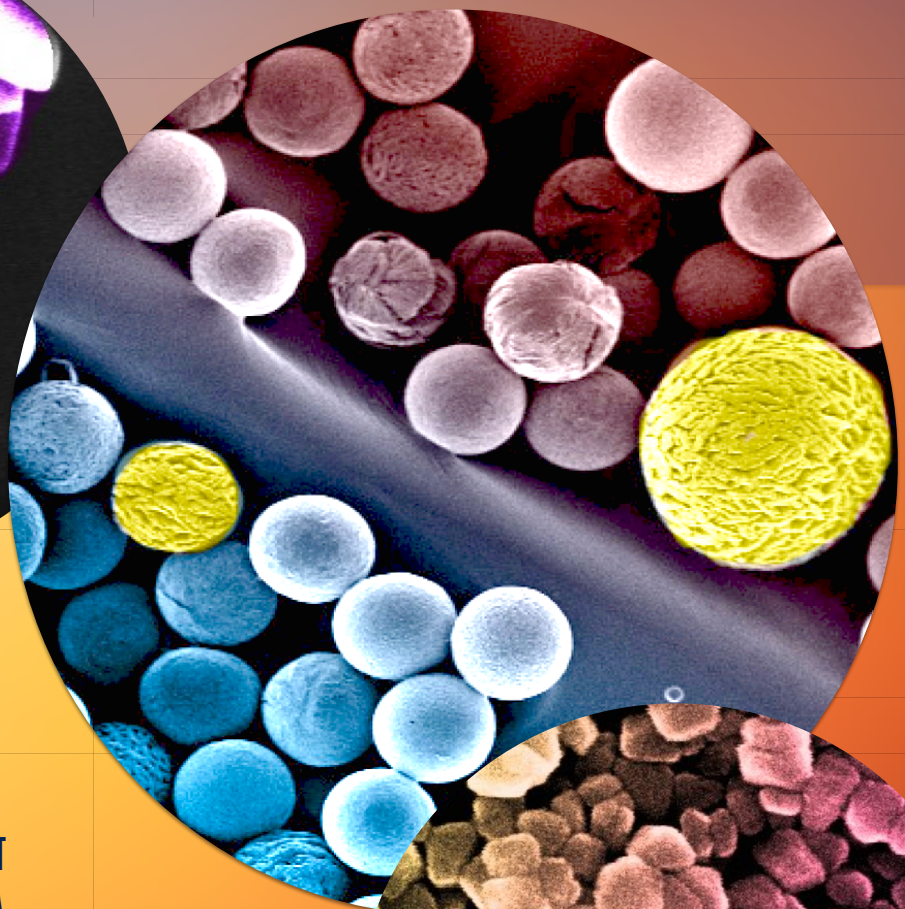
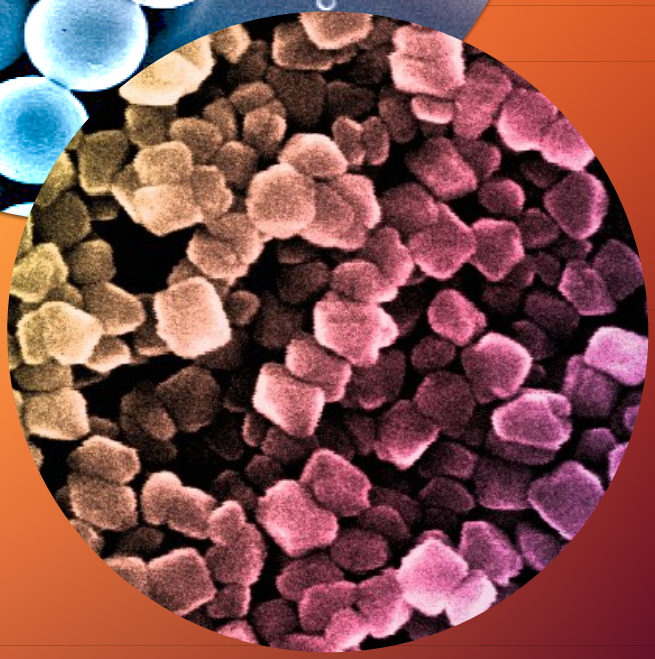
12th International Conference on the SCIENTIFIC AND CLINICAL APPLICATIONS OF MAGNETIC CARRIERS

COPENHAGEN, DENMARK
MAY 22-26 2018





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Coffee cup design by Cristina Rodriguez-Rodriguez @ 2018

Welcome Message

It is our great pleasure to welcome you all to the 12th International Conference on the Scientific and Clinical Applications of Magnetic Carriers. We once again have a wonderful program full of new and great "things" that magnetic particles can do.

Copenhagen is a wonderful place to be hosting the conference and we are very fortunate to be holding our meeting in the Maersk Tower, the newest, state-of-the-art research building at the University of Copenhagen's Nørre campus. We are sure the innovative architecture of the Maersk Tower will make discussing new ideas and collaborations especially fruitful. Magnetic particles are fascinating and there are no limits in their applications.

Your organizers,

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

12th International Conference on the Scientific and Clinical Applications of Magnetic Carriers - Copenhagen, Denmark

Tuesday, May 22, 2018				
18:00	**Registration desk opens** in the Maersk Tower (Mærsk Tårnet), Blegdamsvej 3B			
18:30	**Informal reception and welcome cocktail (Apero) in the Maersk Tower, with the Jacob Venndt's Fabulous Swing Trio - generously sponsored by chemicell**			
22:00	**End of reception**			

Wednesday, May 23, 2018				
08:00	**Registration desk opens** - at the Niels Jerne Auditorium, Mærsk Tårnet (Maersk Tower), Blegdamsvej 3B			
	Opening Session			
09:00	Holst Nissen, Mogens	Opening of the conference and welcome address by the Vice Dean of Research, University of Copenhagen	Copenhagen, Denmark	
09:05	Hafeli, Urs	Short review of the last 2 years of magnetic carriers research	Copenhagen, Denmark	Talk 0
	Session 1	**Hyperthermia I**	*Chair: Silvio Dutz*	
09:35	Chiu-Lam, Andreia	Spatially Controlled and Image Guided Nanoscale Thermal Therapy	Gainesville, FL, USA	Talk 1
09:50	Kostevsek, Nina	Magneto-liposomes as theranostic agents	Ljubliana, Slovenia	Talk 2
10:05	**Coffee break**			
	Session 2	**Hyperthermia II**	*Chair: Silvio Dutz*	
10:30	Beola, Lilianne	Differences in cell death mechanism after magnetic hyperthermia treatment depending on the nanoparticle location	Madrid, Spain	Talk 3
10:45	Mefford, Thompson	Glycoconjugate-Functionalized Magnetic Nanoparticles: A Tool for Selective Killing of Targeted Bacteria Via Magnetically Mediated Energy Delivery	Clemson, SC, USA	Talk 4
11:00	Torres, Teobaldo	Intracellular distribution of magnetic nanoparticles and magnetic hyperthermia effects: a Focused Ion Beam study	Zaragoza, Spain	Talk 5
11:15	Grazú, Valeria	Nanobiohybrids for the thermoactivation of a therapeutic enzyme by magnetic hyperthermia	Zaragoza, Spain	Talk 6
11:30	Pankhurst, Quentin	Optimal formulation properties and physico-chemical characteristics of iron oxide magnetic nanoparticle heating agents for clinical applications	London, UK	Talk 7
11:45	Spirou, Spiridon	Recommendations for in-vitro and in-vivo testing of Magnetic Nanoparticle Hyperthermia combined with Radiation Therapy	Athens, Greece	Talk 8
12:00	Merida, Fernando	Ultrasonic Potentiation of Magnetic Fluid Hyperthermia/Pifithrin-μ Combined Therapies in Ovarian Cancer Cells	Mayaguez, Puerto Rico	Talk 9
12:15	**Lunch**			
	Session 3	**Magnetic Drug Delivery / Magnetic Targeting I**	*Chair: Christoph Alexiou*	
13:15	Waldorff, Erik	Low Frequency Pulsed Electromagnetic Field Applications: Bone and Soft Tissue Repair	Lewisville, TX, USA	Invited Talk 10
14:00	Yagci Acar, Funda	Discovery of self-luminescent polyethyleneimine coated SPIONs and their theranostic applications	Istanbul, Turkey	Talk 11
14:15	Shipunova, Victoria	A novel method of nanoparticle surface biomodification for the development of effective agents for cancer theranostics	Moscow, Russia	Talk 12
14:30	Yoon, Jungwon	Development of a Real-Time Two-Dimensional Navigation System of Magnetic Nanoparticles for Targeted Drug Delivery	Gwangju, Korea	Talk 13

14:45	**Coffee break**			
	Session 4	**Magnetic Drug Delivery / Magnetic Targeting II**	*Chair: Quentin Pankhurst*	
15:15	Alexiou, Christoph	Upscaling of Iron Oxide Nanoparticles for Biomedical Applications; The SEON Concept	Erlangen, Germany	Talk 14
15:30	Bianco, Vincent	A parallel, multi-phase, multi-physics numerical simulation code for development of magnetic fluid control strategies	New York, NY, USA	Talk 15
15:45	Fellows, Benjamin	Heparin Coated Magnetic Nanoparticles for Treatment of In-Stent Restenosis and Prevention of Late Stent Thrombosis	Clemson, SC, USA	Talk 16
16:00	Jafari, Sahar	Magnetically-enabled rapid intra-nasal drug delivery to the brain	North Bethesda, MD, USA	Talk 17
16:15	Baun, Olga	Mag-Guider: A permanent magnet system to guide and image super-paramagnetic nanoparticles	Mainz, Germany	Talk 18
16:30	Nosrati, Zeynab	Fabrication and Characterization of Magnetic Embolising Microspheres with Potential Application in Magnetic Resonance Navigation Technology	Vancouver, BC, Canada	Talk 19
16:45	Liu, Chih-Hsin	Intravenous Delivery of Tissue Plasminogen Activator by Thermosensitive Magnetoliposomes for Target Thrombolysis	Taoyuan, Taiwan	Talk 20
17:00	**Poster session I (Posters 1-115) with Beer and Snacks** - generously sponsored by micromod / free evening thereafter			

Thursday, May 24, 2018				
08:30	**Registration desk opens** - at the Niels Jerne Auditorium, Mærsk Tårnet (Maersk Tower), Blegdamsvej 3B			
09:00	Kim, CheolGi	Integrated Magnetophoretic Platform for Precise Manipulation of Living Cells	Daegu, Korea	Invited Talk 21
	Session 5	**Analytical Techniques**	*Chair: Petr Nikitin*	
09:45	Connolly, Colin	High-Throughput Single-Nanoparticle Magnetic Analysis Platform Using Diamond Magnetic Imaging	Somerville, MA, USA	Talk 22
10:00	Baffa, Oswaldo	Development of an Optical Pumped Gradiometric System to Detect Magnetic Relaxation of Magnetic Nanoparticles	Ribeirao Preto, Brazil	Talk 23
10:15	Fock, Jeppe	Measured correlation of nanoparticle magnetic moment and hydrodynamic size	Copenhagen, Denmark	Talk 24
10:30	**Coffee break**			
11:00	Frandsen, Cathrine	Electron holography studies of individual maghemite nanoflowers	Copenhagen, Denmark	Talk 25
11:15	Nikitin, Maxim	Smart Materials Based on Magnetic Nanoparticles for Biosensing and Drug Delivery	Moscow, Russia	Talk 26
	Session 6	**Nanotechnology I**	*Chair: Frank Wiekhorst*	
11:30	Abakumov, Maxim	Protein coated magnetic nanoparticles for cancer treatment and diagnostics	Moscow, Russia	Talk 27
11:45	Fratila, Raluca Maria	Clickable magnetic nanoparticles; a new tool for magnetic hyperthermia	Zaragoza, Spain	Talk 28
12:00	Jedlovszky-Hajdu, Angela	Magnetic gelfiber based scaffold for theranostic applications	Budapest, Hungary	Talk 29
12:15	**Lunch**			
13:15	Glückstad, Jesper	IDEAS TALK: Light Robotics and Its Potential for Integrating With Magnetic Carriers	Copenhagen, Denmark	Invited Talk 30
	Session 7	**Nanotechnology II**	*Chair: Thompson Mefford*	
14:00	Tasci, Tonguc Onur	Magnetic Microlassos For Single Cell Capture, Manipulation And Cargo Transport	Boston, MA, USA	Talk 31
14:15	Löwa, Norbert	3D-printing of novel magnetic composites based on magnetic nanoparticles and photopolymers	Berlin, Germany	Talk 32
14:30	Frenea-Robin, Marie	Electroactive Magnetic Nanoparticles for Electrochemical Signal Amplification under Magnetic Attraction on a Microchip Device	Lyon, France	Talk 33
14:45	Schotter, Joerg	NIL-fabricated multifunctional magnetic nanoparticles as probes for homogeneous label-free biosensing	Vienna, Austria	Talk 34
15:00	Ronti, Michela	Structural motifs in Self-Assembling Dipolar Spheres	Vienna, Austria	Talk 35
15:15	**Poster session II (Posters 116-256) with coffee and cake**			

17:15	**Bus leaves to Carlsberg Brewery**
17:30	**Tour of "Visit Carlsberg" followed by gala dinner at the Carlsberg mansion and museum; photograph of all the participants**
21:30	**End of the evening**

Friday, May 25, 2018				
08:30	**Registration desk opens** - at the Niels Jerne Auditorium, Mærsk Tårnet (Maersk Tower), Blegdamsvej 3B			
	Session 8	**Nanoparticle Synthesis I**	*Chair: Etelka Tombacz*	
09:00	Kasparis, Georgios	Synthesis and Application of Zinc Ferrite Nanoparticles as Agents for Cancer Thermotherapy	London, UK	Talk 36
09:15	Thanh, Nguyen	High Magnetisation, Monodisperse and Water-dispersible CoFe@Pt Core/shell Nanoparticles	London, UK	Talk 37
09:30	Bender, Philipp	Revealing dipolar-coupled moment correlations in clusters of superparamagnetic nanoparticles	Santander, Spain	Talk 38
09:45	Lak, Aidin	Iron deficiencies and structural defects favor magnetic hyperthermia performance of magnetite nanocubes in viscous media	Genoa, Italy	Talk 39
10:00	**Coffee break**		lak	
	Session 9	**Nanoparticle Synthesis II**	*Chair: Kathy Saatchi*	
10:30	Mefford, Thompson	Quantitative Measurement of Ligand Exchange on Iron Oxides via Radioanalytical Techniques	Clemson, SC, USA	Talk 40
10:45	Secret, Emilie	Synthesis and functionalization of magnetic nanoparticles for remote control of differentiation and oriented growth of neuronal cells	Paris, France	Talk 41

