticles are designed to overcome physical and intrinsic barriers that reduce efficiency of drug delivery and confer drug resistance in human cancers. To minimize immune response to targeted nanoparticles after repeated administrations, we used species specific recombinant natural ligands and single chain antibodies that bind to cellular receptors with a highly affinity. By targeting to cellular receptors that are highly expressed in tumor cells, angiogenic endothelial cells, and active tumor stromal cells, these IONPs allow the drug to overcome the physical barrier in stroma-rich tumors, such as pancreatic cancer and triple negative breast cancer (TNBC), by serving as carrier vehicles for passage through the tumor endothelial cell layer and stromal fibroblasts, thereby increasing the efficiency of delivery into tumors but not into normal tissues. Based on the surface functionalization of the IONPs and chemical properties of drug molecules, we developed approaches for encapsulating to the IONPs, resulting in theranostic IONPs which carry one or multiple therapeutic agents. Various pH- dependent drug release mechanisms were also designed to ensure the efficient release of the drug molecules following receptor-mediated internalization. Targeted delivery, drug release, tumor growth inhibition, and MRI of drug delivery and response have been demonstrated in orthotopic breast and pancreatic and ovarian cancer animal models. Additionally, the retention of theranostic nanoparticles conjugated with near infrared dyes in the tumor cells that are resistant to the therapy allows optical image-guided surgery to remove drug resistant tumors. This presentation will report our progress in the development of the theranostic nanoparticles

One-pot Synthesis and Morphological Control of Janus Superparamagnetic Composite Nanoparticles

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Described is a one-pot colloidal reaction strategy to synthesize Janus magnetic composite particles (JMCPs) with high anisotropy, consisting of Fe_3O_4/silica inorganic hybrids and polystyrene nodule epitaxially arranged in a heterodimer or capped morphology. The key to obtaining the Janus composite particle pairs with high magnetic contents was the combination of miniemulsion polymerization and the sol-gel reaction in a batch process. Because of controlled phase separation between two inorganic components and polymer part induced by polymerization or catalyst addition, the morphological control of the magnetic composite particles was achieved in a straight forward fashion by adjusting the processing parameters. The Janus nanomaterials with superparamagnetic and amphiphilic properties obtained by the facile and effective way will have significant potential applications in the biomedical field.

With the typical Asymmetric shape and surface property, they could be functionalized with two kinds of chemical groups on each side of the composites, such as carboxyl group on polymer component and amine group on silica shell. This kind of nanomaterials may be used in magnetic immunoassay.

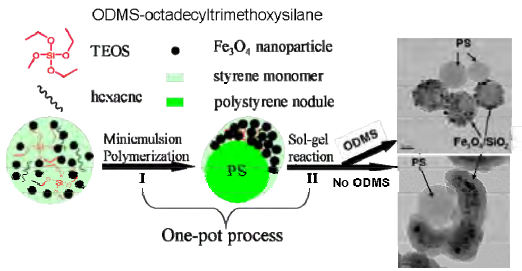

Figure. A novel one-pot colloidal reaction strategy is developed to synthesize Janus magnetic composite particles with high anisotropy, consisting of Fe_3O_4/silica inorganic hybrids and polystyrene nodule epitaxially arranged in a heterodimer morphology or capped shape.

A Robust Protein immobilization Approach onto GMR Biosensors

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In the development of biosensors, the immobilization of biomolecules (e. g. antibodies, antigens, and nucleic acids, etc.) onto the active surface of sensors is crucial. Many functional groups, such as NHS ester and epoxy, etc, have been deposited on biosensor surface to capture proteins. However, these groups are very sensitive to water or moisture, so the storage of biosensors with functionalized surfaces for the on-site and handheld applications has been a big challenge. Herein, we developed and demonstrated a modification method that could be applied to silicon wafer and GMR biosensors, which both have a SiO_2 layer outside. In the first step their surface was modified by 3-aminopropyltriethoxy silane (APTES), then functionalized with glutaraldehyde (Glu) resulting in the surface with aldehyde group. Different antibodies (IL-6, Nectin-4, and PAPP-A) have been efficiently bond to aldehyde terminated silicon wafer, and the three bond antibodies show a high affinity to their antigens. Additionally, APTES-Glu modification also has a more efficient binding to IL-6 antibodies than APTES-EDC coupling. In the following figure (left), sandwich binding was successfully demonstrated on silicon wafer using APTES-Glu under fluorescent microscope. GMR biosensor works with magnetic nanoparticles' binding, and the 30nm Iron oxide nanparicles' binding on the stripe type GMR sensor with APTES-Glu modification is also shown in the right SEM image.

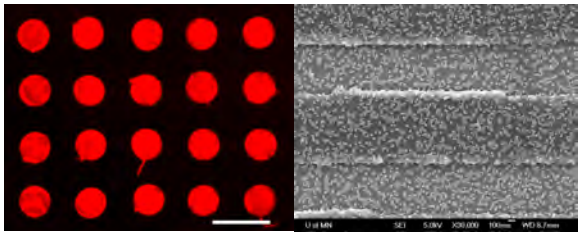

Figure. Both silicon wafer and GMR sensor were modified by APTES-Glu. After PAPP-A capture antibody, antigen, and biotinylated detection antibody binding, AF555-Streptavidin was added and bond to silicon wafer (left, scale bar is 400um),and 30nm streptavidin labeled nanoparticles was added and bond to GMR sensor (right).

Magnetic Field-Flow Fractionation using Parallel-Plate Channels and a Linear Halbach Array of Permanent Magnets

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The most successful implementation of magnetic FFF to date has used a quadrupole electromagnet with a thin helical channel mounted axisymmetrically in its aperture. The success of this approach may be attributed to the efficient use of the uniform constant field gradient within the aperture of the quadrupole. The control of the current to the electromagnet also allows for programmed field gradient decay during sample elution, which is essential for the analysis of polydisperse samples. The quadrupole magnet is large, heavy, and difficult to construct, however. It uses four copper wire coils, each of ~1900 m length, and steel plate for the solenoid cores and magnetic flux return yoke. The pole pieces must be precision-machined to hyperbolic profiles, and the channels also require precise machining using a specialized lathe.

The proposed approach will use a linear Halbach array of permanent magnets and a parallel-plate channel. In 1973, Mallinson proposed that certain magnetization patterns in a planar magnetic structure could result in the magnetic flux escaping from one of the surfaces and none from the opposite surface. Klaus Halbach and others subsequently developed the concept using discrete magnet elements for circular and linear arrays. The advantage of the linear Halbach array for magnetic FFF is that the magnetic field and field gradient is approximately constant at a fixed distance from the array, and both field and field gradient decay exponentially with distance from the array. The field is therefore perfectly suited to implementation of magnetic FFF using conventional parallel-plate channels, with field decay programming being accomplished simply by translation of the magnet array away from the channel. The magnetic field has been modeled around a linear Halbach array and this allows the optimization of magnet block and magnet array dimensions. The model predicts that an instrument based on this approach should equal or exceed the capabilities of the current quadrupole system.

Synthesis and Application of Magnetic Silica Composite with Optical Nanoparticle Layer

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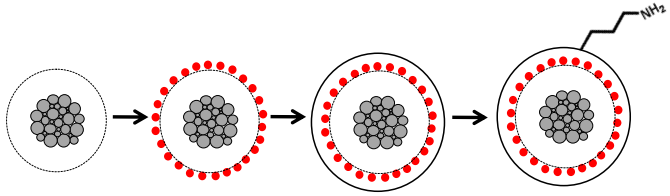
The submicron- to micron-sized multifunctional silica composites containing various nanoparticles can provide much better alternative to their individual nanoparticles in the fields of application, due to their advantages such as robust structure, well-known surface chemistry, and easy handling.

Here, we report the synthesis of submicron-sized magnetic silica composite beads encapsulating quantum dot (QD) layer and gold nanoparticle layer. In the first step, the magnetic cluster composed of magnetite (Fe_3O_4) nanoparticles was synthesized and encapsulated with silica using Stöber method with a slight modification, yielding magnetic silica (MS) composite. Then, the surface of MS composite was functionalized by using 3-aminopropyltrimethoxysilane (APTMS). The self-assembly of carboxy-terminated optical nanoparticles on the silica bead was controlled homogeneously *via* electrostatic interaction and the successive encapsulation by silica was nicely done. The final product will be designated by MSQS for QDs and MSAS for Au nanoparticles.

The MSQS and MSAS composites were characterized by TEM, XRD, UV/Vis absorption spectroscopy, photoluminescence spectroscopy, FT-IR spectroscopy, and alternating gradient magnetometer.

The MSQS showed enhanced photoluminescence compared to its corresponding initial QD solutions. The MSAS showed red-shifted absorption spectrum compared to its corresponding initial Au nanoparticle solutions. Both the MSQS and MSAS were reversibly dispersible, physicochemically stable, robust, and easy to handle. The MSQS was tested for a feasibility of FRET sensor application with Cy5-DNA, displaying an optimistic result. Further investigation is underway and will be published elsewhere.



Schematic representation of the synthesis of MSQS and MSAS.

Antibody conjugated magnetic iron oxide nanoparticles for cancer cell separation in fresh whole blood

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A highly efficient process using iron oxide magnetic nanoparticles (IO)-based immunomagnetic separation of tumor cells from fresh whole blood has been developed. The process involved polymer coated 30 nm IO that was modified with antibodies (Ab) against human epithelial growth factor receptor 2 (anti-HER2 or anti-HER2/neu) forming IO-Ab. HER2 is a cell membrane protein that is overexpressed in several types of human cancer cells. Using a HER2/neu overexpressing human breast cancer cell line, SK-BR3, as a model cell, the IO-Ab was used to separate 73.6% (with a maximum capture of 84%) of SK-BR3 cells that were spiked in 1 mL of fresh human whole blood. The IO-Ab preferentially bound to SK-BR3 cells over normal cells found in blood due to the high level of HER2/neu receptor on the cancer cells unlike the normal cell surfaces. The results showed that the nanosized magnetic nanoparticles exhibited an enrichment factor (cancer cells over normal cells) of 1:10,000,000 in a magnetic field (with gradient of 100 T/m) through the binding of IO-Ab on the cell surface that resulted in the preferential capture of the cancer cells. This research holds promise for efficient separation of circulating cancer cells in fresh whole blood.

Observation of the assembly of magnetite clusters with different sizes in SiO_2 coating process

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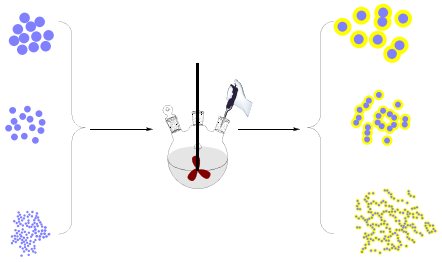
Owing to their fascinating magnetic properties, magnetite nanomaterials have recently shown important utilization in various fields. To promote the application, scientists pay particular attention to the study of their dispersion in solution.

According to previous work, magnetite nanoparticles can self-assemble into dipolar structures, such as strings and chains, both in magnetic field and zero-field. Nanoparticles with larger sizes are much easier to form these structures. These phenomena have already been systematically studied. However, the assemblies of magnetite microspheres or clusters composed of many nanoparticles have been observed in the magnetic field only.

In this work, magnetite clusters with different sizes are coated with SiO_2 under mechanic stirring in zero-field. The SiO_2 shell can preserve the morphology of the assembled clusters in solution. Under the same reaction condition, the clusters with smaller sizes are easier to form rings and chains from TEM observation, which is probably induced by magnetic attractive force between the clusters. To study magnetic dipolar attraction between the clusters in zero-field, we have measured the ac susceptibility of water suspensions of clusters. The results indicate that the residual magnetic moment of clusters is large enough for them to form chains. Our work may help to understand the behavior of magnetite clusters in solution.



Scheme of assembly of magnetite clusters with different sizes in SiO_2 coating process

PEI-coated magnetite nanoparticles linked with anti-Fas for combination of antibody and hyperthermia therapies

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Fas is a kind of protein and has been reported to play an important role in apoptosis [1]. And it is widely known that several cancer cells including HeLa cells overexpress Fas. Anti-Fas monoclonal antibody CH11 binds Fas and induces apoptosis.

Fe_3O_4 (20-30 nm) nanoparticles were coated with Polyethylenimine (PEI). The secondary size of the particles was 320 nm which was measured by dynamic light scattering (DLS). After binding the nanoparticles with CH11, the size of the complexes was 340 nm. *In vitro* growth inhibitory effect of the complexes and the viability of HeLa cells after hyperthermia treatment were studied

After incubating the HeLa cells for 24 hours, three sample groups were prepared: 1) exposed with the nanoparticles only at a concentration of 300 μg/ml (without CH11), 2) exposed with the complexes at 100-300 μg/ml (nanoparticles with CH11) and 3) control cells without any nanoparticles or CH11. Figure 1 shows the number of HeLa cells counted every 24 hours for 3 days. The number of the control sample increased double in 24 hours and saturated after 2 days. The number of the cells with the nanoparticles increased as same as the control sample, whereas that with the complexes was clearly dropped. This drop significantly depended on the concentration of the complexes, which suggested apoptosis induced by CH11.

As for hyperthermia, an ac magnetic field of 210-250 Oe at 120 kHz was applied to HeLa cells for 60 min after 24 hours from exposing the nanoparticles or complexes. The temperature of the cell pellet was increased from 37 deg. C to 40-45 deg. C depending on the field intensity. Figure 2 shows the number of living cells counted by a trypan blue exclusion method after 24 hours of applying a magnetic field. The number of living cells exposed with the complexes was less than half of that with the nanoparticles heated at 45 deg. C. These results suggest effectiveness of combination of antibody and hyperthermia therapies.