11:00	Mickoleit, Frank	Generation of nano-magnetic hybrid materials by genetic engineering and functionalization of bacterial magnetosomes	Bayreuth, Germany	Talk 42
11:15	Pividori, Maria Isabel	Magnetic Molecularly Imprinted Polymers - Synthesis and Applications	Barcelona, Spain	Talk 43
11:30	Morales, Maria del Puerto	Assembling magnetic iron oxide cores to produce well-controlled hydrophilic multicore structures	Madrid, Spain	Invited Talk 44
12:15	**Lunch**			
	Session 10	**Magnetic Imaging**	*Chair: Patrick Goodwill*	
13:15	Diamond, Solomon	Progress Towards a Magnetic Nanoparticle Imaging System for Immunohistochemistry	Hanover, NH, USA	Talk 45
13:30	Paysen, Hendrik	Towards functional magnetic particle imaging of endothelial cells	Berlin, Germany	Talk 46
13:45	Frellsen, Louise	Dynamical monitoring of magnetic markers using quantum diamond magnetometry	Copenhagen, Denmark	Talk 47
14:00	Millán, Angel	Avoiding RES retention of magnetic nanoparticles with a dense PEG coating. A biodistribution study by MRI, SPECT, gamma-counting and TEM	Zaragoza, Spain	Talk 48
14:15	Corte-León, Héctor	Quantitative magnetic force microscopy for single magnetic bead characterization	Teddington, UK	Talk 49
14:30	**Coffee break**			
15:00	Goodwill, Patrick	Magnetic Particle Imaging Emerges into Preclinical Research: Hardware, Nanoparticles, and Animal Models	Alameda, CA, USA	Invited Talk 50
15:45	Goodwill, Patrick	Online Demo of the Momentum™ Magnetic Particle Imaging system from Magnetic Insight Inc.	Alameda, CA, USA	Demo
	Session 11	**Magnetic Separation**	*Chair: Ivo Safarik*	
16:15	Zborowski, Maciej	the Point Of Care	Cleveland, OH, USA	Talk 51
16:30	van Silfhout, Alex	Colloidal Stability of Ferrofluids for Magnetic Density Separation	Utrecht, The Netherlands	Talk 52
16:45	Dempsey, Nora	Efficient capture of highly diffusive magnetic nanoparticles using micro-magnet arrays	Grenoble, France	Talk 53
17:00	**Touristic walk to the boat / with graduate students as guides**			
18:30	**Boat tour from "Ved Strand" across from Christiansborg Castle / with drinks and snacks**			
20:00	**Drop off at Nyhavn / free evening**			

Saturday, May 26, 2018				
08:30	**Registration desk opens** - at the Niels Jerne Auditorium, Mærsk Tårnet (Maersk Tower), Blegdamsvej 3B			
	Session 12	**Biological Applications I**	*Chair: Peter Hore*	
09:00	Roig, Anna	Magnetic Nanocapsules for Brain Repair after a Stroke	Barcelona, Spain	Invited Talk 54
09:45	Weidner, Andreas	Protein coated magnetic nanoparticles for medical applications	Ilmenau, Germany	Talk 55
10:00	Gigoux, Véronique	Targeted Magnetic Intra-Lysosomal Hyperthermia produces lysosomal reactive oxygen species and causes Caspase-1 dependent cell death	Toulouse, France	Talk 56
10:15	**Coffee break**			
	Session 13	**Biological Applications II**	*Chair: Thanh Nguyen*	
10:45	Dapprich, Johannes	Superparamagnetic Particle Scaffold for Regenerating Damaged Neural Tissue	Lawrenceville, NJ, USA	Talk 57
11:00	Lisse, Domenik	Magnetogenetic Activation of Small GTPases with MagIcS Nanoparticles Inside Living Cells	Osnabrück, Germany	Talk 58
11:15	Van de Walle, Aurore	Differentiation status of stem cells impacts the biotransformations of internalized magnetic nanoparticles: in situ investigations using the magnetic imprint	Paris, France	Talk 59
11:30	Gonçalves, Ana	Magnetic actuated SPCL scaffolds dopped with iron oxide magnetic nanoparticles as mechano-instructive platforms for tendon tissue engineering	Barco, Portugal	Talk 60
11:45	Hannon, Gary	A Systematic Approach to Endotoxin Contamination Assessment of Iron Oxide Nanoparticles for Theranostics applications	Dublin, Ireland	Talk 61
12:00	**Lunch**			
	Session 14	**Biosensors**	*Chair: Anna Roig*	
13:00	Hore, Peter	Disruption of Magnetic Compass Orientation in Migratory Birds by Radiofrequency Electromagnetic Fields	Oxford, UK	Invited Talk 62
13:45	Wiekhorst, Frank	Development of a method to analyse the dynamic magnetic behavior of magnetic nanoparticles during cellular uptake with high temporal resolution	Berlin, Germany	Talk 63
14:00	Su, Diqing	A Handheld Platform Based on Wash-Free Magnetic Bioassays for the Early Diagnosis of Influenza A Virus	Minneapolis, MN, USA	Talk 64
14:15	Zabow, Gary	Magnetic microparticles for new NMR-based force sensing	Boulder, CO, USA	Talk 65
14:30	Leliaert, Jonathan	Thermal noise magnetometry: a zero-field magnetic nanoparticle measurement method	Ghent, Belgium	Talk 66
14:45	Teran, Francisco	Taking advantages of nanomagnetism for detecting biomarkers dispersed in biological fluids	Madrid, Spain	Talk 67
15:00	**Closing Comments and Announcement of the NEXT MEETING: Urs Hafeli / Thanh Nguyen**			
15:15	**Meeting ends**			

Social Program

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

Tuesday, May 22

A welcome reception will be held at the Maersk Tower, Blegdamsvej 3B, and will be open to all participants of the conference. It will start at 6:30 PM and go till 10 PM. Conference registration will be available throughout the reception. This reception is made possible by our sponsor Chemicell GmbH - thank you very much!

Wednesday, May 23

During the day, we will have a spouse tour starting at 10 AM. This tour is complimentary and always fun!
After the talks, there will be a poster session with Beer and Snacks, graciously sponsored by micromod Partikeltechnologie GmbH. The rest of the evening is free. Go and explore! In fact, you don't have to go very far, there is quite a few eateries and restaurants available in Nørrebro or downtown Copenhagen.

Thursday, May 24

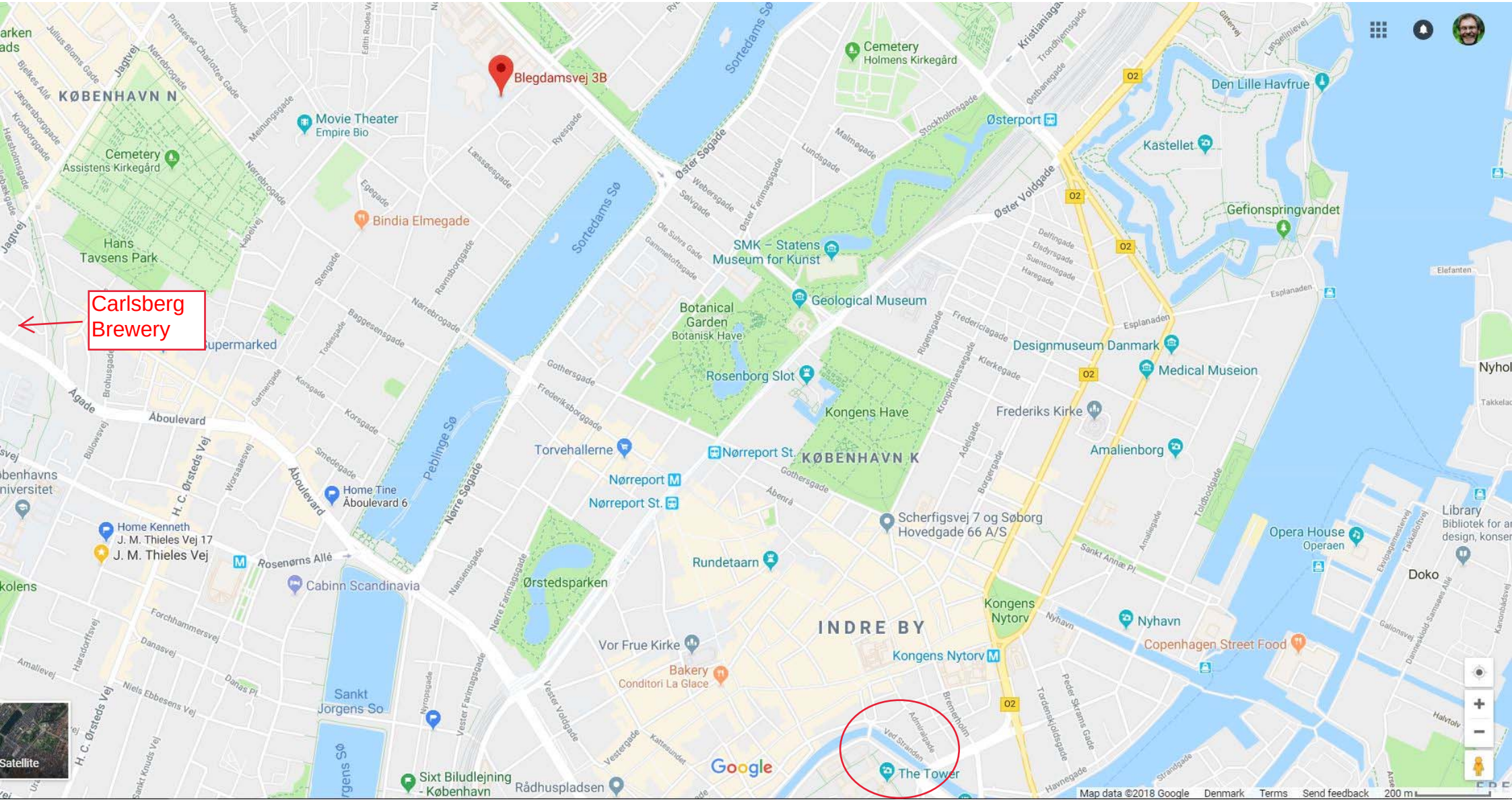
In the afternoon there will be a poster session with cake and coffee.
On this evening, we will have a very special conference dinner. We will visit the Carlsberg brewery, and will then dine in Carl's villa, which is the original Glyptotek. It is essentially like dining in a museum. Enjoy!

Friday, May 25

On this evening, we will have our traditional boat trip. We will walk to the harbour through the botanical garden, and will also pass on the way the Rosenborg Castle, the Frederiks Dome, and the Queen's home Amalienborg. There will be some drinks and snacks on the boat. The end of the boat trip will be at Nyhavn, where everybody can go wild and enjoy the evening with friends.

Saturday, May 26

The meeting will end at 3:15 PM. Please take the opportunity and explore beautiful Denmark on your own after the end of the conference! There is many things to see in and around Copenhagen.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Movie Theater Empire Bio
Bindia Elmegade
Hans Tavsens Park
Sortedams Sø
Peblinge Sø
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Kastellet
Den Lille Havfrue
Gefionspringvandet
SMK – Statens Museum for Kunst
Geological Museum
Botanical Garden
Botanisk Have
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Kongens Have
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Medical Museion
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Spatially Controlled and Image Guided Nanoscale Thermal Therapy

Andreina Chiu-Lam[1,*], Rohan Dhavalikar[1], Zhi Wei Tay[2], Prashant Chandrasekharan[2], Steven M. Conolly[2], and Carlos Rinaldi[1,3]

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Magnetic nanoparticles (MNPs) can be made to dissipate heat in response to an alternating magnetic field (AMF)[1] and are used in magnetic fluid hyperthermia (MFH)[2], where this heat is used to treat cancer. A major advantage of MFH over other nanoparticle cancer thermal therapies is that the required AMF is unaffected by tissue depth. However, systemically delivered MNPs accumulate in nontargeted organs, potentially leading to nonspecific heating during whole body AMF application.[3] Spatial control of MNP heating can be achieved by superimposing a static magnetic field gradient and a AMF, creating a region at the center with small static field where MNPs dissipate heat. Another challenge is being able to track and visualize where MNPs accumulate to determine the location and best time for treatment. Magnetic particle imaging (MPI)[4] is an emerging molecular imaging technology where imaging signal is generated solely by MNPs. Advantages of MPI are that it can provide tomographic, three-dimensional, quantitative distribution of MNPs. The combination of MPI with MFH has potential to overcome the nonspecific heating problem and provide non-invasive feedback through MPI images. Here we show the first *in vitro* and *in vivo* experiments of spatially focused nanoscale thermal therapy. *In vitro*, MDA-MB-231 cells were seeded in a 6x16 array of a 384 well plate and treated with AMF or AMF with selection gradient magnetic field. Polyethylene glycol coated iron oxide was added to each well and treated for 1 hour at 45°C. MNPs were removed and metabolic assay performed two days later. Figure 1 shows that by using static magnetic field gradient we can spatially control the region of heating and cell death. *In vivo*, athymic nude mice were injected with MDA-MB-231-luc subcutaneously in two locations.[5] As the tumor grew to size, MNPs were directly injected into the tumors. MPI imaging and bioluminescence was taken before AMF application. The bottom tumor was targeted for AMF with selection gradient magnetic field and temperature was recorded for both tumors and the rectum with Neoptix™ fiber optic temperature probes for 1 hour during treatment. Figure 2 demonstrates the process of MPI imaging and gradient localized heating and provides compelling evidence for the combination of MPI with MFH to selectively apply nanoscale thermal therapy to a target area.

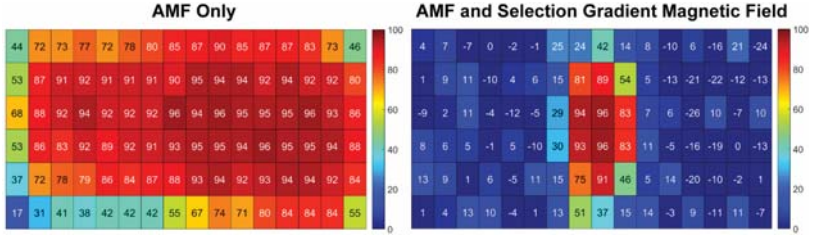

Figure 1. Heatmap for percentage cell death in each well for AMF and AMF with selection gradient magnetic field.

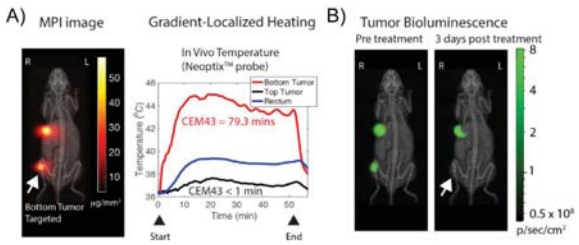

Figure 2. Spatially focused MFH using a bioluminescence MDA-MB-231-luc xenograft model. A) MPI image of dual tumor, both loaded with MNPs. Bottom tumor was targeted and AMF was applied. Temperature profile during treatment shows that the targeted bottom tumor heated to 45°C while the top tumor and the rectum did not heat. B) Bioluminescence before and 3 days after treatment showing significant bioluminescence reduction in the bottom targeted tumor and not the top tumor.[5]

[1]A. Chiu-Lam and C. Rinaldi, Advanced Functional Materials **26**, 3933 (2016) [2]B. Kozissnik, A.C. Bohorquez, et al., International Journal of Hyperthermia **29**, 706 (2013) [3]C. Kut, Y. Zhang, et al., Nanomedicine 7, 1697 (2012) [4]B. Gleich and J. Weizenecker, Nature **435**, 1214 (2005)
[5]Z.W. Tay, P. Chandrasekharan, et al. ACS Nano (accepted 2018)

Magneto-liposomes as theranostic agents

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Due to the high mortality cancer has become one of the major issues of an aging population. In order to improve the patient survival, reduce the treatment time and the unwanted side effects, radically new approaches in treatment and diagnostics are needed. Despite the severe side effects due to the non-specific action of cytotoxic drugs, chemotherapy remains the main choice for the treatment of most cancers. Following the systemic administration, cytotoxic drugs are eliminated rapidly from the blood stream, which results in poor drug accumulation at the target site and, therefore, several consecutive high-dose treatments are needed to achieve the therapeutic effect. By encapsulating the drug in a smart drug delivery system with the prolonged circulation time, the efficiency of the therapy can be strongly increased with decreasing potential side effects. In this way, drug is inactive and non-toxic until it reaches the target cancer cells, where is remotely released in a controlable manner using the external stimulus. Liposomes are spherical vesicles consisting of phospholipid bilayer. Their biocompatibilty and biodegradabililty arise from their composition (natural phospholipids) therefore are assumed to be one of the safest drug delivery systems proposed so far.[2] Their special structure allows the binding of a whole range of different molecules, hydrophilic molecules in the core of the liposomes as well as the hydrophobic ones in the lipid bilayer. By adding different active components either in the core or in the bilayer of the liposomes a safe and efficient multifunctional drug delivery system can be prepared. **To realize this, we developed a temperature-sensitive liposomes (TSLs) as an innovative "theranostic" system containing magnetic nanoparticles (NPs) in the bilayer and a chemotherapeutic drug in the core.** We have demonstrated that photo-thermal therapy can be very efficiently used to induce heating of the magneto-liposomes to achieve mild hyperthermia and, consequently, the drug release from the core of the liposomes. To the best of our knowledge, this is the first report about the use of the magneto-liposomes for the photo-thermal therapy. In addition, MRI experiments revealed that magneto-liposomes exhibit much higher relaxivities than the "free" magnetic NPs, which puts as-prepared magneto-liposomes high on the list of the most promising candidates to be used as the contrast agent in MRI. Furthermore, use of magnetic NPs in the bilayer of TSL containing any lysolipid and/or PEG-based lipid for MRI was reported for the first time. Therefore, as-prepared magneto-liposomes represent a complete novelty in this field of research and thus open new options for the use of TSL liposomes in the medicine.

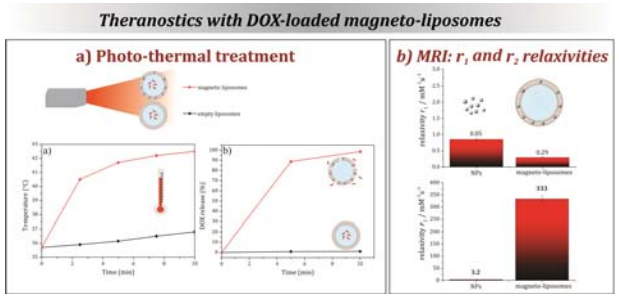

Figure 1: A) Laser-induced DOX burst release from magneto-liposomes: Temperature profile empty- and magneto-liposomes when irradiated with NIR laser (0.55W/cm², 10 min) and corresponding DOX release profiles for empty- and magneto-liposomes. B) **Magneto-liposomes as a promising MRI T_2 contrast agent:** longitudinal r_1 and transverse r_2 relaxivities for hydrophilic IO NPs and magneto-liposomes.

Differences in cell death mechanism after magnetic hyperthermia treatment depending on the nanoparticle location.

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Magnetic hyperthermia is a promising therapy for the localized treatment of cancer. Under the exposure to an external alternating magnetic field, magnetic nanoparticles (MNPs) act as heating agents inducing cell death in the treated region. Understanding the molecular mechanisms involved in the cellular damage generated by this treatment is crucial for the successful application of this therapy.

In this study, 12 nm spherical MNPs coated with PMAO (poly (maleic anhydride-alt-1-octadecene) and functionalized with glucose were prepared by thermal decomposition. In order to evaluate the influence of the nanoparticle location in the treatment efficacy, two different 3D cell culture models, based on collagen gels, were prepared using a macrophage cell line, RAW264.7, (Figure 1). The first model (Model 1) kept all the particles inside the cells while the second model (Model 2) had particles both inside and outside the cells. The first model mimics a scenario where MNPs are administered intravenously with an active targeting, and the second one mimics intratumoral administration. The MNPs uptake and cell death mechanisms induced after the hyperthermia treatment (377 kHz, 13kA/m and 30 minutes, DM100-DM3 nB nanoscale Biomagentics,) were evaluated by flow cytometry. Interestingly, the cell death pathway was different depending on the MNPs location. Necrosis was observed 24h after magnetic hyperthermia application in the model where the nanoparticles are located just inside the cells. In contrast, apoptosis was detected in the model where the particles are inside and outside the cells. In addition, in the second model, our results evidenced an enhancement of the nanoparticles uptake after the exposure to the alternating magnetic field. This observation could justify the repetition of the treatment to obtain a better antitumor effect.

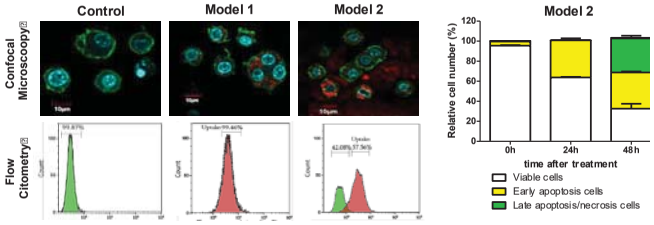

Figure 1. *Left. Confocal microscopy and flow citometry data showing the diferent location of the particles. Model 1 containing particles just inside the cells, while Model 2 containing them both inside and outside. Right. Evolution with time of the cell death mechanism after hyperthermia treatment in Model 2.*

Our results demonstrate the potential efficacy of magnetic hyperthermia in the treatment of malignant tumours. In addition, the use of 3D cell culture models for the optimization of hyperthermia treatments (type and dose of MNPs, repetition cycles, field amplitude and frequency, etc) presents several advantages, allowing the obtention of information in a more realistic way than monolayer cell cultures and reducing the number of animals required for preclinical tests.

Glycoconjugate-Functionalized Magnetic Nanoparticles: A Tool for Selective Killing of Targeted Bacteria Via Magnetically Mediated Energy Delivery.

Benjamin D. Fellows[1], Yash S. Raval[2], Jamie Murbach[1], Yves Cordeau[1], Tzuen-Rong J. Tzeng[2], and O. Thompson Mefford[1]

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New technologies utilizing nontraditional antibiotic mechanisms are urgently needed to combat the increasingly common appearance of multi-drug resistant bacteria. Drug resistant bacteria is a worldwide problem as the number of drug resistant pathogens grows with each year. The World Health Organization developed the Global Action Plan on Antimicrobial Resistance (AMR) in 2015 to control the spread and impact of antimicrobial resistance. In addition, the economic cost of AMR to be approximately 3.1% of global output gross domestic product. To put this in prospective, the cost of cancer represents 1.5% of global GDP. This work explores the feasibility of using magnetically mediated energy delivery (MagMED) to selectively kill enterotoxigenic Escherichia coli strain K99 (EC K99) as a model for antibiotic resistant bacteria.

Click ready magnetic nanoparticles were synthesized and functionalized with bacteria-specific glycoconjugate for adherence to EC K99. When mixtures containing both EC K99 and the GM3-MNPs were exposed to alternate magnetic fields (31 KA/m, 207 KHz), a clinically relevant 3-log reduction in colony forming units (CFUs) of EC K99 was achieved in 120 minutes. Bacterial selectivity of the treatment was shown using a mixed culture experiment including both recepter positive EC K99 and recepter negative EC O157. Targeted cell death of the EC K99 was seen after treatment of the mixed culture with minimal damage to EC O157. Cell death of EC K99 was further supported by the intracellular adenosine triphosphate (ATP) levels which were considerably reduced when incubated with GM3-MNPs and treated with MagMED. These results suggest that GM3-MNPs induced glycoconjugate targeting along with MagMED can be potentially used as a targeted nontraditional antibiotic treatment platform to inactivate/kill bacterial pathogens, with minimal impact on normal microflora and the affected body region/tissue.

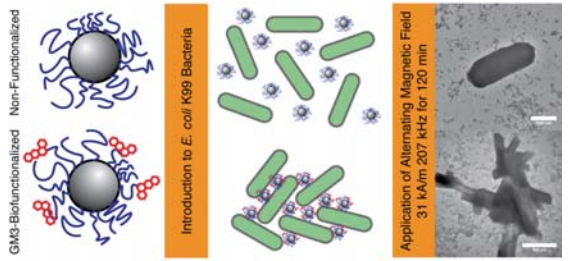

Figure 1: (left) Cartoon representation on functionalized and non-functionalized magnetic nanoparticles (center) representation of aggregation of the GM3-functionalized, but no aggregation with the non-functionalized particles (right) TEM image of cellular destruction following targeted treatment of E. coli K99.

Intracellular distribution of magnetic nanoparticles and magnetic hyperthermia effects: a Focused Ion Beam study.

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Magnetic hyperthermia (MHT) a strategy to achieve selective heating of target tissues is based on the use of magnetic nanoparticles (MNPs) as nano-heaters, through the coupling between an external alternating magnetic field (AMF) and the magnetic moment of the MNPs. Since the MNPs uploaded into the target cells are the heating source, their amount and final distribution within the intracellular space are two parameters of relevance in any physical model of cell death induced by MHT. Imaging analysis through electron microscopies has proven a potent tool for establishing the intracellular biodistribution. This work presents FIB-SEM a study on the intracellular distributions of different MNPs (Fe_3O_4, $MnFe_2O_4$ and $CoFe_2O_4$) within neuroblastoma and microglial cells before and after MHT. The MNP-loaded cells were seeded and fixed directly on coverslips, then were cross-sectioned by FIB, and analyzed by FIB-SEM in order to explore the final intracellular MNPs distribution. Embedding the MNP-loaded cells after the application of an ac magnetic field with an epoxy-resin allowed us to use the "slice and view" protocol to identify the changes on cell morphology and membrane integrity through 3D reconstruction.

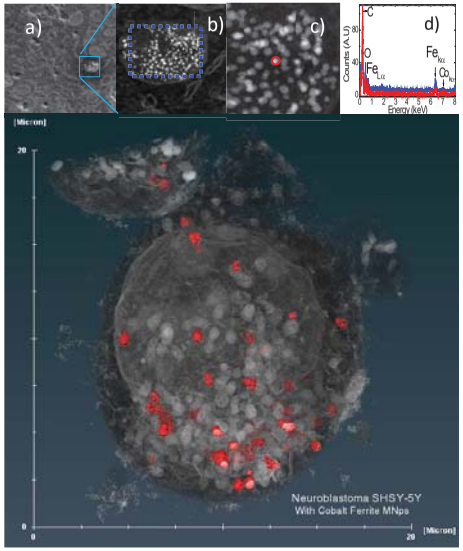

Figure 1 a) STEM image of SH-SY5Y cells incubated with $CoFe_2O_4$ nanoparticles at 20 μgml^{-1} for 24 hours. b and c) are zooms of the region select on a). d) EDX spectrum (in blue) of the small group of MNPs selected in b) and EDX spectrum (in red) of the particle selected in c. Both show the presence of iron and cobalt in the MNPs. e) Shapshot of the 3D cell reconstruction incubated with $CoFe_2O_4$ at 100 μgml^{-1} for 24 hours . Red spots correspond to MNP aggregates.