Reference [1] Zhang et al., Biochem. Biophys. Res. Com. 377, 1205, 2008.

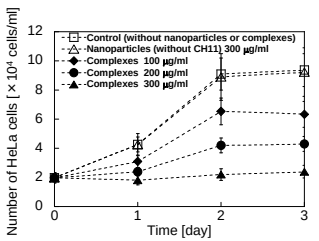

Fig. 1 Numbers of HeLa cells exposed to nanoparticles and complexes.

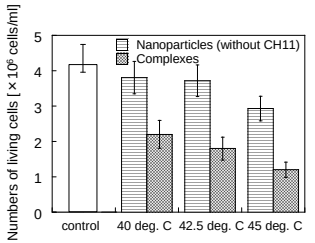

Fig. 2 Numbers of living cells after therapeutic treatment by magnetic nanoparticles and applied magnetic field.

Magnetic-field enhanced relaxation rates of protons in ferrofluids characterized with high-T_c SQUID-detected nuclear magnetic resonance spectrometer

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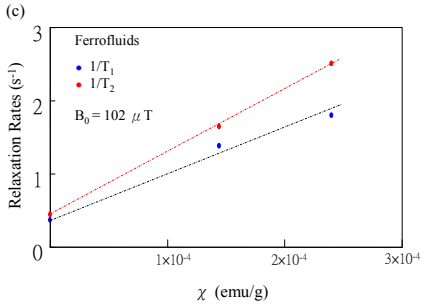
We investigated the magnetic-field enhanced relaxation rates of protons in ferrofluids by using high-T_c SQUID-detected nuclear magnetic resonance spectrometer. The longitudinal relaxation rate $1/T_1$ is slower than the transverse relaxation rate $1/T_2$ for both distilled water and ferrofluids in the same measuring field. This is due to the fact that the $1/T_1$ process involves returning the magnetization to the z-direction, which automatically involves the loss of magnetization in x-y plane governed by the $1/T_2$ process. Additionally, $1/T_1$ increases linearly when the strength of strength of measuring field increases. We attribute this to the magnetic-field gradients generated from magnetic nanoparticles that accelerate the T_1-relaxation more in high strength of magnetic fields than they are in low strength of magnetic fields. It was also found that both $1/T_1$ and $1/T_2$ increase when the magnetic susceptibility of ferrofluids increases. Characterizing the relaxation rates of ferrofluids and clarify their mechanisms will be helpful for their future biological applications such as the MR imaging in low magnetic fields.



$1/T_1$ and $1/T_2$ as a function of χ in B_0 = 102 μT.

The effect of the coating and clustering on the specific absorption rate of magnetic nanoparticles in alternating magnetic field

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The therapeutic benefits of magnetic nanoparticles used for hyperthermia can be increased with the use of nanoparticles systems that have higher heat generation rate which is quantified as specific absorption rate (SAR) at smaller applied power. Hence, it is essential to understand the factors that control the heat generation of magnetic nanoparticle in order to design fluids with optimized functionality. To understand these factors, experimentally measured SAR is compared here against theoretical predictions. SAR can be predicted from first law as $SAR = \frac{\mu_0 \pi \chi''}{\rho_{particle}} H^2 f$, where χ'' is magnetic fluid susceptibility, μ_0 is permeability of free space, $\rho_{particle}$ is particle density and f and H is the frequency and intensity of the applied magnetic field. For this study magnetic susceptibility was evaluated experimentally as well as theoretically employing Debye model.

This exercise was carried out on commercially available iron oxide nanoparticles with various coatings or uncoated. The coatings selected are highly relevant to biomedical applications and include amine and carboxyl functionalization as well as bioaffine ligands such as protein and biotin. The particle and cluster size was determined from transmission electron microscope (TEM), X-Ray diffraction (XRD) and Dynamic light scattering (DLS). XRD results showed that the particle sizes were around 10nm. TEM and DLS studies suggested that relatively clusters exist in ligands coated samples and small aggregates are present in Amine functionalized sample as well as uncoated sample, while minimal clustering appeared in Carboxyl functionalized sample.

The AC magnetic susceptibility of the suspensions was measured as a function of frequency with an in-house made apparatus. The magnetization was measured with vibrating sample magnetometer (VSM). Finally SAR was determined by heating the suspensions in a commercial induction system and measuring the temperature rise as function of time with a fiber optic sensor. The normalized predicted and experimental SAR values for all samples are shown in Table1.

The results show that normalized experimental SAR ($NSAR_E$) agrees relatively well with calculated SAR using experimental susceptibility ($NSAR_{C-}\ \chi''_E$) for Amine and uncoated samples; poor agreement was found when experimental susceptibility was substituted with calculated one ($NSAR_{T-}\ \chi''_C$) using Debye model, which is developed for non-interacting magnetic particles. These results suggest that agglomeration, which was present in both samples, may lead to dipolar interaction between nanoparticles and enhancement in power losses. Good agreement between measurements and predictions based on Debye model were obtained for Carboxyl samples where agglomeration was minimal.

For uncoated sample and Amine coated sample, both $NSAR_E$ values are lower than their $NSAR_{C-}\ \chi''_E$ values, which may due to the potential measurement errors in SAR. However, for protein and biotin coated sample, $NSAR_E$ values are higher than their $NSAR_{C-}\ \chi''_E$ values, an effect that has yet to be fully understood. One possible explanation could be the loss of superparamagnetic character at measuring frequency and an opening in hysteresis loop which may increase the dissipated power above that coming from relaxation heat losses.

Table 1. The comparison of properties and SAR of samples

Coating	Volume fraction(%)	Single particle diameter (nm)	Cluster diameter (nm)	$NSAR_E$	$NSAR_{T-}\ \chi''_C$	$NSAR_{C-}\ \chi''_E$
None	0.244	9.5	75±5	$0.71*10^{-8}$	$2.35*10^{-10}$	$1.34*10^{-8}$
Amine	0.230	9.3	85±5	$0.58*10^{-8}$	$2.21*10^{-10}$	$1.02*10^{-8}$
Carboxyl	0.066	6.2	30±5	0	$1.34*10^{-11}$	0
ProteinA	0.053	8.7	150±5	$0.61*10^{-8}$	$1.57*10^{-11}$	$0.11*10^{-8}$
Biotin	0.355	9.1	140±5	$0.36*10^{-8}$	$1.06*10^{-10}$	$0.31*10^{-8}$

* the units of NSAR are all $Wg^{-1}Oe^{-2}Hz^{-1}$ in the table 1

Characterization of neuron interaction with magnetic nanoparticles actuated by alternating magnetic field

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Superparamagnetic iron oxide nanoparticles are frequently utilized within magnetic resonance imaging (MRI), while other emerging applications include targeting vehicles for drug delivery and cancer hyperthermia. In the recent years there is an increasing interest in employing magnetic nanoparticles for cancer treatment primarily because of minimal side effects associated with hyperthermia. Use of magnetic nanoparticles for hyperthermia treatment may be especially useful for treating brain tumors. While it is well understood that cancer cells are damaged by temperatures above 42° C, little is known about the heating effects on healthy brain cells. In this context, the impact of increased heat generated by biofunctionalized iron oxide nanoparticles in alternating magnetic field on cortical neurons was investigated here. Particles with two types of functional coatings were used for this study.

Iron oxide nanoparticles coated with aminosilane (FA) and starch (FD) were purchased from Chemicell. The particle size was obtained from x-ray diffraction (XRD) and morphology was observed through transmission electron microscopy (TEM). The results showed that those two samples have the similar particle size and both have aggregates in the solutions. Cortical neurons were isolated from embryonic day seven chicks and cultured on poly-L-lysine (PLL) coated culture dishes (1,000,000 cells/ml) for two days prior to addition of particles. 20% v/v of particles to cell culture were added. The RF magnetic field was produced by an induction heating system. Iron oxide nanoparticles generated heat when placed in RF magnetic field, which increased the temperature of the cell culture. The temperature variation was monitored by fiber optic sensor.

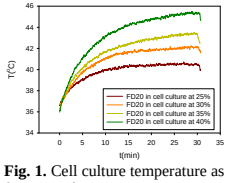

Fig. 1. Cell culture temperature as function of time.

Four power levels (25%, 30%, 35% and 40% of 3kW) were used to reach different rates of temperature increase (as shown in Fig.1). The cortical neuron viability was assessed using calcein-AM and propidium iodide and imaged using a epi-fluorescent microscope (at 10x). The calcein-AM images are shown in Fig.2. Green and red represent alive and dead cells respectively. The results show that heating, under theses specific heating conditions, does not reduce viability as compared to the control. The neurons can withstand temperatures as high as 45°C with virtually no effect on their viability. Studies on the effect of repeated heating sessions and the long term effects on viability are currently ongoing.

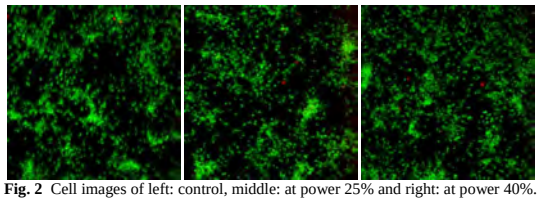

Fig. 2 Cell images of left: control, middle: at power 25% and right: at power 40%.

Complexes of magnetic nanoparticle-adenoviral vectors encoding therapeutic gene enhanced cardiac regeneration

Y. Zhang[1,*], W. Li[1,2,*,#], L. Ou[1], W. Wang[1,2], E. Delyagina[1], C. Lux[1], H. Sorg[3], G. Steinhoff[1,*] N. Ma[1,2,*]

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In this study we formed a stable complexes of magnetic nanobeads (MNBs) /adenoviral vectors (Ad) encoding hVEGF gene (AdhVEGF) via streptavidin and biotin interaction and assessed and whether the complexes administrated intravenously could regenerate ischaemically damaged hearts under the control of an external magnetic field in a rat acute myocardial infarction model.

Adenoviral vectors were conjugated to MNBs with the Sulfo-NHS-LC-Biotin linker. Transduction efficacy of MNBs/ Ad encoded marker gene, cytotoxicity and specificity were evaluated in rat mesenchymal stem cells under magnetic field stimulation. In vivo, MNBs/AdhVEGF complexes were injected intravenously and an epicardial magnet was employed to attract the circulating MNBs/AdhVEGF complexes in a rat acute myocardial infarction (AMI) model.

In vitro, compared with Adluc alone, MNBs/Adluc complexes induced folds higher transduction efficiency under the magnetic field while the cell viability kept above 80%. High specificity was demonstrated by a well-defined spots pattern of reporter gene expression. In vivo, epicardial magnet effectively attracted MNBs/AdhVEGF complexes and resulted in strong therapeutic gene expression in the heart. When compared to other MI treated groups, MI-M+/AdhVEGF group significantly improved left ventricular function ($p<0.05$) assessed by pressure-volume loops after 4 weeks. Also MI-M+/AdhVEGF group exhibited higher capillary than other MI treated groups ($p<0.05$).

Magnetically targeted delivery of complexes of magnetic nanoparticle-adenoviral vectors encoding VEGF gene to an infarcted heart could enhance cardiac function. This novel method to improve gene therapy outcomes in AMI treatment offers the potential into clinical applications.

Fabrication of Environmental Response Block Polymer Magnetic Microspheres and Their Application in Controlled Release of Drug

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Controlled radical polymerization based on 1, 1-diphenylethylene (DPE method) was used to prepare pH-responsive and thermosensitive magnetic composite microspheres. By this method, Fe_3O_4/Poly (acrylic acid-*stat*-methyl methacrylate-*block*-(2-dimethylamino) ethyl methacrylate) (Fe_3O_4/P(AA-MMA-DMA)) microspheres and (Fe_3O_4/P(AA-MMA-NIPAM)) microspheres microspheres were prepared via emulsifier free emulsion polymerization using 1, 1-diphenylethylene (DPE) as radical control agent in the presence of Fe_3O_4 nanoparticles.

The structure and properties of Fe_3O_4/P (AA-MMA-DMA) and (Fe_3O_4/P(AA-MMA-NIPAM))microspheres were characterized by IR, ^{1}H NMR, SEC-MALLS, TEM, TGA, VSM and DLS. The application of Fe_3O_4/P (AA-MMA-DMA) microspheres in controlled release of drug was also investigated.

It was found that (Fe_3O_4/P(AA-MMA- DMA)) microspheres obtained were pH-responsive and (Fe_3O_4/P(AA-MMA- NIPAM)) microspheres are thermosensitive as shown in Figure 1 and 2. Perfect sphere-shaped morphologies of two microspheres are presented in Figure 3. Superparamagnetism with a saturation magnetization of 14.36 emu/g, 13.0 emu/g are achieved. Moreover, Fe_3O_4/P (AA-MMA-DMA) microspheres could control the release of phenolphthalein in a buffer solution by adjusting the pH value.