NANOBIOHYBRIDS FOR THE THERMOACTIVATION OF A THERAPEUTIC ENZYME BY MAGNETIC HYPERTHERMIA

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Due to their size-tunable physical and chemical properties, **magnetic nanoparticles** (MNPs) have demonstrated a wide range of applications in healthcare and life science. In particular, the well-known property of remotely inducing local heat when an alternating magnetic field (AMF) is applied has opened a new area of research called **Magnetic Hyperthermia**. Traditionally, the activation of these MNPs as nanoheaters has been used seeking the destruction of tumoral cells[1]. **In this work**, however, we want to show that it is possible to use the heat generated by AMF for a **different purpose**: ***to locally reach an optimal temperature*** *(45ºC)* ***that enhances the conversion of a prodrug*** *(indole-3-acetic acid)* ***into a drug*** *(peroxylated radicals)* ***by a therapeutic enzyme*** *(horseradish peroxidase, HRP).*

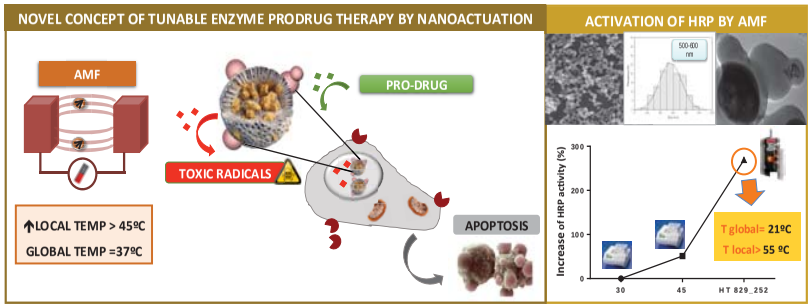

Figure 1. The new Enzyme Pro-Drug Therapy concept proposed based on the thermo-activation of HRP by nanoactuation.

For this, the co-encapsulation of MNPs together with HRP in biomimetic silica nanoparticles has been optimized. Different **biohybrids (biosilica+MNP+HRP)** were prepared using polyethylenimines of different molecular weight (1300 and 60000 daltons) as amino catalysts for the formation of the silica nanoparticles. Besides, the combination of enzyme crosslinking and the addition of polyols during encapsulation, provided a 250 times increase in the half-life time of the enzyme at 50°C. After carrying out and extensive physicochemical characterization (SEM, TEM, DLS, SQUID, ICP-OES...), it was demonstrated that by applying AMF it is possible to reach local temperatures higher than 45°C within the enzymes microenvironment without an increase in the overall temperature of the reaction medium. These results show that it is possible to think about developing a **novel "on/off" switch approach for the remote conversion of pro-drugs in cancer therapy** by enzymes of thermal origin that show little or no activity at 37ºC. This would allow a precise remote control of the therapy that solves a limitation of the current strategies that could not avoid its activation outside the target site.

[1]Dutz S et al. Nanotechnology 2014; 25:452001.

Optimal formulation properties and physico-chemical characteristics of iron oxide magnetic nanoparticle heating agents for clinical applications

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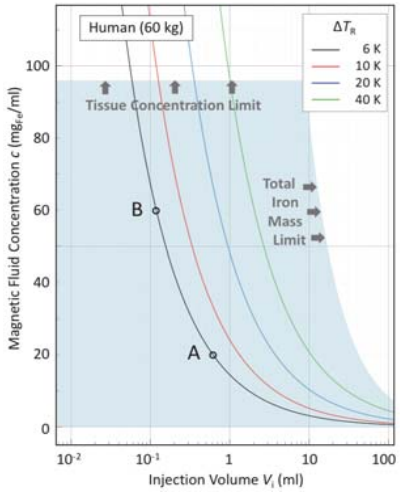
In a recent publication [1] we have reviewed and commented on the clinical and preclinical dosage limits of interstitially administered magnetic fluids for therapeutic hyperthermia based on current clinical practice and on theoretical models of heat transfer in tissue.

A key outcome of that work is the figure opposite, which shows bioheat model calculations in the steady-state, zero perfusion, zero metabolic heat generation limit, for a 60 kg human [2]. Although the model assumptions are non-physical, nevertheless the lessons to be learned from the figure are valid in real world applications.



Of particular note are the dosage limitations imposed by the clinically acceptable limits on both the local tissue (denoted by the 'tissue concentration limit') and the body as a whole (the 'total iron mass limit'). In the figure, the range of acceptable doses – in terms of both the injection volume V_i and the magnetic fluid concentration c – are indicated by the region shaded in grey. Furthermore, if one's objective is to achieve a certain degree of magnetic heating, as measured by a certain elevation of the local tissue temperature, then it may be preferable to adopt a more concentrated formulation of the agent.

This is illustrated by Points A and B on the figure, both of which mark a temperature rise of 6 K. Point A is for a fluid of concentration c = 20 mg_{Fe}/ml_{fluid}. To achieve the 6 K temperature rise, 0.61 ml_{fluid} must be administered, corresponding to a mass of iron of 12.2 mg_{Fe}. At Point B, just 0.12 ml_{fluid} of the more concentrated c = 60 mg_{Fe}/ml_{fluid} fluid is needed – corresponding to a lower mass of iron, *viz*. 7.2 mg_{Fe}. Depending on the intended clinical application, it may be preferable to use the lower injected dose of iron to achieve the same degree of heating at a given site.

In this work we discuss these and other considerations as to the optimal formulation properties of magnetic heating agents, and their physico-chemical properties. We also present and discuss a new iron oxide heating agent, RCL-01, that has been developed by Resonant Circuits Limited with a view to meeting current clinical needs for high concentration, high performance agents.

[1] Southern & Pankhurst, Int. J. Hyperthermia, 2018 (at press; author's preprint: http://tinyurl.com/y7f88gh8).
[2] Key parameters assumed in the figure: particles with intrinsic loss power *ILP* = 3.0 nHm^2/kg_{Fe}; dispersion factor $v = V_d/V_i = 2.4$; thermal conductivity of the surrounding medium λ_2 = 0.52 W/Km; and activation field amplitude H = 5 kA/m and frequency f = 300 kHz. Formulation parameters assumed: an agent of relatively high retention ($\mathcal{R}$ = 0.85) at the injection site, and a high degree of systemic tolerance ($\mathcal{F}$ = 0.85) in the body.

Recommendations for in-vitro and in-vivo testing of Magnetic Nanoparticle Hyperthermia combined with Radiation Therapy.

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*Corresponding author

Magnetic nanoparticle (MNP)-mediated hyperthermia (MH) coupled with radiation therapy (RT) is a novel approach for cancer treatment. It is an attractive approach because it has the potential to overcome various practical difficulties encountered in previous attempts to combine hyperthermia and RT in the clinic. In this work we present recommendations for the *in vitro* and *in vivo* testing and application of the two treatment techniques. These recommendations were developed by members of Working Group 3 of the RADIOMAG COST action TD 1402. The purpose of the recommendations is not to provide definitive answers and directions but, rather, to outline those tests and considerations that a researcher must address in order to perform *in vitro* and *in vivo* studies.

The recommendations are divided into five parts. The first part, *in vitro* evaluation of MNPs, addresses colloidal stability, sterilization, homogeneity, and endotoxin contamination of the aqueous MNP solution. The second part, *in vitro* evaluation of MNP-cell interactions, describes cell viability, cell proliferation, and distinguishing between necrotic and apoptotic cells. The third part, *in vivo* evaluation of the MNPs, concerns pH considerations, osmolarity, the behaviour of MNPs in an applied magnetic field, heating rates, frequency- and dose-response curves and possible effects on the MNPs themselves. The fourth part, MH combined with RT focuses on modelling and simulations, the choice of the pre-clinical cancer models, thermal dosimetry, treatment sequence, blood flow issues, thermotolerance, heating profiles, study endpoints, toxicity and treatment evaluation. Lastly, the fifth part discusses the pharmacokinetic and biodistribution studies of MNPs, including radiolabeling of the MNPs, the effects of particle shape, size and charge, the nature of the coating polymer, the injected quantity, the requirements for animal experimentation, the route of administration, and metabolism and excretion. Synthesis and characterization of the MNPs, as well as RT protocols are beyond the scope of these recommendations.

Ultrasonic Potentiation of Magnetic Fluid Hyperthermia/Pifithrin-μ Combined Therapies in Ovarian Cancer Cells

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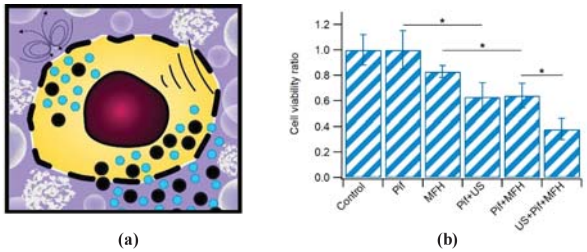
In cancer treatment, a recurrent approach to improve the effects of magnetic fluid hyperthermia (MFH) is the conjugation of targeting ligands and drug molecules onto magnetic nanoparticles. However, molecule conjugation in active targeting is complex, expensive, and has led to reduced circulation times in blood *in vivo*. Sonoporation is the process by which ultrasound waves promotes pore formation and permeabilization of cell membranes, facilitating the passage of drug molecules and other agents to intracellular environments. This process is depicted in Figure 1a. In this work, we propose the use of sonoporation rather than active targeting to enhance the cellular uptake of the drug Pifithrin- μ along with magnetic nanoparticles, thus improving the effects of MFH. Ovarian cancer cells have been exposed to different ultrasound regimens in the presence of ultrasound contrast agent microbubbles, using ultrasound intensities up to 6 W/cm^2 and exposure times up to 60 seconds. Iron oxide magnetic nanoparticles were synthesized in our laboratory using an optimized synthesis method and coated with polyethylene glycol (PEG). Nanoparticles and Pifithrin-μ were added to cells prior the application of ultrasound, using a focused transducer. Once sonicated, cells were exposed to alternating magnetic fields with intensities of 11 - 15 kA/m to achieve sustained temperatures of 41 for 30 minutes. Our preliminary results, shown in Figure 1b, demonstrate that combined Pifithrin-μ/MFH treatments led to increased cancer cell death when ultrasound exposure was carried out in the presence of both drug and magnetic nanoparticles. In addition, the cell killing profile was higher than that of individual treatments when cells are treated with drug or MFH, respectively, and also higher than that of combined MFH/drug without exposure to ultrasound. We attribute the increased cancer cell death to ultrasound-enhanced drug uptake by cells, potentiated by thermal effects achieved with magnetic hyperthermia. The study of additional ultrasound and MFH experimental conditions is currently underway to evaluate further improvements in drug/nanoparticle uptake by cells. Hence, it is expected that the use of ultrasound as an external stimulus for MFH applications has a great potential in nanoscale cancer therapy, becoming an effective sono-thermo-chemotherapy in the treatment of ovarian cancer.



(a) (b)

Figure 1. a) Graphical representation of microbubble-mediated cell sonoporation for the enhanced uptake of magnetic nanoparticles and drug molecules. Black, blue and grey circles represent nanoparticles, drug and microbubbles, respectively. b) Viability ratios of HeyA8 cells when exposed to various regimens of the drug Pifithrin-μ (Pif), magnetic fluid hyperthermia (MFH) and ultrasound (US) either as individual or combined treatments.

Invited Talk

Low Frequency Pulsed Electromagnetic Field Applications: Bone and Soft Tissue Repair

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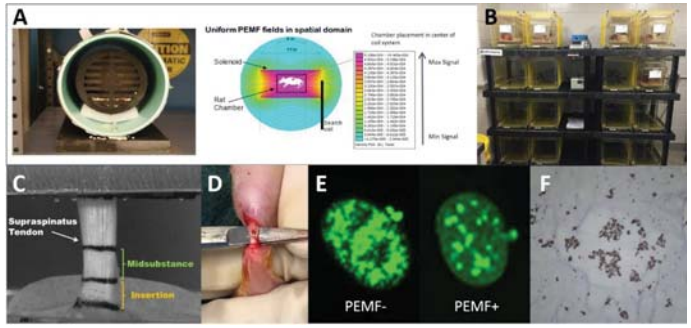
Currently the only Class III pulsed electromagnetic field (PEMF) devices approved by the US Food & Drug Administration (FDA) have been within the category of bone growth stimulation/osteogenesis stimulation for treatment of long-bone non-unions and as an adjunct treatment to lumbar and cervical fusion surgery [1-3]. With the approval of PEMF for osteogenesis stimulation, research has been directed towards optimization of the PEMF signals for improvement in efficacy for current indications in addition to expanding the types of applications that PEMF can be utilized for.

Orthofix sponsored research on osteoporosis reversal has shown that in a rodent osteoporosis reversal model (OVX treatment) different PEMF slew rates (10-300 T/s) yield a bi-phasic biological response with the optimal slew rate (30 T/s) showing similar efficacy in reducing bone loss as a bisphosphonate (alendronate) [4]. In addition PEMF research on the healing of soft tissues such as rotator cuff and Achilles tendon has shown up to a 100% increase in tendon modulus in rodents at early time points (4 weeks) following supraspinatus tendon transections with immediate repair. Increases in tendon stiffness, humeral head bone mineral content, and tendon collagen organization were also observed. This effect was shown to be present with as little as 1 hour of daily PEMF treatment [5,6]. Subsequent rodent Achilles tendon studies showed opposite effects for full thickness tears while partial tears showed minimal improvement. The combined studies shows that PEMF can be effective for soft tissue repair but is dependent on the location of application [7,8]. Lastly, investigations have been performed showing real-time anti-inflammatory effects of PEMF on human intervertebral disc (IVD) cells with a subsequent in-vivo acute inflammation rodent IVD model showing similar results with decreases in inflammatory gene expressions [9-11]. In an effort to enhance this anti-inflammatory effect co-cultures with magnetic micro-rods and –cubes showed little synergistic effect with a standard commercial PEMF signal but further studies could be warranted.

The PEMF research we have undertaken shows that effects are waveform-, duration-, tissue- and location-dependent warranting the need for careful pre-clinical studies prior to translation to the clinic.



A: OVX high-slew rate PEMF system and field simulation. B: Standard multi-subject PEMF system. C: Rodent rotator Cuff mechanical testing. D: Rodent Achilles surgery (partial tear). E: Real-time imaging of IL-6 gene expression in disc cell. F: Nucleus pulposus c0-culture with magnetic micro-cubes.

Reference: [1] Garland et al, Contemp Orthop. 1991, 22(3):295-302. [2] Mooney et al, Spine. 1990, 15(7):708-712. [3] Foley et al, Spine J. 2008, 8(3):436-442. [4] Androjna et al, ORS (Orthopaedic Research Society) 2017, Poster #: 1670. [5] Tucker et al, Journal Orthopaedic Research. 2017, 35(4):902-909. [6] Huegel et al, Journal of Shoulder and Elbow Surgery (JSES), 2018, 27(3):553-560. [7] Boorman-Padgett et al, ORS (Orthopaedic Research Society) 2018, Poster #: 1454. [8] Huegel et al, ORS (Orthopaedic Research Society) 2018, Poster #: 1458. [9] Miller et al, The Spine Journal. 2016, 16(6):770-6. [10] Tang et al, Journal of Orthopaedic Research. 2018, 36(2):778-787. [11] Tang et al, ORS (Orthopaedic Research Society) 2017, Poster #: 832

Discovery of Self - luminescent Polyethyleneimine Coated SPIONs and Their Theranostic Applications

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Exploitation of superparamagnetic iron oxide nanoparticles (SPIONs) as theranostic materials is highly popular. Combination of SPIONs with fluorescent entities such as dyes or quantum dots are also popular to achieve dual imaging modality. Yet, SPIONs have strong absorbance in the visible window where most widely used luminescent probes show activity. We have discovered that SPIONs produced by aqueous co-precipitation method in the presence of branched polyethyleneimine (bPEI), demonstrate a very strong blue luminescence.

Branched polyethyleneimine (bPEI) is the most widely used cationic polymer for gene delivery. Interestingly, it does have a weak blue luminescence, which has been unrecognized until recently. The origin of this luminescence is not exactly known but seen in different amine rich systems, sometimes misinterpreted as the luminescence of the inorganic nanoparticle that it exists together with. This is intriguing since SPIONs have strong absorbance in the visible region. In order to understand the major effectors of this phenomenon, systematic analysis of the reaction variables, investigation of alternative synthetic methods, detailed XPS and EPR studies coupled with DFT calculations were performed. All of these studies indicate that, this strong luminescence of bPEI occurs only if bPEI-SPIONs are produced via co-precipitation process in which oxidation of some amine groups and reduction of Fe^{3+} ions on the SPION surface take place. This oxidation of bPEI was determined as the major cause of such dramatic enhancement of its typical blue luminescence (1200 times stronger). Emission from bPEI-SPION is excitation wavelength dependent, which is quite practical, and increases dramatically and irreversibly with acidification. Such fine chemical changes in the magnetic core and the bPEI did not harm the superparamagnetic nature of the core or the transfection ability of the coating: Luminescent bPEI-SPIONs did not only generate T2 contrast in the aqueous solutions but also strong intracellular optical signal *in vitro* as an indication of label-free optical detection. These nanoparticles effectively delivered a model oligonucleotide, PIC, to HeLa cells, demonstrating a significant transfection potential, as well.

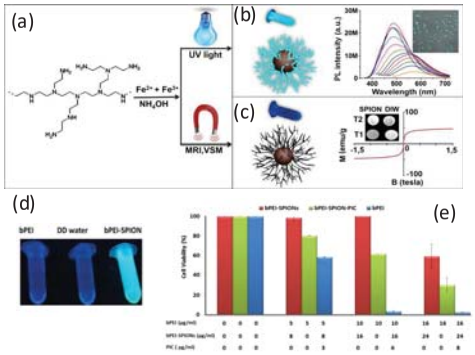

Figure 1. (a) schematic of bPEI/SPION synthesis, (b) optical properties (inset: intracellular blue luminescence), (c) magnetic properties (inset: T1 and T2 weighted images), (d) luminescence under UV lamp, (e) cytotoxicity and poly(I:C) transfection.

A novel method of nanoparticle surface biomodification for the development of effective agents for cancer theranostics

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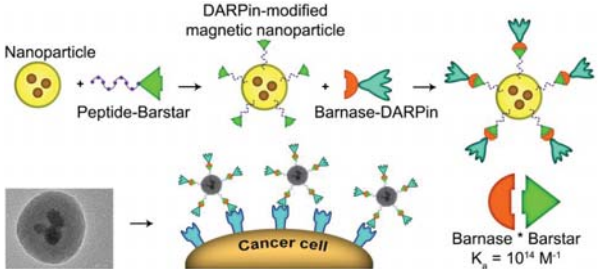
Targeted delivery – the concept of delivery of certain drugs and diagnostic compounds to the specific tissue sites within the living organism, is one of the key points in the development and progress of personalized medicine. Now significant attention of researchers is being directed toward one certain area of personalized medicine, namely, *theranostics*, which implies the combination of diagnostic and therapeutic approaches on a single platform. It seems promising to use different multifunctional supramolecular systems based on nanoparticles for theranostic applications, because different nanostructures, due to their physico-chemical properties, possess a number of properties that are impossible to obtain using different components individually. This comprehensive effect on the tumor tissue allows realizing the principle that the whole is greater than the sum of its constituent parts.

Here we show the development of magnetite nanoparticles capable of selectively binding to cancer cells overexpressing the HER2/neu oncomarker. This oncomarker is overexpressed in ~20-30% cases of breast cancer and has important clinical value. For the first time, a number of unique approaches for the biomodification of nanoparticles with the scaffold protein DARPin9.29, which highly specifically recognizes HER2/neu (K_D = 3.8 nM), has been developed. These nanoformulations were obtained through the unnatural peptides capable of binding the nanoparticles surface as solid phase, and with the use of the Barnase*Barstar module as a "molecular glue" between nanoparticles and scaffold protein as schematically illustrated in Figure. Ribonuclease Barnase (12 kDa) and its natural inhibitor Barstar (10 kDa) are proteins of bacterial origin, which exhibit extremely fast kinetics and high affinity of binding ($K_{aff} \sim 10^{14}$ M^{-1}), which is comparable only to well-known streptavidin*biotin pair. DARPins (Designed Ankyrine Repeat Proteins) are the novel class of recognition molecules of a non-immunoglobulin nature that are obtained by ribosomal or phage display methods. This class of proteins is an ideal candidate for carrying out genetic engineering manipulations and incorporating them into complex molecules with several functions for the following reasons: 1) small size; 2) absence of cysteines in the structure, resistance to aggregation, correct folding, good expression in bacteria; 3) thermodynamic stability; 4) high (up to picomolar values) affinity for receptors. The proposed bioengineering platform for nanoparticle biomodification is rapid (minutes-scale), preserves the protein functionality and orientation, and enables excellent control over the number and ratio of attached molecules.

The obtained particles retained aggregative and sedimentation stability under physiological conditions and selectively interacted with HER2/neu-positive cells, as demonstrated by a number of molecular biological methods. Binding of the obtained nanoparticles with target cells was quantitatively estimated by the original previously developed MPQ-cytometry method [V. Shipunova et al., ***Nanoscale***, 2016; M. Nikitin et al., ***Nature Nanotechnology***, 2014]. The method is based on the non-linear magnetization properties of magnetic nanoparticles and allows real-time measurement of a very small relative variation of magnetic susceptibility up to 10^{-8} at room temperature, thus providing sub-nanogram sensitivity of nanoparticles in 20 µl volume.

The composition of such targeted nanoparticles makes it possible to use them for diagnostic purposes now, and the further direction of this work is to impart a therapeutic modality to these complexes, namely the inclusion of toxic modules (e.g. pseudomonas exotoxin). Thus, this study is a step towards the creation of nanostructures for the therapy and diagnostics of oncological diseases. Application of such targeted nanoparticles opens up new possibilities for designing new effective agents for cancer theranostics.

The work was supported by the Russian Science Foundation grant № 17-74-20146.



Development of a Real-Time Two-Dimensional Navigation System of Magnetic Nanoparticles for Targeted Drug Delivery

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Magnetic nanoparticles (MNPs) have been applied successfully for drug delivery in the treatment of Alzheimer's disease by our group [1]. However, we cannot accurately observe the effectiveness of treatment in real time. A 1D Magnetic Particle Imaging (MPI)-based navigation system was developed in [2], but a 2D MPI-based navigation system should be completed to apply it for treatments and in-vivo tests.

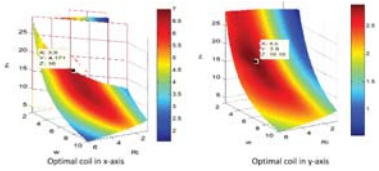

Fig 1. The optimal coil

Several limitations existed in the previously introduced 1D system [2] to implement a 2D MPI-based navigation system. First of all, the magnetic field gradients were not strong enough for steering MNPs in both directions in the Electro-Magnetic Actuator (EMA) mode, and the system couldn't apply drive field with a high frequency due to the utilization of a DC power supply for the drive and selection fields. Thus, in this paper, the design, configuration, and specifications of the real-time 2D MPI-based navigation system are optimized by using Comsol™ optimization module, and using a soft magnetic material to enhance both the steering force and the image quality for MNP monitoring. After optimization, the maximum gradient of the optimized system in x-, y- axes are 6.6 T/m and 2.88 T/m, respectively as shown in Fig.1. With these high gradient fields, less than 1 mm monitoring resolution can be achieved. Besides, the drive coil is separated from the EMA coil to implement a higher frequency which will improve the density of FFP scanning trajectory. The system workspace is kept at 3*3 cm^2 which is suitable for lab-mouse experiments. The designed 2D navigation system has 0.5s MPI time and 0.5s EMA time, achieving 1Hz for the hybrid system, allowing a real-time position control of MNPs with Resovist™ particles. The movement of MNPs cluster can be observed from both cameras and MPI image as shown in Fig.2. Real-time implementation of the system and experiments in a vascular network will be studied in future works.

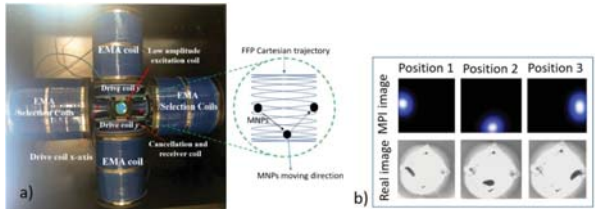

Figure 2. (a) Real 2D MNP Navigation System. (b) MPI is showing the positions of Resovist ™ particles in the update rate of 1Hz.

[1] F. U. Amin, A. K. Hoshiar, T. D. Do, Y. Noh, S. A. Shah, M. S. Khan, J. Yoon, and M. O. Kim, "Osmotin-loaded magnetic nanoparticles with electromagnetic guidance for the treatment of Alzheimer's disease," *Nanoscale*, 2017.

[2] X. Zhang, T.-A. Le, and J. Yoon, "Development of a real time imaging-based guidance system of magnetic nanoparticles for targeted drug delivery," *Journal of Magnetism and Magnetic Materials*, vol. 427, pp. 345-351, 2017.

Upscaling of Iron Oxide Nanoparticles for Biomedical Applications – The SEON Concept

C. Alexiou, S. Lyer, C. Janko, M. Pöttler, H. Unterweger, I. Cicha, R. Singh, R. Friedrich, M. Mühlberger, E. Schreiber, J. Band, B. Weigel, R. Tietze

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A broad range of different nanoscaled objects have been developed so far and especially iron oxide nanoparticles have drawn considerable attention due to the wide range of applications. These are found in the fields of mechanical engineering, electronics and above all biomedical technology. Another reason for the popularity of iron oxide nanoparticles is the large number of different synthesis options that allow a wide variety of product configurations. Iron oxide nanoparticles in the field of biomedicine have a convincing application potential in in-vitro diagnostics, imaging, drug delivery and many other areas. This is based on the intrinsic material properties of superparamagnetism, which allows the relatively free configuration of the surface. In our group we work intensively on new methods and applications of these materials. In recent years, for example, particle systems based on iron oxide nanoparticles have been developed for drug delivery of oncological and cardiovascular diseases, regenerative medicine and imaging [1]. However, clinical translation would always remain unsurpassed if no further development could take place after basic research. This means first and foremost the production in larger volumes and under quality controlled conditions. This involves small-scale parameterization, the implementation of adequate site analysis and extensive, intensive and lengthy work on process optimization. Without these, however, the products cannot be manufactured under GMP conditions, which, together with successful regulatory toxicity studies, is a prerequisite for entering into clinical trials.

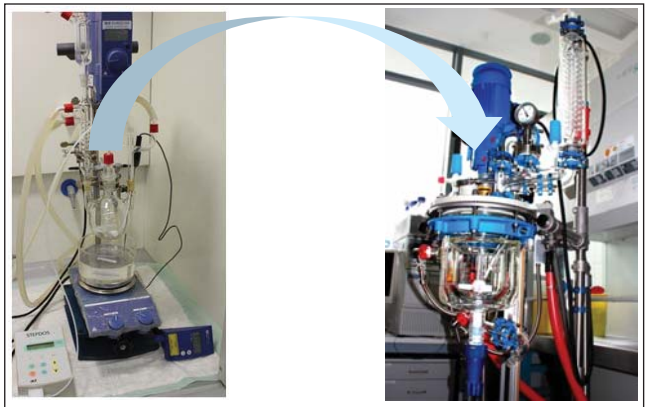

Fig. 1: Transfer of the lab synthesis (left picture) to the quality controlled clean room facility operating a 2 liter pilot plant (right picture).

In our group we operate a manufacturing unit (Fig. 1) in cooperation with the pharmacy of the Universitätsklinikum Erlangen under the regulatory conditions necessary to achieve the goal of GMP compliant product quality for iron oxide nanoparticles.

[1] Lyer S, Tietze R, Unterweger H, Zaloga J, Singh R, Matuszak J, Pöttler M, Friedrich RP, Dürr S, Cicha I, Janko C, Alexiou C. : Nanomedical Innovation: The SEON-concept for an Improved Cancer Therapy with Magnetic Nanoparticles. Nanomedicine (Lond) 10: 3287-304, 2015.