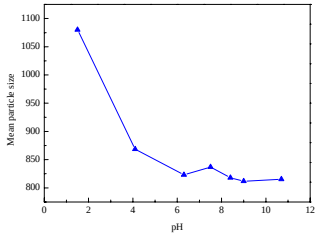

Fig. 1 The pH dependence of the mean particle size of Fe_3O_4/ P (AA-MMA-DMAEMA) microspheres at 37 ℃

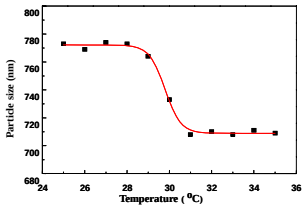

Fig. 2 The temperature dependence of the mean particle size of Fe_3O_4/ P (AA-MMA-NIPAM) microspheres

Poster 171

Rapid Lateral Flow Test Strips for Detection of Anti-*Treponema Pallidum* Antibody Using GoldMag® Nanoparticles as a Carrier

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[2] Xi'an GoldMag Nanobiotech Co., Ltd., Zhang Ba Wu Rd., Xi'an, China, *E-Mail: yali_cui@mail.com

Lateral flow immunochromatographic assay (LFIA) is a point-of-care testing (POCT) that has been widely used in medical diagnosis due to its inherent advantages of simple, fast-response and inexpensive. While the traditional test strips presented results on the basis of presence/absence, test strips design has progressed toward quantitative assays and the integration of readers. The strips using colloid gold as a carrier can be semi-quantitatively detected by a scanner, however, only signal from top 10 μm layer can be detected. Thus, the signal generated below was inevitably missed, making this method less sensitive.

The quantitative test of analytes can be achieved by measuring magnetization of whole volume of superparamagnetic labels in the detection zone. These devices have a higher sensitivity over those conventional optical methods. We have focused on the research on Fe_3O_4/Au composite nanoparticle (GoldMag®) for ten years. This new kind of nanoparticles has the advantages of the optical visible, chemical stability, efficient coupling for biomolecules and fast-acting to an external magnetic field. The GoldMag® nanoparticles with sunflower-like structure (Fig.1) was modified by sodium polystyrene sulfonate (PSS), and then the monodispersion nanoparticles could be used in the detection of biomolecule in the lateral flow test strips. The quantitative LFIA test was established in sandwich reaction format which is used for detection of antibodies of *T. pallidum* (Fig.2). The concentration of anti-*T. pallidum* antibody was determined by the Magnetic Assay Reader (Fig.3). The result shows that the antibodies' titer is positively correlated to the magnetic signal intensity, the higher antibodies' titer the stronger magnetic signal. Therefore, GoldMag® is promising for a wide range of point-of-care applications in the fields of medical diagnostics, food and environmental monitoring in the near future.

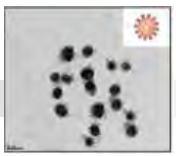

Fig.1 The TEM image of sunflower like Fe_3O_4/Au nanoparticles

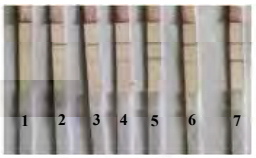

Fig.2 LFIA test strips for detection of antibodies to *T. pallidum* using GoldMag® as labels (Strip 1 is health human pooled sera; Strips 2-5 are control serum with 0.5, 1.0, 4.0 and 66.0 NCU respectively; Strips 6-7 are clinical positive serum)

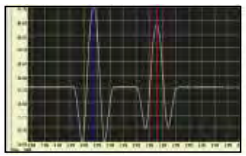

Fig.3 The magnetic signal of test line (blue) and control line (red) in LFIA measured by MAR

Poster 172

Drug-loaded magnetic nanocomposite devices for cancer magnetic thermochemotherapy

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Nanothermotherapy is a novel and effective approach based on magnetic nanoparticles (MNPs) for cancer treatment, which can be achieved by applying nanoscaled metallic particles that convert electromagnetic energy into heat. Currently, clinical trials at phase II are now under investigations on patients in Germany and Japan and demonstrate very inspiring for cancer therapy. In oncology clinical, hyperthermia is usually applied as an adjunct treatment to an already established treatment modality such as chemotherapy, as hyperthermia can effectively enhance the cytotoxicity of various antineoplastic agents (thermal chemosensitization). In recognition that MNPs can be acted simultaneously as mediators for magnetic nanothermotherapy as well as drug carriers, it is thus highly feasible to design and fabricated drug incorporated magnetic nanocomposite devices (MNDs) for multimodal cancer treatment of thermochemotherapy, to realize the possible thermal enhancement to drug cytotoxicity. In the current study, various drug-loaded MNDs, including magnetic nano-drugs, solar-planet structured MNDs and drug-loaded magnetic nanocomposite implant for cancer thermochemotherapy will be discussed.

MNPs were synthesized by chemical co-precipitation or thermal decomposition method. Epirubicin, a water soluble anthracycline drug was used for the fabrication of the magnetic nano-drug. The drug molecules were immobilized onto the surface of amino-coated MNPs through linker chemistry. For the preparation of the solar-planet structured MNDs, docetaxel loaded PLGA nanoparticles in the PLGA nanoparticles were first prepared by the single emulsion method, MNPs were further conjugated with the docetaxel loaded polymeric nanoparticles by EDN/NHS approach. Docetaxel-loaded magnetic nanocomposite implant was fabricated by the solvent cast method with Poly(DL-lactide-co-1, 3-trimethylene carbonate) (PLA-PTMC) (50:50,Mw 300,000 Da) as the metrics. Inductive heating property of the nanocomposite film was evaluated by exposing the sample under the alternative magnetic filed (AMF) of 300kHz. In vitro drug release profile was analyzed by the HPLC assay. Cytotoxicity and in vivo antitumor effect of the magnetic thermochemotherapy mediated by the nanocomposite implant were evaluated by CCK-8 assay on U251 human glioma cells and xenograft tumour model.

Fig.1 shows the illustrations of the structures of the MNDs fabricated as mentioned in this paper.The heating profiles of the suspensions of magnetic nano-drug and magnetic nanocomposite implant under alternative magnetic field (AMF) of 300kHz were shown in Fig.2. As can be seen in Figure 2 higher particle concentration or higher field intensity can result in a greater increase in the temperature. The desired hyperhtermic temperature can be achieved by appropriate adjusting the MNPs concentration or parameter of the AMF.

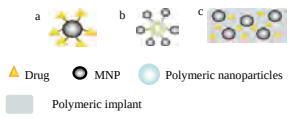

Fig.1 Illustration of various MNDs (a: Magnetic Nano-Drug, b: solar-planet structured MND; c: magnetic nanocomposite implant)

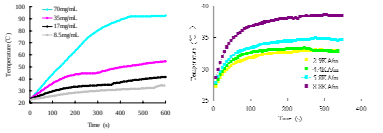

Fig.2 Heating profiles of magnetic nano-drug (left) and magnetic nanocomposite implant (right) subjected to AMF of 300kHz.

Sustained drug delivery can be realized by the above mentioned magnetic nanocomposite devices. Moreover, magnetic heating by exposing the MND suspension under the AMF would facilitate the drug release. In vitro cytotoxicity and in vivo antitumor effect of magnetic thermochemotherapy mediated by the various MNDs indicated that thermochemotherapy can significantly inhibit the tumor growth as compared with the mono-treatment of chemotherapy or hyperthermia, and consequently, thermochemotherapy can remarkably increase the life span of tumor bearing mice over that observed for control group and mono-treatment groups.

The MNDs exhibit advantageous feature for a facilitated drug delivery from the nano-carriers and the magnetic heating potential is adequate for hyperthermic treatments. We thus conclude that even though further detailed investigations are still necessary, tentative use in local tumor therapies aiming at a specific chemotherapeutic release in combination with magnetic heating is promising and feasible in the long term.

Tutorial on Magnetic Particles in Immunoassays

With Dr. Peter Hawkins

Tests based on immunoassays now make a major contribution in the diagnosis of diseases and magnetic particles play an important part in the sample extraction and purification stages of automated, immunoassay analysers in clinical chemistry laboratories. Magnetic particles can also be used as the label in the immunoassay which has the potential of developing one-step, near-patient analysers. Prof. Hawkins is a named inventor on several patents of such an analyser and in the 3 half-hour lectures will describe some of the different approaches that have been used to make these devices and the problems experienced.

Magnetic Particles in Immunoassays

Peter Hawkins

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Introduction and Summary.

•Immunoassays use antibodies as analytical reagents and have had a major impact in medicine as well as in veterinary and forensic sciences, agriculture and national defence.

•Magnetic particles (or beads) already play an important part in automated immunoassays and are been used in the development of simple, near-patient, diagnostic devices.

• The lectures will cover the following topics:

1. An introduction to antibodies, antigens and different immunoassay formats including the use of magnetic particles as solid phases and as a label.
2. A review of some of the published immunoassay measurement techniques using magnetic particle labels.
3. A description of the applications of the particles in lateral-flow format immunoassays and in immunohistochemistry.

Immunoassays.

•Specific and sensitive analytical techniques.

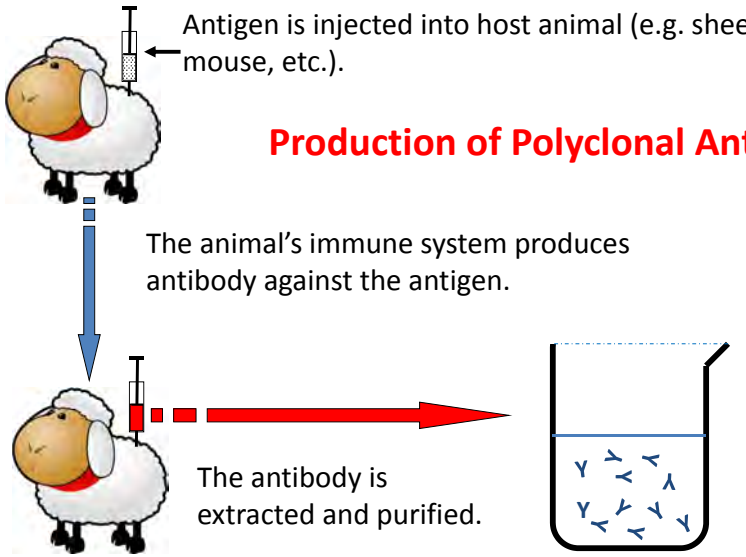
•Thousands of immunoassays have been developed over the last 50 years.

•Use reaction between an antigen, Ag (usually the analyte) and its antibody, Ab.

$$Ag + Ab \underset{k_d}{\overset{k_a}{\rightleftharpoons}} Ag.Ab\ complex$$

•Affinity = $\frac{k_a}{k_d}$

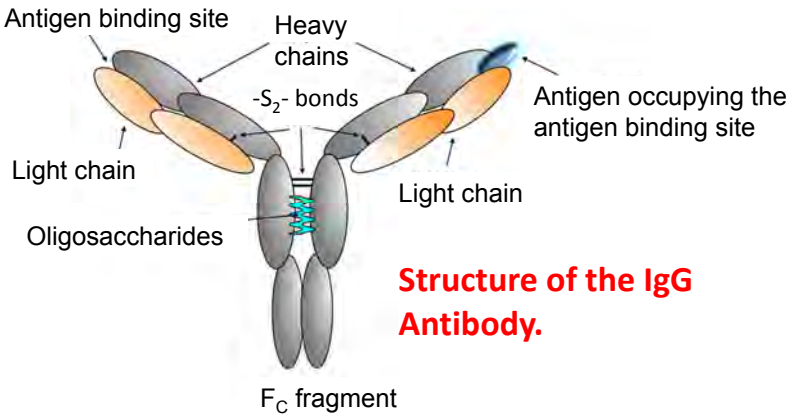
Production of Polyclonal Antibodies



Polyclonal and Monoclonal Antibodies.

•The immune response produces many different antibodies to the antigen (e.g. IgG, IgA, IgM, IgE and IgD, where Ig is immunoglobulin).

•IgG is the most commonly used in immunoassays.



Structure of the IgG Antibody.

•Antibody binds with an epitope on the antigen.
•Some antigen have two or more epitopes to which different polyclonal antibodies may bind.
• Monoclonal antibodies are very specific and bind to just one epitope on the antigen. They are more expensive to produce.

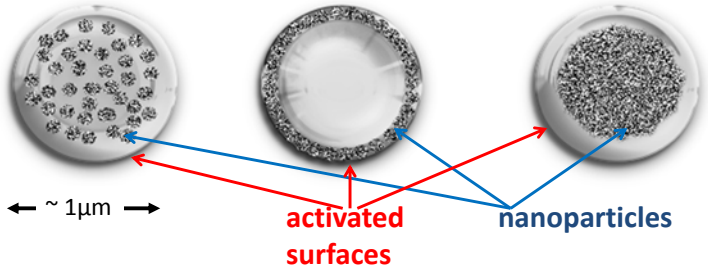
Quantifying Immunoassays.

•Numerous, very sensitive, immunoassay techniques have been developed.

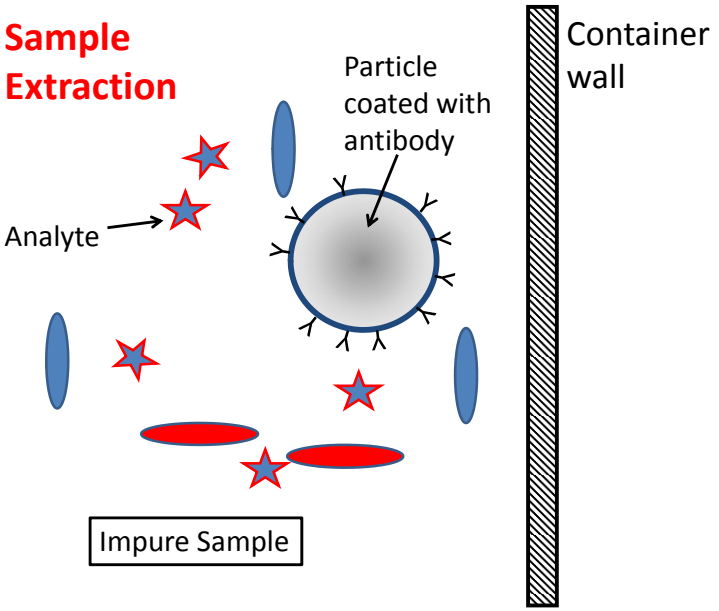
•Many automated analysers use magnetic particles in a sample extraction and purification stage.