Acknowledgement: DFG-AL 552; Cluster of Excellence Engineering of Advanced Materials; Margarete Ammon Stiftung, München; Manfred-Roth-Stiftung, Fürth.

A parallel, multi-phase, multi-physics numerical simulation code for development of magnetic fluid control strategies

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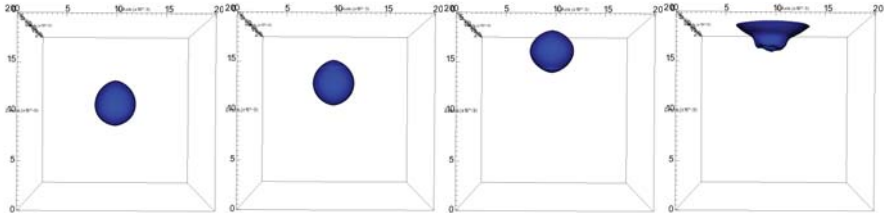
Strategies for directing magnetic nano-particle laden fluid *in vivo* are costly and complex to develop using animal models or clinical trials. This hampers the finding of optimal strategies for contrast agents, magnetic drug targeting or hyperthermia. An efficient simulation platform that is flexible enough to adapt to evolving needs is sought. We are developing a robust, easily-extendable, parallel code that is both state-of-the art algorithmically and efficient. Our first design goal is to apply realistic external magnetic fields to assist in optimizing control of magnetizable fluid volumes.

Our code is based on the Parallel Robust Interface Simulator (Paris), a parallel finite volume code that can solve immiscible multi-phase flows and be readily adapted for multi-physics problems. Paris uses a Volume-of-Fluid (VOF) approach for interface advection and a height function method for interfacial curvature and normal vector computations. With the VOF approach, single-fluid Navier-Stokes equations are solved by treating the two fluids as a single fluid with a jump in material properties across the interface. In a multi-fluid system with a magnetic and a nonmagnetic fluid, magnetic forces can be localized onto the fluid-fluid interface as a result of the jump in magnetic permeability. This allows us to implement the magnetophoretic force as an interfacial force akin to surface tension acting on the droplet. This interfacial approach, in conjunction with the VOF method, avoids the mass and momentum conservation issues that are prevalent in the advection-diffusion approaches.

We present simulation results that demonstrate the flexibility and efficiency of Map-Paris; for a super-paramagnetic ferrofluid droplet suspended in water we impose (a) a uniform vertical magnetic field; (b) a vertical magnetic field with a uniform vertical gradient; and (c) the 3-dimensional magnetic field that results from the arbitrary placement of two or more electromagnets (coils), whose current and geometric properties may also be selected. Code validation is performed based on uniform magnetic fields of 1.25×10^5 A/m, 2.5×10^5 A/m, and 5.0×10^5 A/m. These show excellent convergence properties at only 128^3 spatial resolution. Tests for linear uniform magnetic field gradients of 6.27 mT/mm, 12.54 mT/mm, and 25.08 mT/mm show magnetophoretic attraction which, in the presence of a wall, will allow a magnetic fluid volume to be held in place. In a companion abstract (this meeting) we show our experimental studies of such a magnetic fluid arrangement, but in a pulsatile flow through a rigid cylindrical tube. Finally, we demonstrate the ability of the code to explore the influence of more complex magnetic fields with and without the presence of flow in the surrounding environment.

Our simulations results show convergence for resolutions upwards of 128^3 computational nodes. To examine the efficiency of these codes, a higher resolution (256^3) simulation was run on three different hardware configurations, with the following runtimes: (i) a typical laptop (∼1 day); (ii) a typical desktop workstation (∼4 hours); (iii) a 96 CPU cluster (∼1 hour).



Simulation of a 2.5 mm radius magnetic fluid droplet ($\rho = 1000\ kg/m^3$, $\chi = 4$) in a vertical field with vertical gradient $\sim 0.002\ T/m$ at $t = 0.03, 0.06, 0.09, 0.105$ seconds.

Heparin Coated Magnetic Nanoparticles for Treatment of In-Stent Restenosis and Prevention of Late Stent Thrombosis

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Neointimal hyperplasia is a condition which develops after a vascular stent has been implanted in a patient. Uncontrolled proliferation of vascular smooth muscle cells causes buildup of vascular tissue leading to the internal arterial wall thickening effectively reversing the effectiveness of the arterial stent. Modern treatment of this uses drug eluting stents which although lead to decreased stent failure rate, cause all cell lines associated with vascular healing to stop proliferation, effectively halting or delaying the healing process. This can lead to late stent thrombosis which is not seen when using traditional stents.

To address this, we have developed heparin loaded magnetite nanoparticles as a targeted treatment for bare metal magnetizable stents. Heparin has been shown to selectively inhibit vascular smooth muscle cells and has been used in treatment for decades. Although ideal, pure heparin must be systemically injected in mass quantity as it is removed quickly from the body. Heparin loaded onto iron oxide nanoparticles offset the dose and targeting issue associated with pure heparin treatment.

In this work we show that magnetic nanoparticles loaded with heparin exhibit the proliferative activity of bulk heparin at concentration as low as 1µg of Fe/ml. Using florescence staining we demonstrate that the suppression of vascular smooth muscle cells is due to a change in phenotype from synthetic to contractile. Initial *in vivo* dosing is well tolerated, shows long biological half-life and confirms circulation.

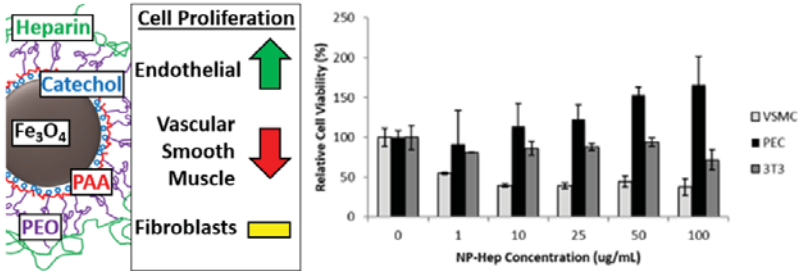

Figure 1: (left) graphical representation of the nanoparticle composition as well as the cellular response to the heparin functionalized particles (right) MTS assay of 3 cell lines commonly associated with neointimal hyperplasia incubated with A) heparin coated nanoparticles

Fellows, B.D., Ghobrial, N., Mappus, E., Hargett, A., Bolding, M., Dean, D. and Mefford, O.T., 2018. *Nanomedicine: Nanotechnology, Biology and Medicine.*

Magnetically-enabled rapid intra-nasal drug delivery to the brain

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The blood-brain barrier provides natural protection to the central nervous system against noxious substances but also prevents access to therapeutics. The nose is the only place where the brain 'meets the outside world'. The olfactory nerve extends directly from the olfactory bulb in the limbic region of the brain to the nose, penetrate the mucosal lining and allow direct contact with the environment [1]. Thus, the nose is considered an attractive route for needle-free vaccination and for systemic drug delivery, especially when rapid absorption and effect are desired.
The goal of this research was delivery of magnetic rods from the nose across the cribriform plate to the brain by aid of magnetic field. The cribriform plate is a part of the ethmoid bone and supports the olfactory bulb. In human, the size of cavities in cribriform plate is a few millimeters which is reduced to millimeter or less by aging [2]. In mice, the size of cribriform cavities are a few hundred microns [3].
Our company has developed methods of propelling drug-loaded magnetic particles through living tissues in vivo. Examples have included the delivery of cancer-fighting drugs into tumors [4] and delivery of magnetic nanoparticles into the dense articular cartilage with 10-nm-wide pores [5].
Nasal delivery experiments were performed by using multi-segmented nano rods of iron (Au-Fe-Au and Au-Fe-Au-Fe-Au rods). The diameter of rods is 250 nm and their lengths are 1-2 microns. Two sets of totally 4 Helmholtz coils were used to apply wiggling magnetic field to pull and rotate the rods. NdFeB magnet was used to provide a static magnetic field of 0.8 T. The rods were administered intra-nasally in mouse cadavers and delivered to the brain by applying both static and wiggling magnetic fields. After the magnetic experiment was done, the whole brain was dissected and observed under optical microscope. The Prussian-blue staining kit was used to stain the rods. Nasal magnetic delivery was investigated at various settings of magnetic field (i.e. frequencies of 100-500 Hz and currents of 1-11 amps), time durations (20-40 minutes), and different magnetic rods loadings to either pull, rotate or pull and rotate the rods. Pulling and Rotating the rods simultaneously resulted in a nearly 100 times increase in delivery of magnetic rods to the brain as compared with no magnetic field and only-pulling conditions. The detailed results of nasal delivery and a low-cost compact interventional MRI system that can image, concentrate, and propel the drug-loaded particles through the nose to the cribriform plate and the brain will be discussed further during the presentation.

[1] Djupesland PG *et al.*. "The nasal approach to delivering treatment for brain diseases: an anatomic, physiologic, and delivery technology overview". Ther Deliv, vol. 5(6), pp. 709–733,2014.

[2] J. K. Kalmey *et al.*, "Age-related size reduction of foramina in the cribriform plate," *Anat. Rec.*, vol. 251, no. 3, pp. 326–329, Jul. 1998.

[3] Q. Ma *et al.*, "Outflow of cerebrospinal fluid is predominantly through lymphatic vessels and is reduced in aged mice," *Nat. Commun.*, vol. 8, no. 1, p. 1434, Dec. 2017.

[4] L. O. Mair *et al.*, "Single-Hole Microshell Particles for Loading Temozolomide," in *IEEE Conference Proceedings, Spring 2014 Symposium: From Lab to Life: Field Based Applications of MEMS & NEMS*, 2014, pp. 8–10.

[5] S. Jafari *et al.*, "Magnetically targeted delivery through cartilage," *Cit. AIP Adv.*, vol. 8, 2018.

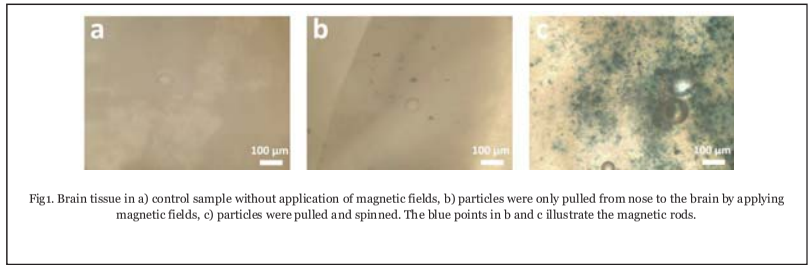

Fig1. Brain tissue in a) control sample without application of magnetic fields, b) particles were only pulled from nose to the brain by applying magnetic fields, c) particles were pulled and spinned. The blue points in b and c illustrate the magnetic rods.

Mag-Guider: A permanent magnet system to guide and image super-paramagnetic nanoparticles

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A new concept of using permanent magnet systems for guiding superparamagnetic nano-particles (SPP) on arbitrary trajectories over a large volume is presented. The same instrument can also be used for magnetic resonance imaging (MRI) using the inherent contrast of the SPP. The basic idea is to use one magnet system which provides a strong, homogeneous, dipolar magnetic field (B_0) to magnetize and orient the particles, and a second constantly graded ($dB/dr = G$) field, superimposed on the first, to generate a force on the oriented particles. As a result, particles are guided with constant force and in a single direction over the entire volume.
A possible realization of this idea is a coaxial arrangement of two Halbach cylinders. A dipole to evenly magnetize and orient the particles, and a quadrupole to generate the magnetic force, F_{mag}, on the SPPs. Dipole and quadrupole can be mechanically rotated relative to each other by an angle α (see Fig. 1a) the force is given by

$$\vec{F}_{mag} = \frac{mG}{\Xi}\begin{pmatrix} G_x + B_0\sin(2\alpha) \\ G_y + B_0\cos(2\alpha) \end{pmatrix} \quad \Xi \equiv \sqrt{B_0^2 + G^2(x^2+y^2) + 2B_0G(x\sin(2\alpha) + y\cos(2\alpha)}$$

where m is the magnetic moment of the SPP.
A simple prototype (cf. Fig. 1b) was constructed to demonstrate the principle in two dimensions on several nano-particles, which were moved along a rough square by manual adjustment of the force angle (see Fig 1c).
The observed velocities of SPPs in this prototype were always several orders of magnitude higher than the theoretically expected value. This discrepancy is attributed to the observed formation of long particle chains as a result of their polarization by the homogeneous field. The magnetic moment of such a chain is then the combination of that of its constituents, while its hydrodynamic radius stays low.

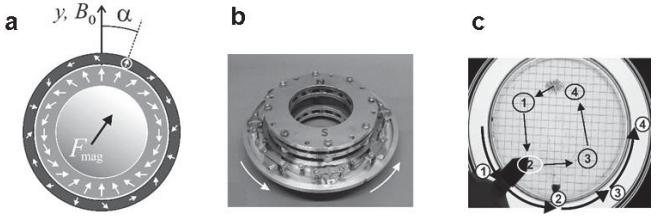

Figure 1: **(a)** Schematic drawing of an ideal Halbach dipole (inner ring in light gray) surrounded by an ideal Halbach quadrupole (darker gay). Since Halbach dipoles have no external (stray)field, the quadrupole can be rotated force free around the dipole by an angel α. The resulting magnetic force points the along 2α. **(b)** Photograph of a simple prototype, made from FeNdB-magnets generating a field B_0=0.1 T and a gradient G=0.2 T/m**. (c)** Some 30 µm size SPP (dark cloud on top) are moved in a rough square by manual rotation of the quadrupole in for steps of α=45°. The particles wander with a speed of ca 5 mm/s to positions marked by the numbered circles.

O. Baun and P. Blümler: "Permanent magnet system to guide superparamagnetic particles", Journal of Magnetism and Magnetic Materials **439** (2017) 294-304.

Fabrication and Characterization of Magnetic Embolising Microspheres with Potential Application in Magnetic Resonance Navigation Technology

Zeynab Nosrati, Tullio V. Esposito, Ning Li, François Michaud, Sahan Ranamukhaarachchi, Stoyan Karagiozov, Gilles Soulez, Sylvain Martel, Katayoun Saatchi, and Urs O. Häfeli*

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Arterial embolization is a minimally invasive treatment for cancerous and non-cancerous diseases including unresectable hepatocellular carcinoma, uterine fibroids and bronchial arteries malignancies. Effective embolization depends on shape, size and delivery method of EMBOLIC AGENTS. Desired size of embolic agents depends the diameter of target vessels and radiographic findings; varying from 40 µm up to 1.2 mm. Targeted delivery of embolic agents improve the therapeutic outcome of embolization by reducing ineffective occlusion of targeted blood supply and undesired necrosis of the surrounding healthy tissues.

Here, we address a high throughput method to produce homogenously large sized magnetic microspheres (MMS) with potential applications in Magnetic Resonance Navigation (MRN) technology. Through MRN, an external magnetic field (similar to clinical MRI scanner) by means of computer programs, effectively magnetize, monitor and navigate MMS to the target location for therapeutic purposes such as drug delivery, chemoembolization and radiation therapy.

A simple 3D printed micro-co-flowing device (Fig.1) was designed to fabricate highly magnetic, biodegradable and biologically compatible MMS consists of poly (lactic-co-glycolic acid) (PLGA) microspheres encasing 50 wt% C_{12}-BP coated magnetic Fe_3O_4 nanoparticles (MNP). This systems is able to control uniformity and size of MMS by adjusting the needle internal diameter as primarily parameter and then the flow rates of the continuous and dispersed phase as secondary parameter.

MMS morphology, mean particle size and size distribution were quantified from SEM images (Fig.2). Magnetic performance of MMS was investigated using a vibrating sample magnetometer (Fig.3). MMS were nontoxic towards HUVEC (human umbilical vein endothelial cells) and HEK293 (human embryonic kidney) cells. The presented co-microflowing method allows for the reliable production of large MMS sized 130-700 µm with narrow size distribution (CV <7%) and magnetic properties useful for MRN. *In vivo* MRN experiments with 250 µm MMS are currently ongoing.

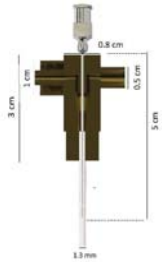

Figure 1. Micro-co-flowing device with inserted exchangeable needle.

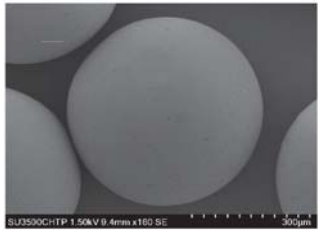

Figure 2. SEM images of MMS encasing 50 wt% MNP

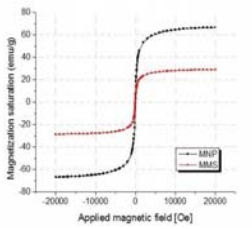

Figure 3. Magnetization curves for C12-BP coated MNPs and 250 µm MMS containing 50 wt % MNPs (red)

Intravenous Delivery of Tissue Plasminogen Activator by Thermosensitive Magnetoliposomes for Target Thrombolysis

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Recombinant tissue-type plasminogen activators (rtPA) may induce hemorrhagic side effects that limit its application for acute treatment of stroke. Target delivery of rtPA may greatly reduce the required dose to achieve pharmacological efficacy and systemic distribution of the drug. Therapeutic efficacy of intra-arterial (*i.a.*), but not intravenous (*i.v.*), delivery of immobilized rtPA on the surface of magnetic nanoparticles (MNPs) as carriers under magnetic guiding has been demonstrated in rats. Nevertheless, *i.v.* administration with magnetic targeting did induce local retention of MNPs that should allow high enough of [rtPA] at the site of thrombus. We asked whether encapsulation of rtPA is required to preserve the enzymatic activity of rtPA from endogenous inhibitors in circulation for effective thrombolysis after *i.v.* delivery. In this study, thermosensitive magneto-liposomes (DPPC:DSPE:CH 90:5:5) with rtPA (TML@rtPA) were prepared, optimized and characterized with good hematological biocompatibility in rats. The elevated release of rtPA with time was achieved at 43°C *vs.* 37°C *in vitro*, whereas application of magnetic field attenuated rtPA release from TML@rtPA. With thromboelastometry, enhanced thrombolysis was also demonstrated by incubation of TML@rtPA (rtPA 1 µg/mL) in whole blood at 43°C *vs.* 37°C, which was associated with reduced clotting time at 43°C, but not 37°C. A novel rat embolic model with a thermo-controlled system was established with high thermal resolution by superfusion of 43°C *vs.* 37°C saline dripping on the left iliac artery with clot lodging. TML@rtPA (*i.a.*; rtPA 0.2 mg/kg) under magnetic guiding with focal hyperthermia of 43°C induced restore of iliac blood flow (IBF) by up to 79% of basal flow 15 min after *i.a.* administration of TML@rtPA, as measured by ultrasonic flowmetry. TML@rtPA administered *i.v.* at 43°C induced significant IBF restore to 62% of the basal level 55 min after drug delivery, which appeared to be much slower than that induced by *i.a.* administration. In contrast, *i.a.* or *i.v.* delivery of TML@rtPA at 37°C did not increase IBF throughout the observation time. The results demonstrated that TML@rtPA under a mobile magnetic field effectively induced target thrombolysis with 20% of regular dose in a novel rat embolic model using a magneto- and thermo-controlled system. To our knowledge, this is the first demonstration of effective and reproducible thrombolysis induced by *i.v.* administered nanocomposite with magnetic targeting.

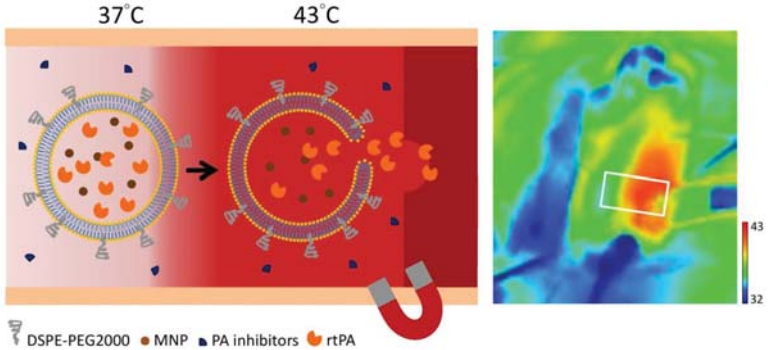

Figure With focal hyperthermia induction and magnetic manipulation of rtPA release, PEGylated thermosensitive magnetoliposomes preserve rtPA activity and serve as carriers for intravenous delivery of rtPA to achieve target thrombolysis in a rat model. A thermal image on the right presents an increased temperature in the focal area of the left iliac artery of a rat. The white contour represents sharp temperature gradient in this model, allowing thermosensitive release of the content in the target site.

Invited Talk

Integrated Magnetophoretic Platform for Precise Manipulation of Living Cells

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The integration of a remotely controllable particles/cells manipulation in a lab on a chip systems promises to play a key role towards the advancement in gene sequencing, single cell analysis and cell separation technology. Particularly, most existing single cell platforms are unable to achieve large scale operation with flexibility on cells and digital manipulation, and thus there is urgent need of innovative techniques to accomplish the automation of single cell manipulation. Recently, the flexibility of magnetic shuttling technology using nano/micro scale magnets for the manipulation of particles has gained significant advances and has been used for a wide variety of single cells manipulation tasks. Here, we have developed a class of integrated magnetic track circuits designed by conventional lift-off technology for executing sequential and parallel, timed operations on an ensemble of single cells for multiplexed analysis [1].

As for the effective collection and monitoring of very low density cells, like as CTC (circulating tumor cell), the assembly of this magnetic tracks into a novel architecture, resembled with spider web network consisted of several radii and spirals was developed, as seen in Figure 1. A planar Hall resistance sensor was integrated at the center of the web networks, and the manipulation and monitoring are achieved *via* superparamagnetic particles with dual functions as a biomolecule carrier for transportation and labels for monitoring [2]. Here, I will include the micro-magnetic force and energy for the magnetic carriers digital cells on chips [3,4], of which novel platform could possibly open a new biological assay system overcomes collection barrier for very low density target cells, and allows the in-vitro rare cells analysis in individual cell levels.

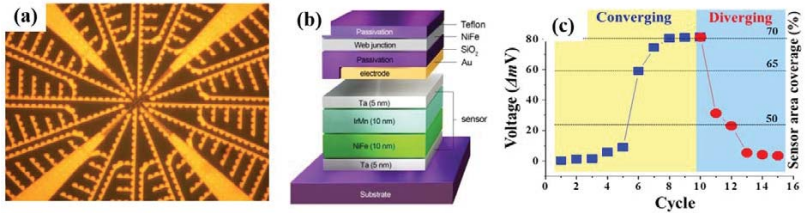

Figure 1. (a) Photo for spintrophoretic spider web, (b) Cross sectional view of sensor, (c) signal variation [2].

References:

[1] B. Lim, et al, *Nature Communications*, 5, 1(2014)

[2] B. Lim, et al, *NPG Asia Materials* (2017) doi:10.1038/am.2017.37

[3] X. Hu et al., Lab Chip., (2016), doi:10.1039/c6lc0066c

[4] B. Lim et al, J. Phys. D: Appl. Phys., 50 (2017) 033002

High-Throughput Single-Nanoparticle Magnetic Analysis Platform Using Diamond Magnetic Imaging

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Magnetic nanoparticles (MNP) are widely-used tools used across a range of industries, with particularly powerful biomedical applications for clinical and research diagnostics, clinical therapy, and basic life science research. Many of these applications require consistent sources for magnetic nanoparticles with narrow distributions of magnetic properties, but no technology is now commercially available for manufacturers or users to quantify single-particle magnetic properties with sufficient throughput to provide efficient and cost-effective quality control.

We have developed a high-throughput MNP analysis platform using magnetic imaging with nitrogen-vacancy (NV) centers in diamond. This general-purpose technology provides sensitive, wide-field images of the magnetic field at the surface of a synthetic diamond sensor. Thousands of MNP may be imaged simultaneously in seconds or minutes without the need for high vacuum, cryogenic temperatures, or other large and expensive apparatus. The particles may be imaged repeatedly while varying an external magnetization field in an automated sequence to construct detailed histograms of single-particle magnetization curve data, such as magnetic susceptibility and magnetic remanence. The broad capabilities of diamond NV centers also allow for AC response and full-vector magnetometry to be integrated in the future.

Rapid image acquisition, a compact footprint, and low-cost components will enable this technology platform to address unmet needs of throughput, efficiency, and cost-effectiveness for industrial quality control of magnetic nanoparticle use and manufacture.

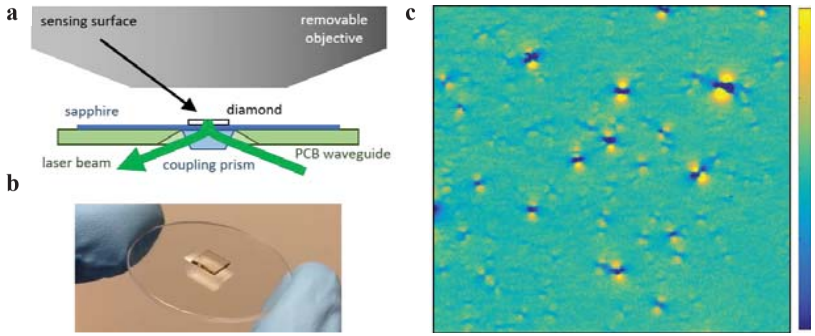

Figure. **(a)** Partial schematic of the diamond magnetic imaging apparatus; **(b)** optical assembly containing the diamond imaging sensor before installation in the instrument; **(c)** example magnetic image of chromium dioxide MNP after magnetization at 200 kA/m in the vertical direction. Some objects in the image are aggregates of many MNP. Field of view is 60 µm. Color scale is ±10 µT.

Development of an Optical Pumped Gradiometric System to Detect Magnetic Relaxation of Magnetic Nanoparticles

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The investigation of magnetic nanoparticles (MNPs) for medical and biological applications is relatively recent and is steadily growing. MNPs can be produced in different shapes, sizes and functionalization endowing them with unique physical and biological properties. When properly functionalized, they can target cancer cells and deliver a drug or a physical action to these cells. MNPs are being investigated in several applications in medicine such as hyperthermia, magnetic particle imaging (MPI), cell separation and magnetofection, *in vitro* and *in vivo* alternating current biosusceptibility, T1 magnetic resonance contrast agent of tissues and magnetorelaxometry [1-3]. In each of these applications, a specific physical property is measured. Magnetorelaxometry relies on the fact that when MNPs are magnetized they can relax by the Brownian and Néel mechanisms. Brownian relaxation involves the physical rotation of the entire nanoparticle relative to the fluid medium, whereas Néel relaxation occurs due to thermal fluctuations of the direction of the magnetic moment relative to the crystal orientation. Both mechanisms depend on the MNP size and for certain conditions one can have a fast relaxation through Brownian compared to Neel mechanism. Thus, this fact can be explored to target cells. When a MNP is free to rotate the biological fluids, they will relax faster than when attached to a cell. This can produce a high contrast to detect magnetic labelled cancer cells making it possible to differentiate normal from cancer tissue. Until very recently SQUIDs were the main detector employed to measure MNPs [4], but Optically Pumped Magnetometer (OPM) appears now as a motivating alternative. OPMs are small, don't need liquid helium and are simpler to operate than SQUIDs. Here, we present the initial steps of the development of an OPM based instrument to measure relaxation of MNP in vitro. The magnetometers were arranged in a gradiometric configuration. MNPs must be magnetized and its magnetization be measured as a function of time with the most sensitive magnetometer to obtain high sensitivity. The diagram shows the main parts of our system a vector zero-field optically pumped magnetometers OPM (QuSpin Inc., Louisville, Colorado, USA)), a Zero Gauss Chambers with three layers of MuMETAL ZG-206 (Magnetic Shield Corporation, Bensenville, IL USA).