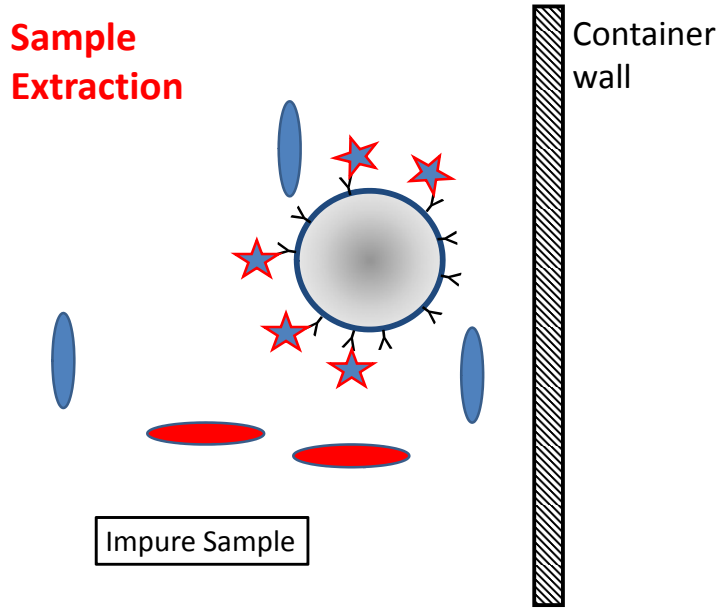
•Direct quantification of the Ag.Ab complex product is possible in a few cases although most assays use a solid phase and a label (marker, tag or reporter).

Magnetic Particles and Solid Reaction Surfaces.



•The particles contain nanoparticles of superparamagnetic magnetite (Fe_3O_4) with diameters of about 20 to 30 nm.
•The nanoparticles are embedded in a polymer matrix (e.g. polystyrene).
•Antibody is immobilised via its Fab fragment to the activated surface.


Sample
Extraction
Particle
coated with
antibody
Container
wall
Analyte
Impure Sample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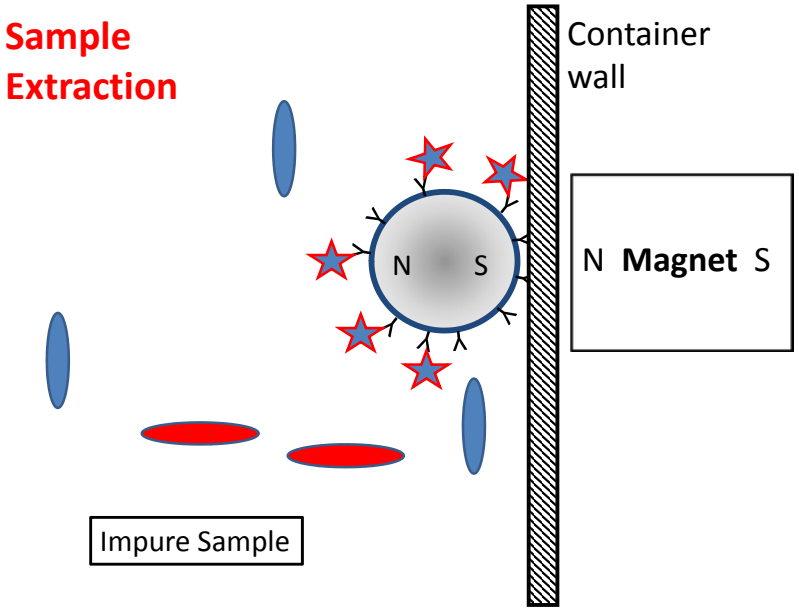
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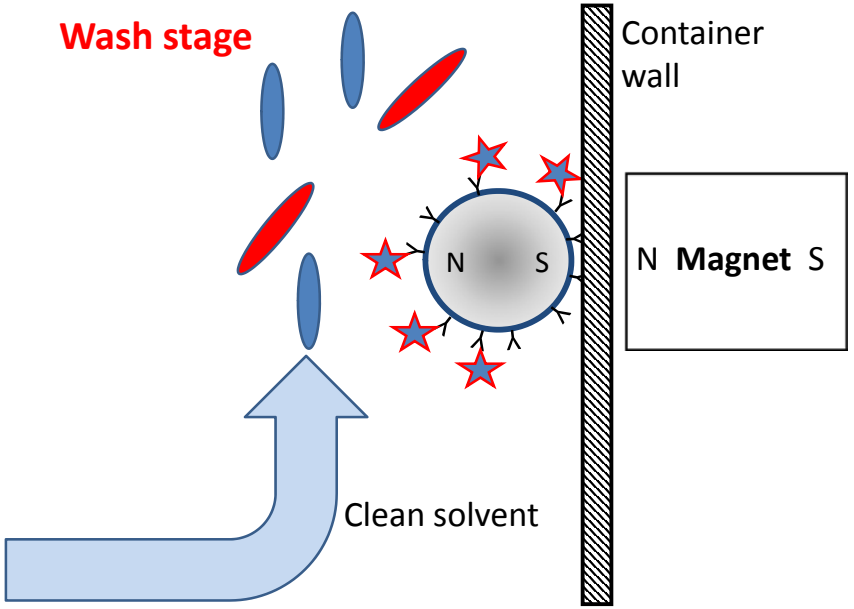
Wash stage
Container
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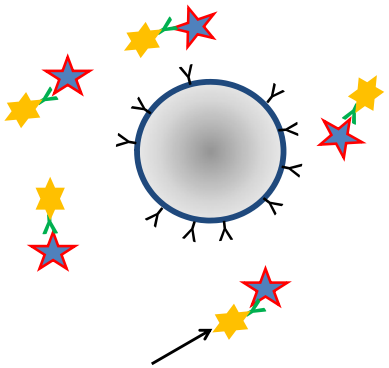
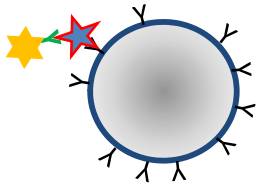
Clean solvent

•Chaotropic agent added to release analyte from particle.
• Second antibody with label added.
•Immunoassay quantified via the label.



Second antibody plus label

Container wall



•The particle could provide a solid surface for a sandwich assay to form between the analyte and the two antibodies.

•Labels used in conjunction with the magnetic particles:
Radioisotopes- now an almost obsolete technique.
Enzymes - enzyme-linked immunosorbent assay, (ELISA).
Chemiluminescent- commonly used in automated analysers.
Fluorescent - quantum dots are becoming popular.
Silver nanoparticles – an electrochemical method used .

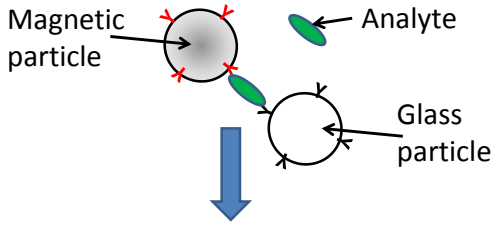
Potential Advantages of using Magnetic Particles as Labels in Immunoassays.

•There is very little magnetic material in most biological samples to interfere with the measurements.
•External magnets can be used to speed up assays by bringing antibody-coated particles to reaction surfaces.
•External magnets can be used to remove excess label.
•Sensing systems have been devised that do not respond to excess label.
•Magnetic materials do not readily degrade so samples can be archived and re-measured later.
•Simple, one step (or homogeneous), immunoassays are possible.

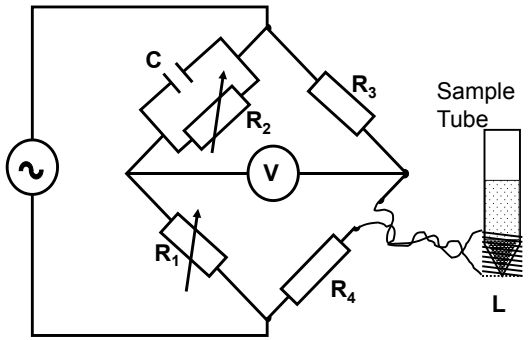
•Several ingenious techniques have been developed to quantify immunoassays using magnetic particles as labels.

1. Technique where the analyte bridges two different particles.

•Technique used by LifeAssays, Lund, Sweden.



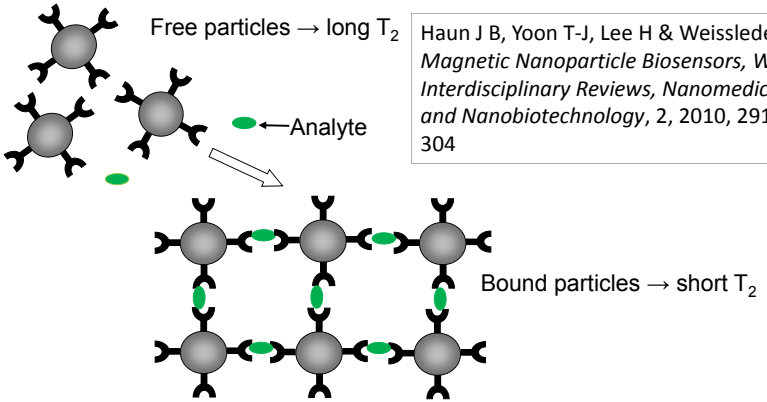
Cross-linked particles sink under gravity.



•Maxwell – Wein Bridge is balanced by adjusting R_1 and R_2.
•Cross-linked particles sink to the bottom of the tube, increasing the inductance of L and unbalancing the bridge.

Kriz C B, Rådevik K & Kriz D, Magnetic Permeability Measurements in Bioanalysis and Biosensors, *Analytical Chemistry,* 68, 1996, 1966-1970.

2. Technique using several cross-linked particles.



Haun J B, Yoon T-J, Lee H & Weissleder R, *Magnetic Nanoparticle Biosensors, Wiley Interdisciplinary Reviews, Nanomedicine and Nanobiotechnology*, 2, 2010, 291-304

Clusters of cross-linked particles (diameter ≈300nm) decrease the transverse time constant T_2 for the proton magnetic resonance of the water molecules. Unbound particles (diameter ≈38nm) have a smaller effect on T_2.

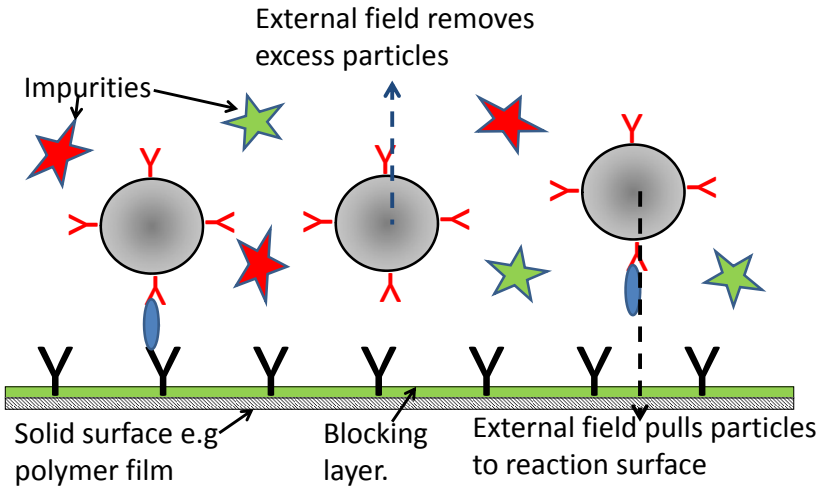
Competitive and Non-competitive Assays on a Reaction Surface.

•Many methods use a polymer, glass or ceramic reaction surface on which capture antibody is immobilised via its F_c fragment.
• A sensor is usually placed beneath the reaction surface.

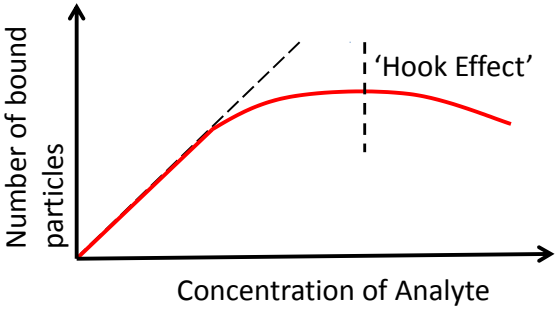
1. Non-competitive (or Sandwich) Assays are used with analytes having at least 2 epitopes.

2. Competitive Assays are used with small analyte molecules having only 1 epitope.

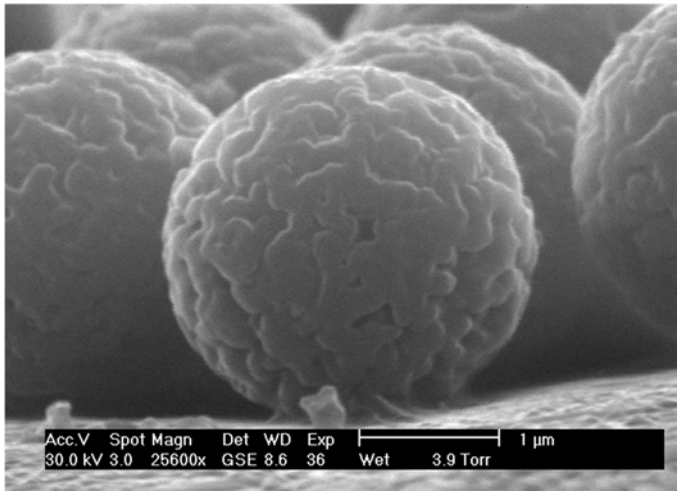
Magnetic particle labels in Sandwich (Non-competitive) Immunoassays.



Typical response of a sandwich immunoassay using magnetic particles as the label.

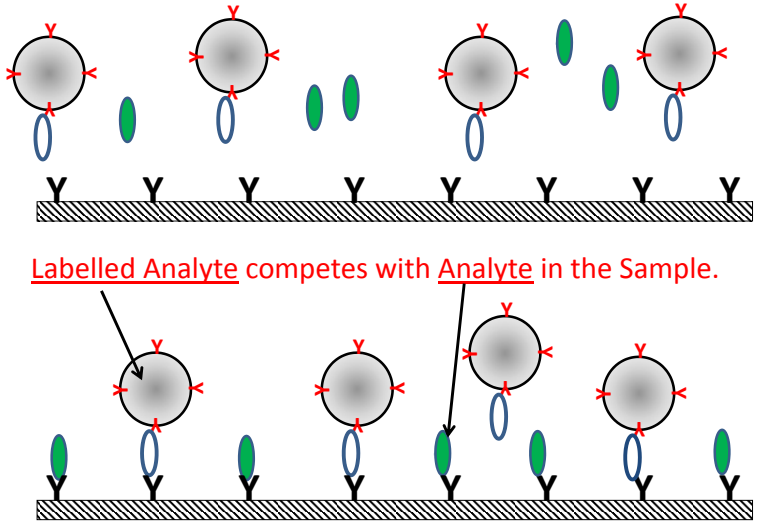


•The response becomes non-linear as capture antibody on the reaction surface is used up.
•'Hook effect': unlabelled analyte competes with labelled analyte for antibody on the reaction surface.

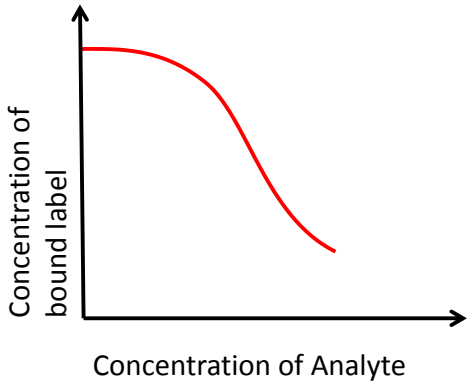


An EM image showing immobilised 2.8 µm-diameter particles on a plastic strip in a sandwich assay.

Magnetic Particles in Competitive Immunoassays



Typical response of a competitive immunoassay using magnetic particles as the label.



•The concentration of bound, labelled analyte decreases non-linearly with increasing concentration of the analyte.

Techniques that can distinguish between particles bound to the surface and unbound magnetic particles.

1. Decay in Magnetic Remanence.

•The particles are aligned by an external magnetic field.

•The field is removed and the particles are randomised by two mechanisms:

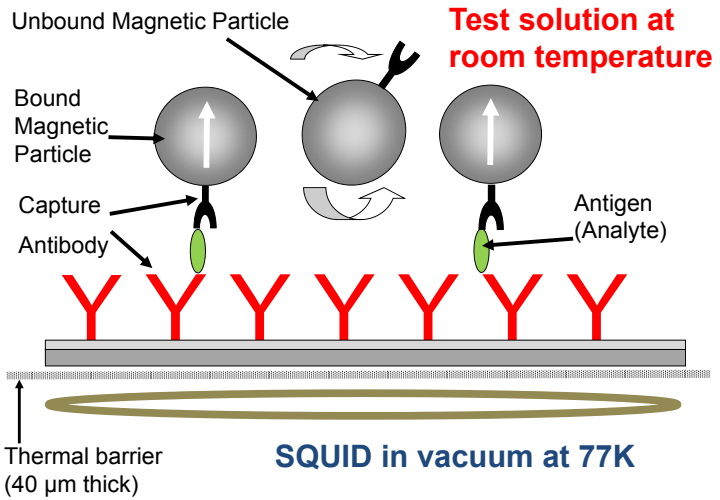
Brownian motion time constant, $\tau_B \approx 1$ ms

Néel relaxation time constant, $\tau_N \approx 1$ s

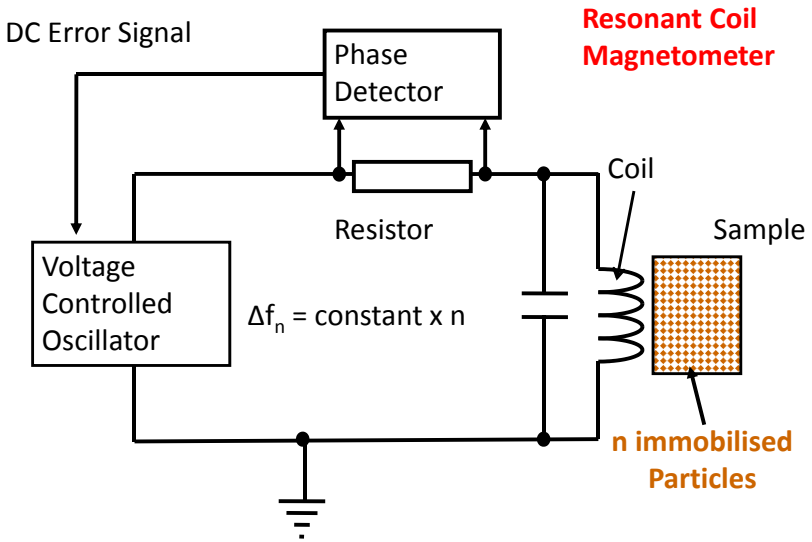
•The bound magnetic particles are not subject to Brownian motion so the slower decay in remanence measured by an external detector comes only from the bound particles.

2. Limited response range of the Detector.

•The response of detectors often falls off rapidly with distance so particles in suspension produce a weaker signal.

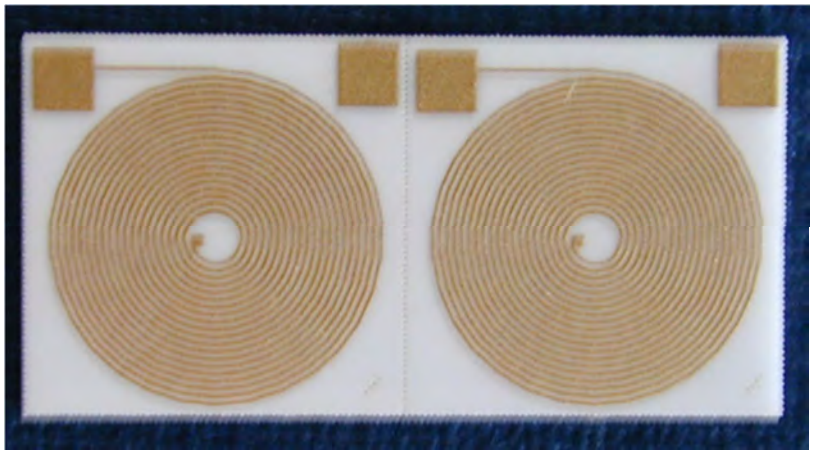


Chemla Y R, Grossman H L, Poon Y, McDermott R, Stevens R, Alper M D, & Clarke J, Ultrasensitive magnetic biosensor for homogeneous immunoassay, *Proceeding of the National Academy of Science of USA*, 97, 2000, 14268-14272



Hawkins P, Luxton R, and Macfarlane J, Measuring system for the rapid determination of the concentration of coated micrometer-sized paramagnetic particles suspended in aqueous buffer solutions, *Review of Scientific Instruments*, 72, 2001, 237-242.

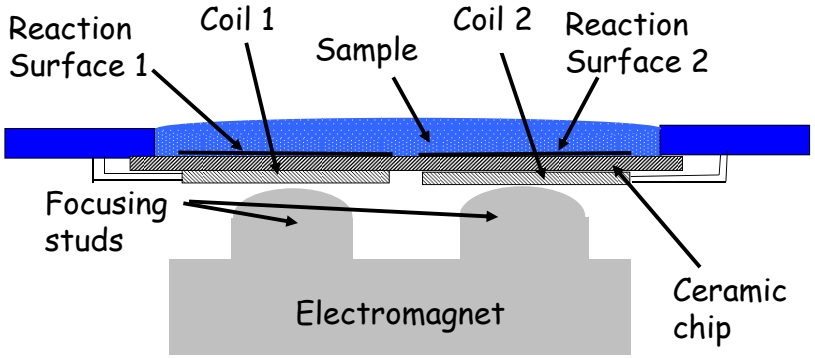
Flat Spiral Coils in a Two-analyte Measuring System.



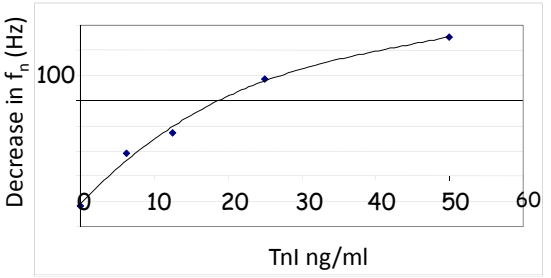
Coil 1 Coil 2

The Reaction Cell

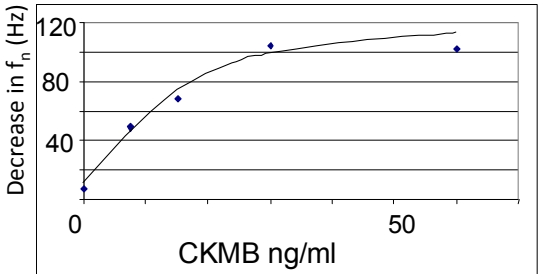
Randox Laboratories,
County Antrim, UK



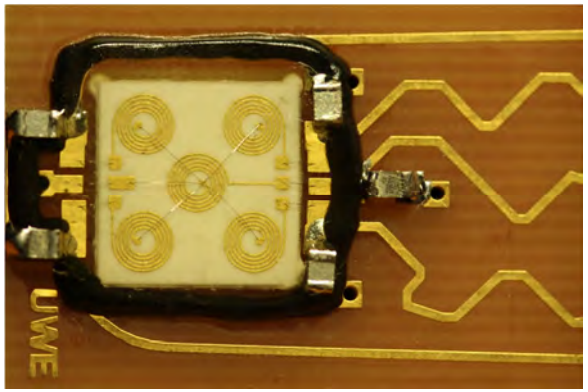
Kiely J, Hawkins P, Wraith P & Luxton R, Paramagnetic particle detection for use with an immunoassay based biosensor, *IET Science, Measurement & Technology*, 1, 2007, 270-275.



Simultaneous Measurements using two Coils

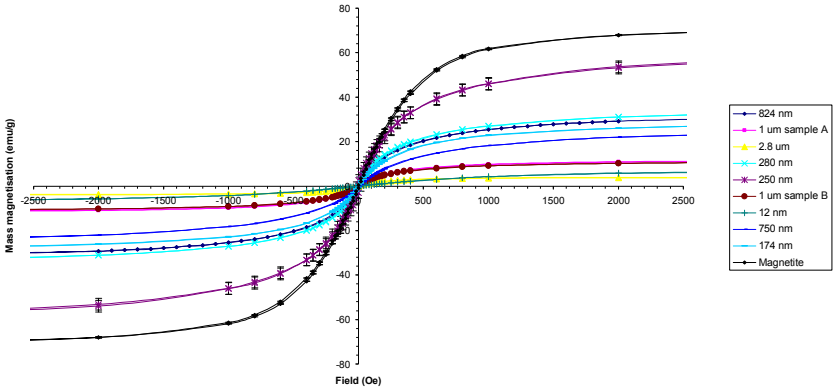


Troponin I and Creatine Kinase-MB are cardiac markers.

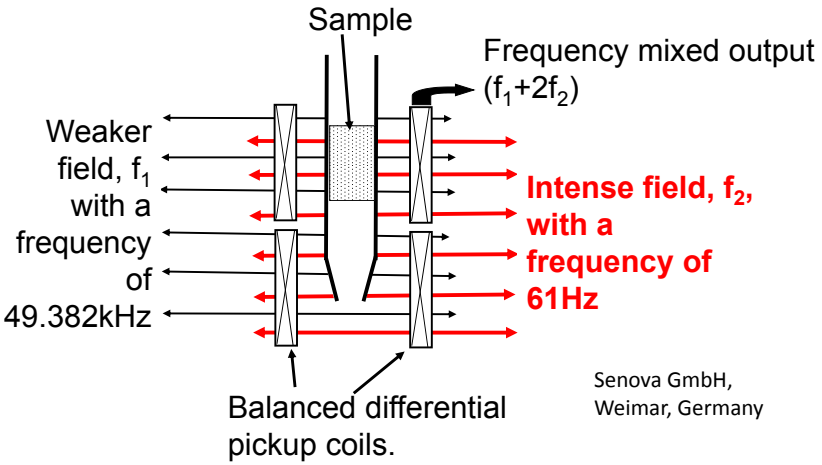


A five-coil measurement system.

Measurement Systems based on Magnetic Saturation.



Commercially available particles saturate at fields greater than about 2000 Oe.



Senova GmbH, Weimar, Germany

Meyer M H F, Hartmann M, Krause H-J, Blankenstein G, Mueller-Chorus, Oster B J, Miethe P & Keusgen M, CRP determination based on a novel magnetic biosensor, *Biosensors and Bioelectronics,* 22, 2007, 973-979.

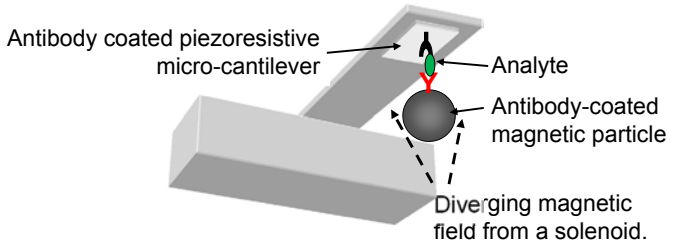
Magnisense, San Francisco, USA

- In a variation of the previous method, a simpler coil arrangement is used.
- f_1 is 24.4 kHz and has a fixed amplitude of 8.88 Oe.
- f_2 is 0.025 Hz and has a variable amplitude, H, up to 452.4 Oe.
- A signal proportional to d^2M/dH^2 is derived.
- The second derivative is related to the composition of the particle.
- Can be used in a multi-analyte assays.

Alphandéry E, Lijeour L, Lalatonne Y, & Motte L, Different signatures between chemically and biologically synthesized nanoparticles in a magnetic sensor: A new technology for multiparametric detection, *Sensors and Actuators B*, 147, 2010, 786–790

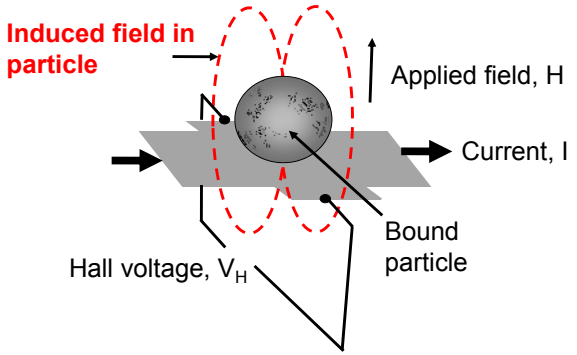
Techniques capable of detecting a Single Particle.

1. Micro Cantilever Based Force Amplified Biological Sensor



Baselt D R, Lee G U, and Colton R J, Biosensor based on force microscope technology, *Journal of Vacuum Science and Technology B*, 14, 1996, 789-793.

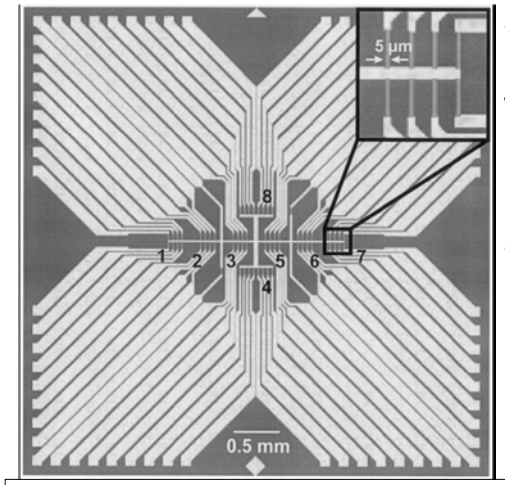
2. Thin-Film Hall Effect Sensors.



$V_H = C\ (I \times H)$, where C is a constant for the sensor

Besse P-A, Boero G, Demierre M, Pott V, & Popovic R, Detection of a single magnetic microbead using a miniaturized silicon Hall sensor, *Applied Physics Letters*, 80, 2002, 4199–4201.

3. Thin film Gigantic Magneto-Resistive Sensors.



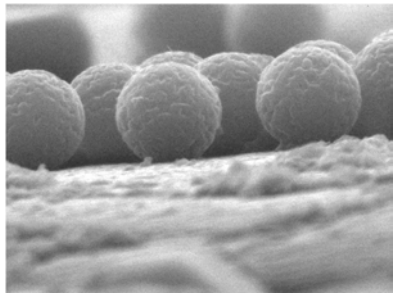
•Several research groups have worked, or are working, on this approach including Diagnostic Biosensors, Minneapolis.

•Used in the US Naval Research Laboratory, cBASS measurement system.

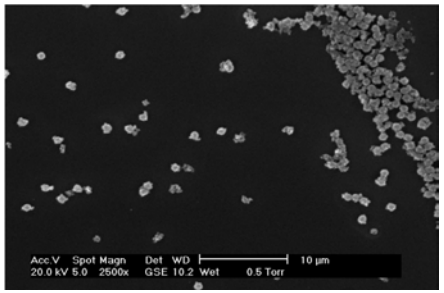
Tamanaha C R, Mulvaney J C, Rife J C & Whitman L J, Magnetic labelling, detection, and system integration, *Biosensors and Bioelectronics*, 24, 2008, 1–13.

Problems associated with Measurement Systems using a small Number of Magnetic Particles.

1. Variations between particles in size, Fe_3O_4 content and amount of bound and active antibody on the surface.



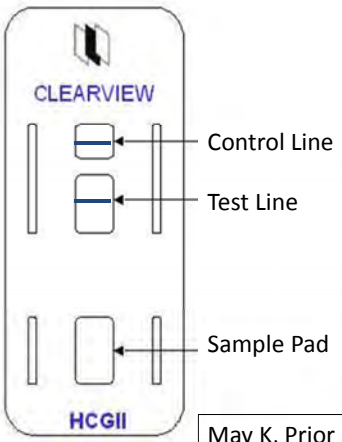
2.8µm diameter particles



760nm diameter particles

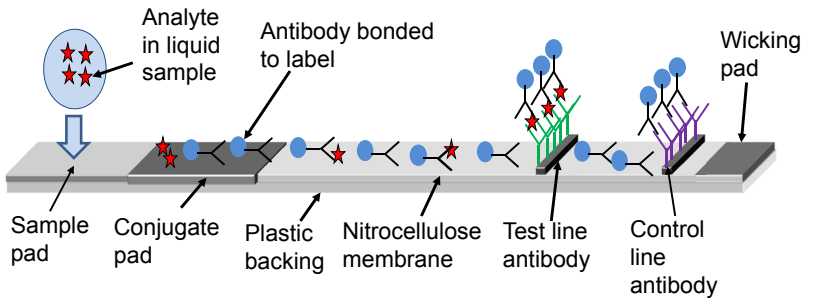
2. Difficulty in directing the magnetic particles and analyte to the measurement site.

Lateral Flow Immunoassays (or Immunochromatography).



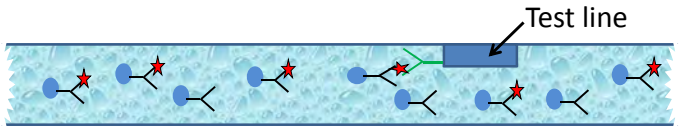
•Simple devices not requiring a skilled operator or sample preparation.
•Mainly used in applications to determine if the analyte concentration is above a threshold value.
•Pregnancy test for human chorionic gonadotropin hormone now widely accepted.

May K, Prior M E & Richards I, *UK patent* GB 2204398, 1988.



Direction of flow of liquid and reagents ----------→

•Sample pad contains a filter to remove unwanted solids.
•Conjugate pad contains the dried label and antibody to the analyte which are reconstituted by the sample liquid.
•Average pore size of membrane ≈ 10 × the diameter of the label.
•Test line contains a second antibody to the analyte.
•Control line contains an antibody to the antibody attached to the label.



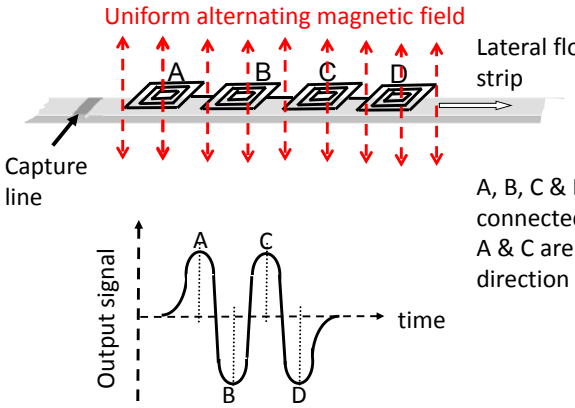
Direction of flow ------->

Some problems in making lateral flow devices more quantitative:

1. Test line does not capture all labelled analyte.
2. Particles trapped in the nitrocellulose membrane cause difficulties in determining baseline measurements.
3. Not all free label is captured by the control line making it difficult to determine total number of particles involved in the assay.

Four-Coil Gradiometer Detection System

MagnaBiosciences, San Diego, USA

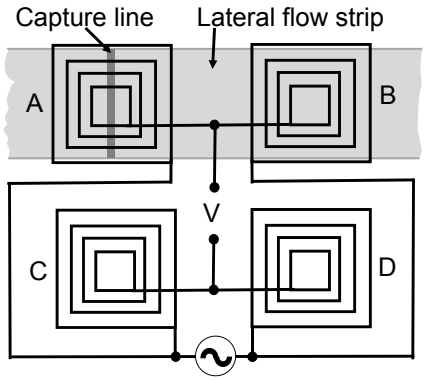


A, B, C & D are identical coils connected in series.
A & C are wound in opposite direction to B & D.

LaBorde R T, & O'Farrell B, Paramagnetic labeling offers an alternative method for analyte detection, *IVD Technology*, April 2, 2002

Four-Coil Balanced Bridge Detection System

Magnasense, Survontie, Finland



A, B, C & D are identical coils connected in a bridge circuit.
A & C are wound in opposite direction to B & D.
The lateral flow strip does not move and coil A is positioned over the capture line.

Mäkiranta J. and Lekkala J, Optimization of a novel magnetic nanoparticles sensor, *XVIII IMEKO World Conference, Metrology for a Sustainable Development, Rio de Janeiro, Brazil, 2006*.

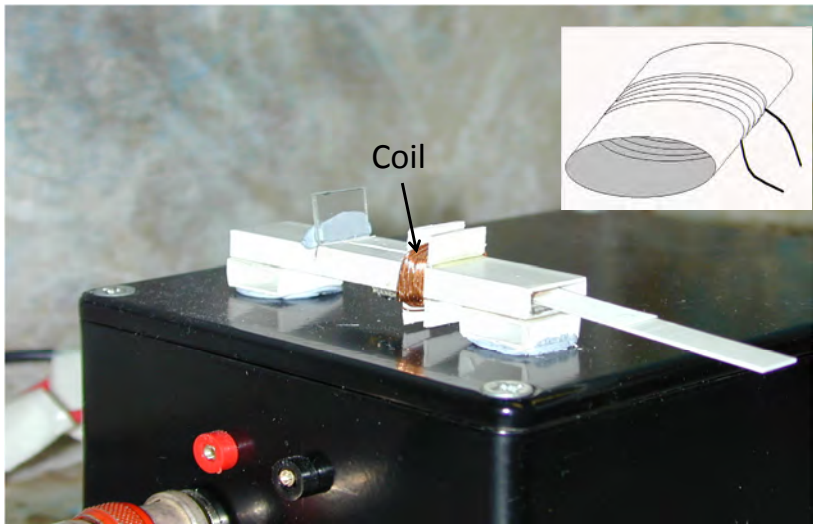
Integrated Giant Magneto-Resistive Strip

Diagnostic Biosensors , Minneapolis, USA

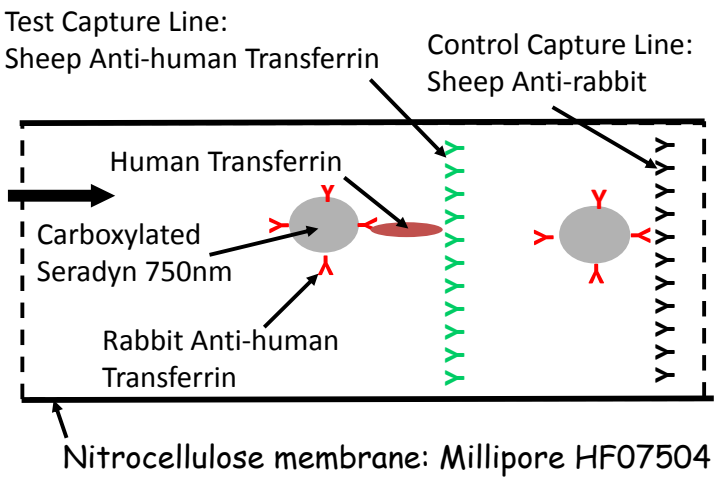
•A GMR strip is integrated into the membrane to detect the presence of magnetic labels in capture spots.

Taton K, Johnson D, Guire P, Lange E & Tondra M, Lateral flow immunoassay using magnetoresistive sensors, *Journal of Magnetism and Magnetic Materials*, 321, 2009, 1679-1682

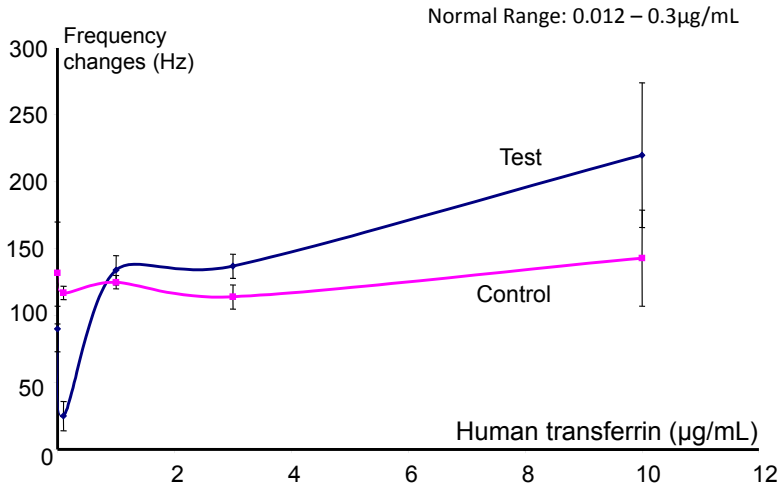
Resonant Coil Magnetometer in Lateral Flow Measurements



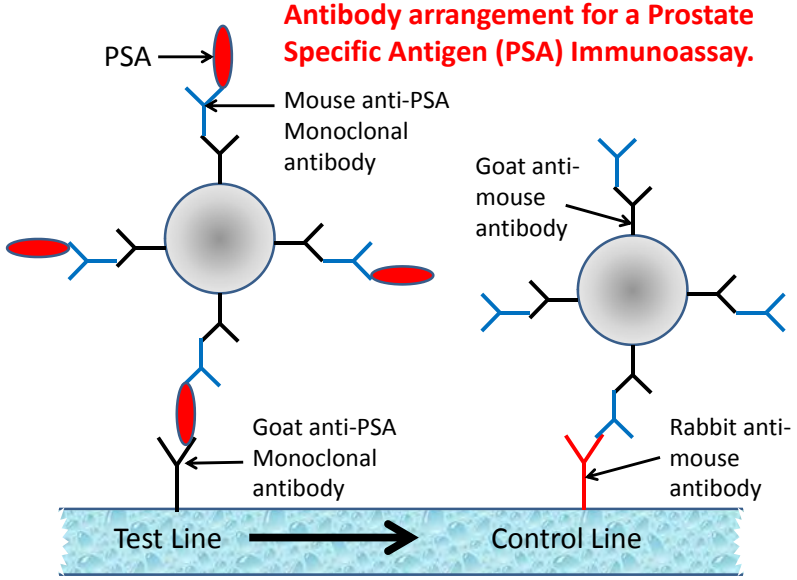
Antibody arrangement for Human Transferrin immunoassay.



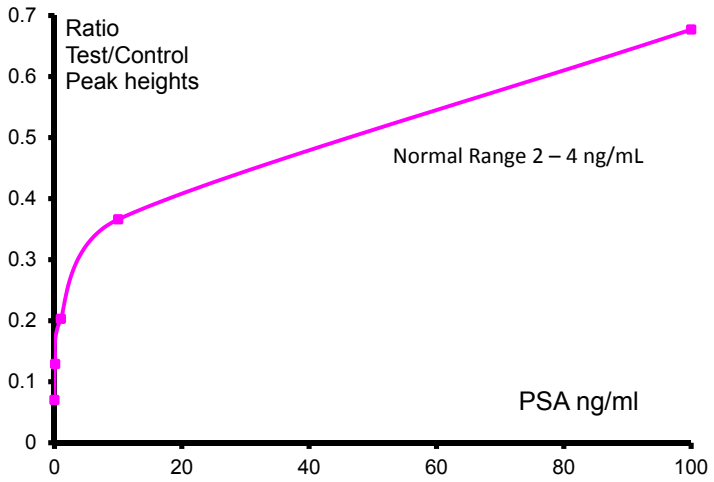
Lateral flow assay for human Transferrin



Antibody arrangement for a Prostate Specific Antigen (PSA) Immunoassay.



Lateral flow assay for PSA



Application of Magnetic Particles in Immunohistochemistry in HER2 breast cancer.

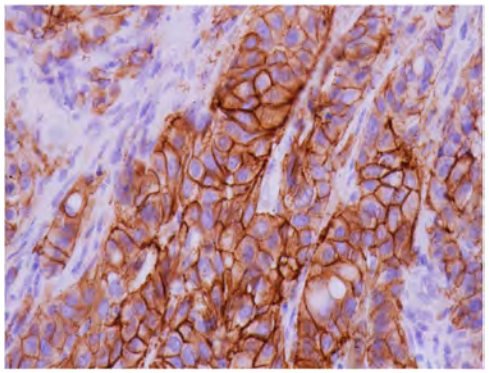
•20-30% of breast cancers are caused by over-expression of the human epidermal growth factor receptor-2 (HER2).

•Advanced stages of HER2 can be treated with monoclonal antibody trastuzumab (marketed as Herceptin). Other treatments will soon be available.

•The disease is diagnosed by making a microscopic slide of a biopsy and applying anti-human HER2 labelled with an enzyme.

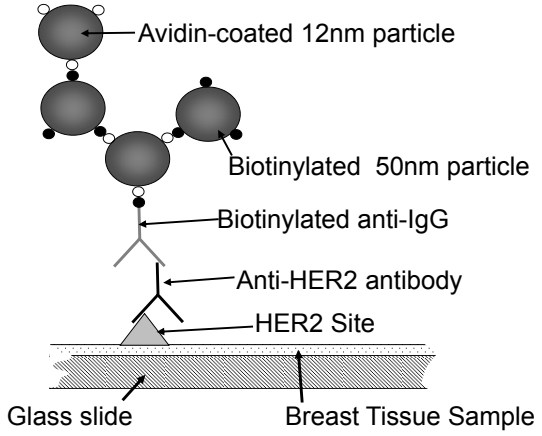
•The enzyme is used to precipitate a brown dye on to the slide.

•The intensity of the staining is determine by eye using a microscope. The brown coloration is scored (0, 1+, 2+ or 3+) which approximately equates to expression level.

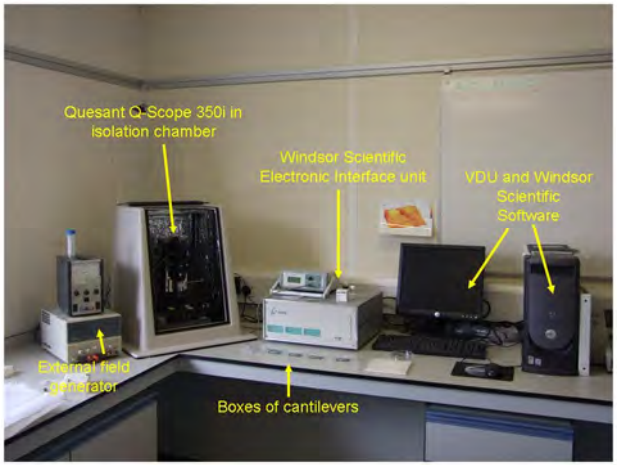


•Slides scoring 2+ or 3+ are assessed further using a fluorescent labelled antibody (fluorescent *in situ* hybridisation, FISH).

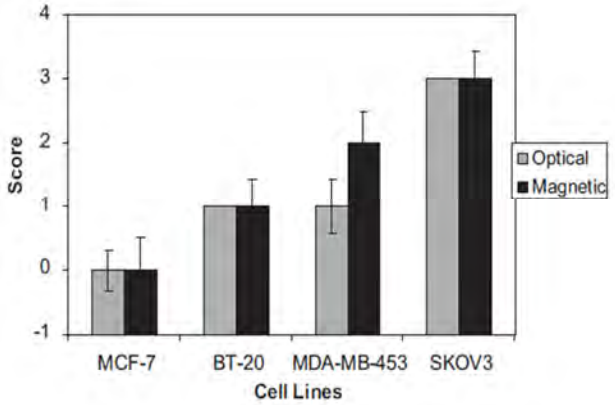
•In this investigation, magnetic particles were used as label and an amplification technique used to produce a stain on commercially-available slides with known scores of HER2.



The slides were then scanned using a magnetic force microscopy in the presence of a magnetic field.



•The normalised results were in good agreement with the optical scoring and known characteristics of the cell lines.



Mitchels J, Hawkins P, Luxton R & Rhodes A, Quantification in histopathology—Can magnetic particles help? *Journal of Magnetism and Magnetic Materials*, 311, 2007, 264–268.

Further Reading:

Magnetic Nanoparticles in Immunoassays, Chapter 9, p 243-276, *Magnetic Nanoparticles From Fabrication to Clinical Applications*, Edited by Nguyen T K Thanh (2012), CRC press.

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Keith Hewett
Julie Richardson
Nicholas Haji-Ioannu
Tony Rhodes
John Mitchels
Patrick Wraith
Jackie Barnett
John Eveness
Andrew Hill
Sabine Barakat
Elham Mohd Sharif
Bing Han
Mark Colclough (University of Birmingham)
+ numerous BSc and MSc Students

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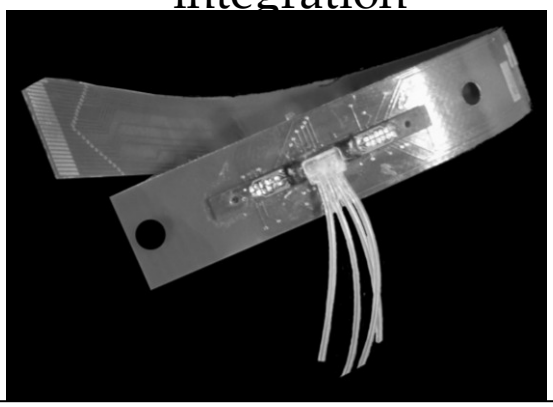
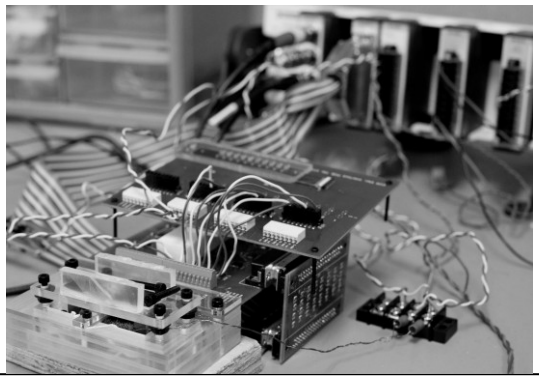
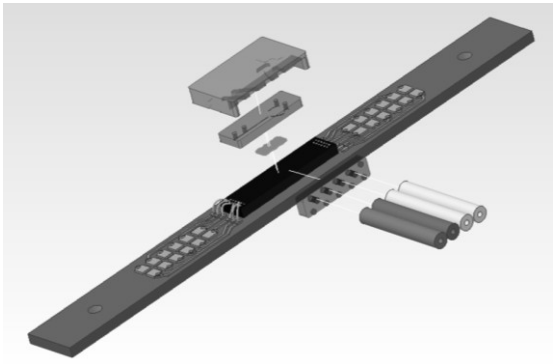
Electronics – Microfluidics integration



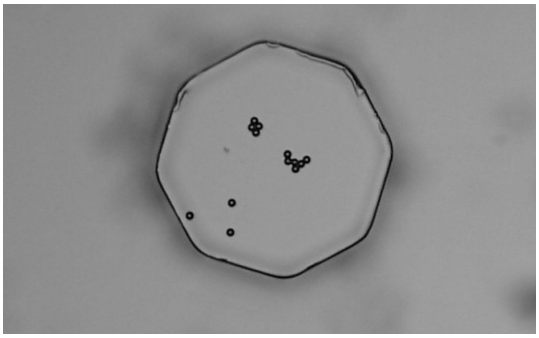
Magnetic – Fluidic Test System



Multilevel Micro-miniature Fluidics



We make nano - structures to design.



Diagnostic Biosensors
1712 Brook Ave. SE ; Minneapolis, Minnesota 55414-2422 USA
Contact: Mark Tondra, Mark@DiagnosticBiosensors.com; +011 612 331-3584
www.DiagnosticBiosensors.com

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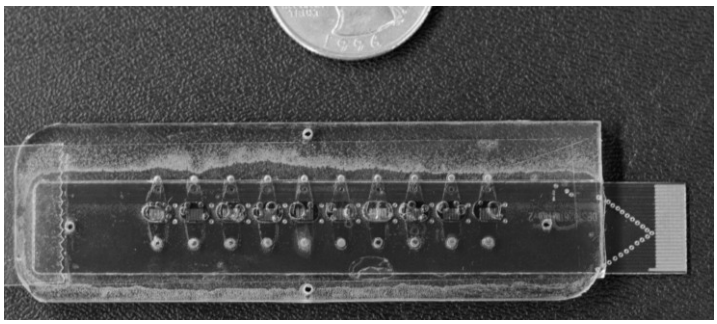
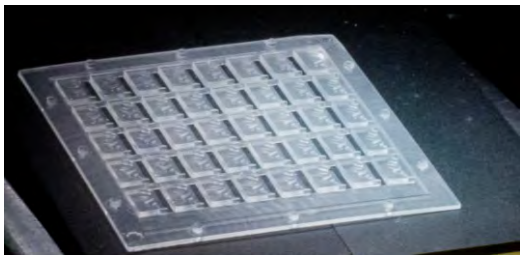
- Microelectronic Cell-based toxicity monitor
- Fluidic and Magnetic test system design



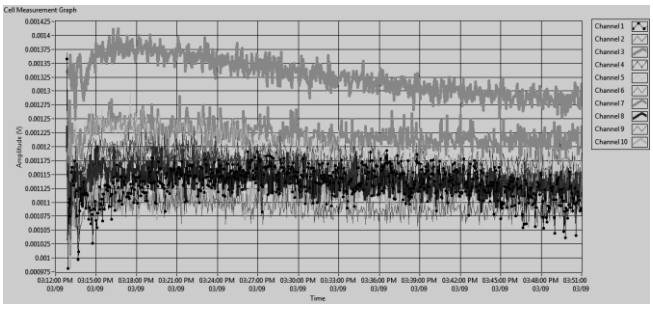
Capabilities:



- Microfluidic – MEMS mold design
- Electronic – microfluidic assembly
- Magnetic detection and actuation sensor and circuit design
- Custom Nanofabrication

Equipment and Software:

- Micromold design, CAD, and embossing and thinning.
- Lab-View Lab control software and electronics.
- Microelectronics schematic, PCB, assemble, and test.
- Micro-assembly alignment tool
- Wet lab



Diagnostic Biosensors

1712 Brook Ave. SE ; Minneapolis, Minnesota 55414-2422 USA

Contact: Mark Tondra, Mark@DiagnosticBiosensors.com; +011 612 331-3584

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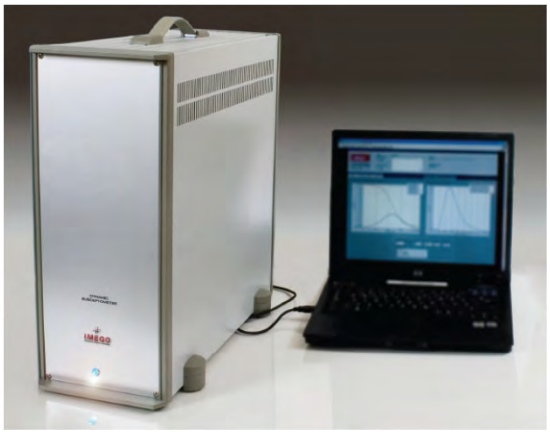
DynoMag

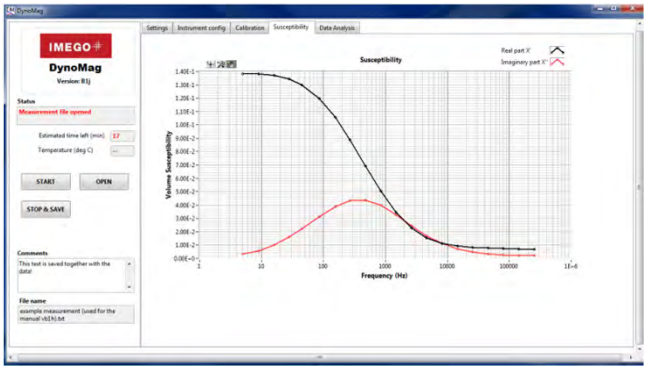
AC susceptometer



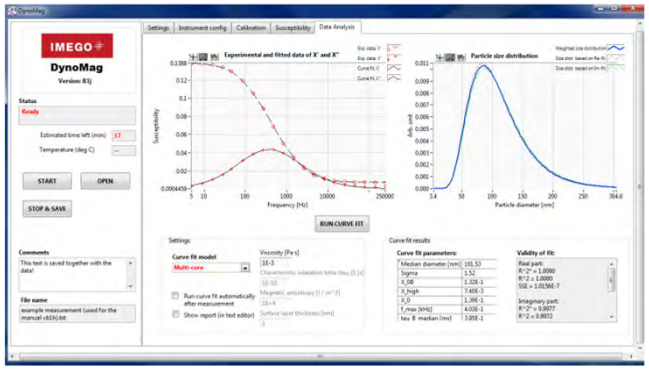
DynoMag is a portable, easy to use AC susceptometer. It measures the real and imaginary components of the AC susceptibility in a wide frequency range. The instrument enables determination of the magnetic properties of liquids, powders or solid samples.

Typical application areas include:

- Measurement of size distribution of magnetic nanoparticles (MNP)
- Quality control during nanoparticle synthesis and manufacturing
- Studies of binding reactions of biomolecules to the surface of MNP



A DynoMag measurement of the AC susceptibility versus frequency for magnetic nanoparticles dispersed in a liquid (Chemicell FluidMag cobalt-ferrite). The decrease in real part and the maximum in imaginary part of the susceptibility around 400 Hz is due to Brownian relaxation of the nanoparticles. The residual non-zero real susceptibility above 10 kHz comes from fast Néel relaxation.



A fit to the measured data in the left figure. With the analysis package you can fit your experimental DynoMag data and determine the particle size distribution from the Brownian relaxation as well as Néel relaxation of single core particles.

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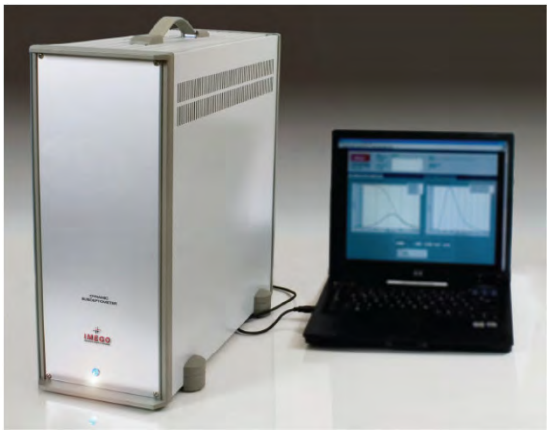
Tel. +46 (0)31 750 1800 Fax. +46 (0)31 750 1801, **www.imego.com www.acreo.se**

DynoMag
AC susceptometer



Property	Value	Comments
Frequency interval	1 Hz - 500 kHz	Measurement accuracy is lower below 5 Hz
Excitation field	0.5 mT = 5 G	The magnetic field strength is constant below 1 kHz, falling off at higher frequencies
Volume susceptibility resolution	$1 \cdot 10^{-6}$	The value is the standard deviation of the volume susceptibility, measured at 1 kHz, with an excitation filed of 5 G and a time constant (measurement time) of 1 s.
Sample size	Cylindrical sample holder with volume 0.2 cm^3	The sample volume can be customized to smaller volumes than 0.2 cm^3
Measurement time	Typically around 15 minutes	Depends on the number of data points chosen, 15 minutes is for 20 points
Operating temperature	Normal lab temperatures	

For further information please contact:
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Jakob Blomgren, PhD
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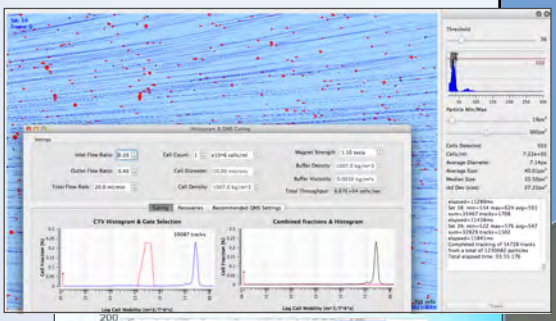

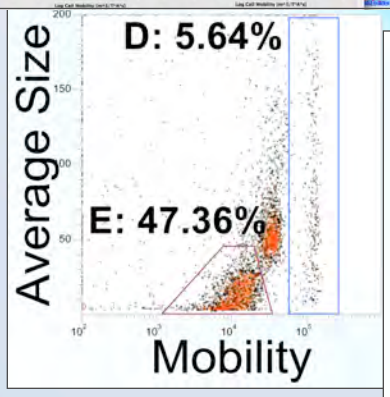

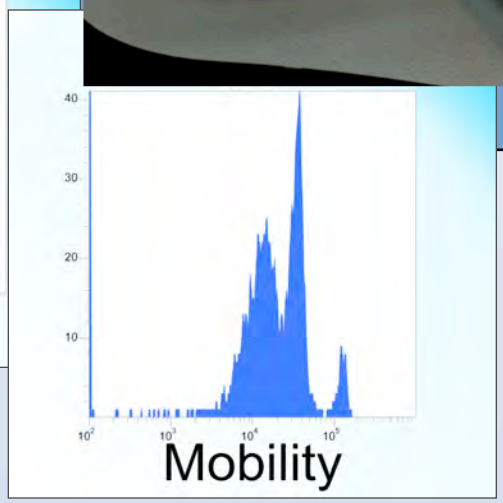

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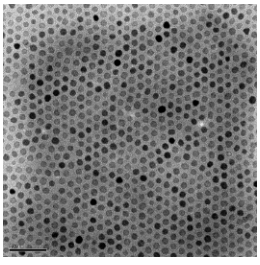

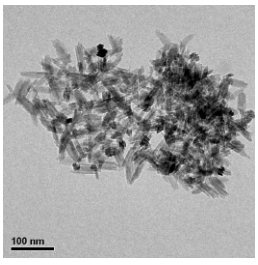

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NANOSIGHT

Multi-Parameter Characterization of Nanoparticle Dispersions by Size, Count and Scattering Intensity

Ray Eby, Duncan Griffiths, William Bernt, Patrick Hole
NanoSight USA Central US ; Tel: 312-545-6527 ; e-mail: ray.eby@nanosight.com

Nanoparticle Tracking Analysis (NTA) is a method that has gained increasing popularity for the study of nanoparticle dispersions, providing high-resolution size analysis as well as a direct measure of concentration by particle counting. By also collecting a measurement of scattered light intensity (along with the basic tracking data for each particle), a powerful combination of information allows for the differentiation of sub-populations within a heterogeneous mixture.

NTA employs a novel illumination method, allowing direct observation of the particles in suspension to be individually imaged and sized by tracking their Brownian motion frame-by-frame within a high resolution video recording. NTA operates within the size range of approximately 10 – 1000 nm. The technique offers significant advantages over existing light scattering techniques (such as DLS- and SLS-based systems) for the characterization of polydisperse populations that have nanoscale particles.

Magnetic Fe-Oxide dispersions are often polydisperse, especially at lower concentrations and where aggregation has occurred. NTA is able to resolve the full breadth of particle sizes present in these systems.

The NTA method is extended to allow not just the characterization of size, but also light scattering intensity on an individual *particle-by-particle* basis. This multi-parameter measurement capability allows sub-populations of nanoparticles of varying composition to be resolved in a complex mixture. All other factors equal, light scattering intensity increases with the sixth power of the size of the particle. However, material refractive index is another primary variable of scattering intensity. As an example, NTA can resolve 90 nm gold from 90 nm polymer latex beads within the same suspension. Using other methods, a simple size measurement shows only one size population, whereas, NTA resolves two distinct populations using the scattering information that is derived along with primary NTA methodology. Within a more complex system, such as viruses in growth media, particle populations might not be so distinct, nonetheless, results still allow some useful identification where a traditional size measurement would be very difficult. This uniquely demonstrates the ability of the technique to differentiate nanoscale materials, not just on their hydrodynamic size but also by their scattering ability.

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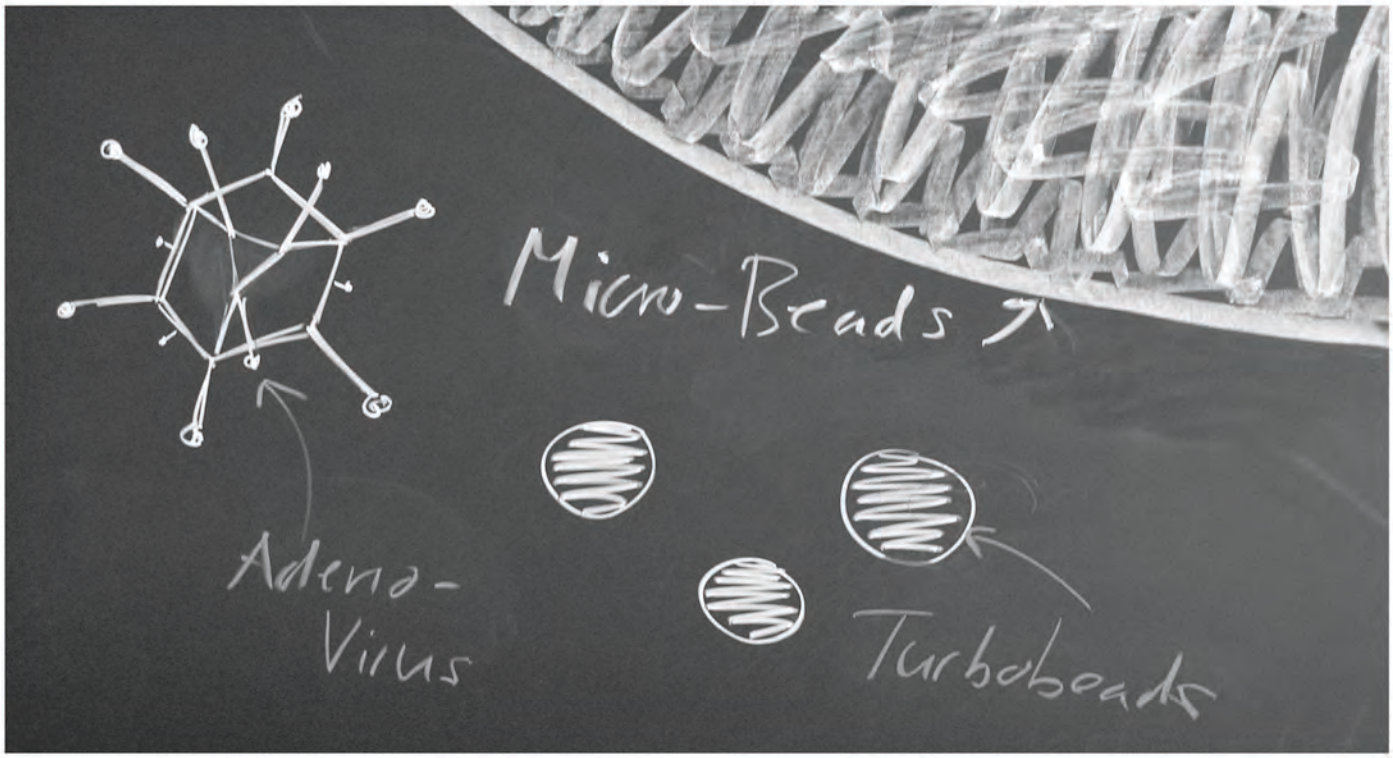

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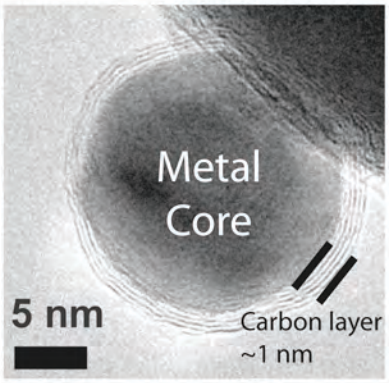

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