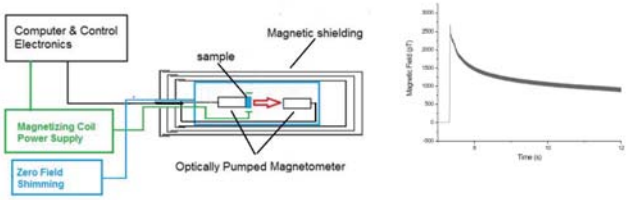

Figure 1: Schematics of the experimental set up (right) and a typical relaxation signal (left).

Acknowledgements: Partial financial support: FAPESP Grant 2016-0232-0, CNPq and CAPES

References:

1- **Kötitz, R., et al.,** Journal of Magnetism and Magnetic Materials, 1999. 201(1): p. 102-104.
2- **Heim, E., et al.**, Journal of Magnetism and Magnetic Materials, 2009. 321(10):1628-1631
3- **De Haro, L.P., et al.**, Biomedical Engineering/Biomedizinische Technik, 2015. 60(5): p. 445-455.
4- **Hincapie Ladino, E.A. et al.,** Supercond. Sci. Technol. 30 (2017) 084007

Measured correlation of nanoparticle magnetic moment and hydrodynamic size

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The hydrodynamic size, V_h, and remanent magnetic moment, m, are of key importance for the properties of colloidal magnetic nanoparticle (MNP) dispersions. Often these parameters are treated independently although it known that they are often correlated. Here, we present a method to determine the distributions of m and V_h as well as their correlation from dynamic magnetic measurements vs. frequency, f, *and* magnetic field strength, H.

We consider AC susceptibility (ACS) or optomagnetic (OM) measurements. ACS measures the magnetic response to an alternating magnetic field of frequency f and amplitude H, whereas the OM technique relies on measurements of the field-induced modulation of the optical transmission signal for MNPs with linked magnetic and optical anisotropies.[1] Using either of these techniques, the distributions of V_h or m can be estimated from low-field measurements vs. frequency or from low-frequency measurements vs. field,[1] but the correlation between V_h and m cannot be obtained. In the method presented here, the complete set of OM and/or ACS measurements are analysed in terms of a bivariate lognormal distribution of m and Brownian relaxation frequencies $f_B = k_B T/(6\pi\eta V_h)$, where η is the viscosity. The bivariate distribution accounts for the distributions of m and V_h and it assumes a power law relation between m and V_h.[2]

Fig. 1a shows ACS and OM measurements on Micromod BNF 80 MNPs vs. f and H. The lines are the fit to the above model with the bivariate distribution shown in Fig. 1b with the correlation $m \propto V_h^{0.5}$.[2] This square-root dependence is characteristic for multi-core particles.[3] As a novel feature, we show that the use of higher harmonics in the analysis extends the measurement window and allows for the robust determination of values of f_B larger than the maximum value of f.

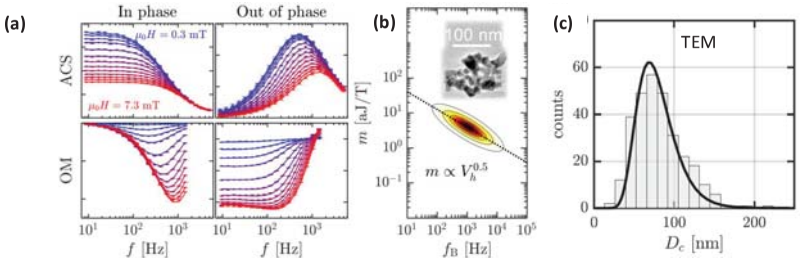

Fig 1(a) ACS and OM measurements (points) vs. H and f on BNF 80 particles. The solid lines are the fit obtained from simultaneous analysis of all data to a bivariate distribution of Brownian relaxation frequencies and magnetic moments. Colours from blue to red correspond to increasing field amplitudes. **(b)** Resulting bivariate distribution function. The inset shows a transmission electron microscopy (TEM) image of a particle. **(c)** Size histogram obtained from TEM. The line is the number-weighted size distribution from **(b)**. Figures were adapted from Fock *et al.*[2]

(1) Fock, J.; Jonasson, C.; Johansson, C.; Hansen, M. F. *Phys. Chem. Chem. Phys.* **2017**, *19* (13), 8802–8814.
(2) Fock, J.; Balceris, C.; Costo, R.; Zeng, L.; Ludwig, F.; Hansen, M. F. *Nanoscale* **2018**, *10* (4), 2052–2066.
(3) Ahrentorp, F.; Astalan, A.; Blomgren, J.; Jonasson, C.; Wetterskog, E.; Svedlindh, P.; Lak, A.; Ludwig, F.; van IJzendoorn, L. J.; Westphal, F.; Grüttner, C.; Gehrke, N.; Gustafsson, S.; Olsson, E.; Johansson, C. *J. Magn. Magn. Mater.* **2015**, *380*, 221–226.

Electron holography studies of individual maghemite nanoflowers

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Flower-shaped multicore nanoparticles – "nanoflowers" (NFs) – of magnetite/maghemite (Fe_3O_4/γ-Fe_2O_3) have in recent studies demonstrated potential to be effective heat agents for magnetic hyperthermia applications. For example, some iron oxide NFs have shown specific power losses, which are an order of magnitude larger than that of single-core particles with same core size [1,2]. The origin of enhanced power dissipation in NFs is not yet fully understood, neither is the underlying magnetic structure of the NFs.

To address the magnetic structure of individual maghemite NFs (Fig. 1a) we applied electron holography, a transmission electron microscopy (TEM) based technique capable of imaging magnetics quantitatively at the nm scale. The phase image obtained from an electron hologram contains information on the magnetic field in the sample plane. We have studied different regions of NFs deposited on a TEM grid, incl. both closely-spaced NFs and more isolated NFs. Fig. 1b shows an electron hologram with a spatial resolution of 10 nm. The field map of the NF in Fig. 1c indicates a quasi-uniform single-domain state. For isolated NFs (with limited influence of dipolar interaction from other NFs), we extracted the magnetic field of the NFs at remanence. Based on the obtained magnetic moment per NF [3] and size of the NFs, we estimate the mean magnetization per NF. We compare this to the saturation magnetization of γ-Fe_2O_3 and use this to evaluate the uniformity of the magnetization within individual NFs. We discuss our results in relation to the cooperative ordering behavior described e.g. in [2].

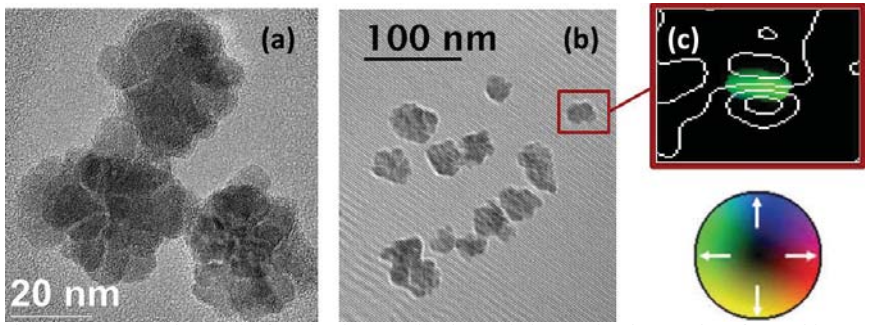

Figure 1 - (a) TEM micrograph of maghemite NFs. (b) Electron hologram showing a region of NFs with different sizes and separations. Interference fringes are seen along the diagonal. (c) Magnetic field map of an isolated NF boxed in b. The white lines represent magnetic contour lines, and the color indicates the magnetization direction according to the color wheel.

1. P. Hugounenq et al. *J. Phys. Chem C* **116**, (2012) 15702
2. L. Lartigue et al. *ACSnano* **6**, (2012) 10935
3. M. Beleggia et al. *Ultramicroscopy* **110**, (2010) 425

Smart Materials Based on Magnetic Nanoparticles for Biosensing and Drug Delivery

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Theranostics, a fusion of two key parts of medicine – diagnostics and therapy of the organism's disorders, promises to bring the efficacy of medical treatment to a fundamentally new level and to become the basis of personalized medicine. Ability to combine multiple functionalities in a single agent and react to external stimuli makes smart nanomaterials the perfect agents for theranostics. Here, we will describe several novel approaches for fabrication of smart materials for biosensing and drug delivery.

The first approach is based on our recently proposed method of fabrication of biocomputing materials that can be programmed to simultaneously analyze the levels of multiple molecular inputs (of virtually any nature) according to the rules of Boolean logic, and implement a variety of output actions based on the biocomputation results [1]. We have developed a multimodal magnetic and optical smart nanoagent (Fig. 1), which is capable of sensing a small-molecule marker and i) switching its affinity to a biomedical target as response to high concentrations of the marker, and ii) reporting the marker's concentration in real-time via changing its optical surface plasmon resonance properties [2].

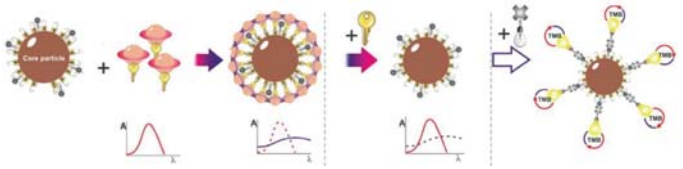

Fig. 1. Design and operation of the magnetic/optical smart nanoagent with input-controllable optical properties and switchable affinity to a target.

Next, we developed novel superparamagnetic nanoparticles with a magnetite core in a porous metal organic framework shell (Fig. 2). These nanoparticles with stimuli-responsive porous shell can transport high amounts of molecular payload and release it via controlled biodegradation under the influence of phosphate ions. The nanoagents are attractive as theranostic agents combining both the diagnostics probe and drug delivery vehicle properties. We demonstrated their application both as a multimodal (MRI contrasting, magnetometric and optical labeling) and multifunctional (in vivo bioimaging, biotargeting by coupled receptors, lateral flow assay) agents.

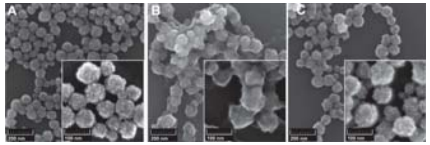

Fig. 2. SEM images: A – citrate capped Fe_3O_4;
B – hybrid Fe_3O_4@MIL-100(Fe) NP;
C – Fe_3O_4@MIL-100(Fe) NP after 1 week in PBS.

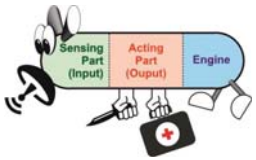

Fig.3. Conceptual architecture of a nanorobot.

The ease of fabrication, controllable bioconjugation properties and low level of non-specific binding indicate high potential of the nanoparticles to be employed as multifunctional agents in biomedical research, advanced biosensing and for development of the next-generation smart materials – nanorobots (Fig. 3) [3].

[1]. M.P. Nikitin, et al., Nature Nanotechnol., 9 (2014), 716–722.
[2]. K.G. Shevchenko, et al., Biosens. Bioelectron., 88 (2017), 3–8.
[3]. I.L. Sokolov, et al., Biochim. Biophys. Acta, 1861 (2017), 1530–1544.

Protein coated magnetic nanoparticles for cancer treatment and diagnostics

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Magnetic nanoparticles (MNP) are in the field of great interest in two past decades. MNP can be used as an effective MRI contrast agents, drug delivery systems and effective formulations for magnetic hyperthermia. Such variety of application in each particular case can be reached only by specific chemical design of magnetic core structure and surface coating. Thus optimization of such parameters allows using these nanoparticles not only in separate application, but also in combined modalities.

For combined drug delivery and MRI imaging we have developed complex system based on iron oxide nanocrystals, coated with human serum albumin (HSA-MNP) with following crosslinking with formation of stable biocompatible shell. Physicochemical properties of HSA-MNP were investigated in details by HAADF-TEM, DLS, AFM, also magnetization and T2 relaxation properties were investigated. HSA serves as a natural transport protein for xenobiotics in blood and can effectively bind drug molecules to surface. Our experiments have shown that HSA-MNP were able to bind doxorubucin, cisplatin and bacteriochlorine *a* molecules, effectively delivery this drugs to tumor cells and tissue. Particularly for doxorubicin loaded nanoparticles we have shown effective imaging of 4T1 mouse breast cancer model accompanied with increase of median survival from 26 to 39 days.

Bacteriochlorine *a* is a heteromacrocyclic organic molecule which can act as photosensitizer (PS). Under light irradiation PS molecules promotes formation of reactive oxygen species (ROS) which damage cell compartments and lead to cell death. However low solubility in water restricts application of these molecules without further modification. To overcome this disadvantage we have developed a method for loading of PS on HSA-MNP surface. PS loaded HSA-MNP has shown similar photoinduced cytotoxicity in comparison with free drug and were stable in water solution for few weeks. Moreover *in vivo* experiments with mice bearing tumors have shown that after *i.v.* injection of PS loaded HSA-MNP we were able to detect PS delivery to tumor by both MRI and *in vivo* fluorescence.

This results allow to propose HSA coated MNP as a perspective tool for drug delivery of different antitumor drugs for cancer treatment.

The study was supported by the Russian Science Foundation (grant No. 17-74- 10169) and NUST MISiS (K2-2018-008).

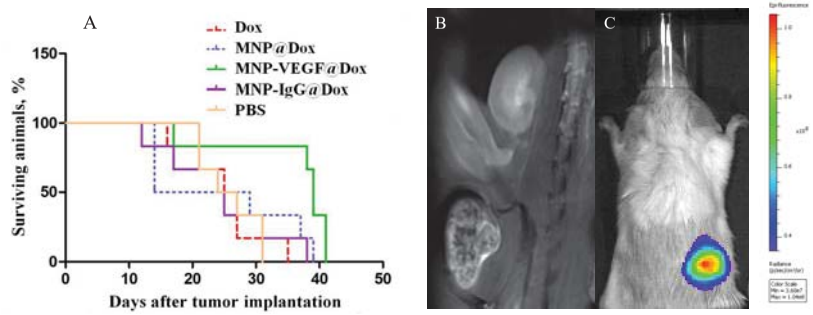

Fig. 1. Survival curves of animals bearing experimental 4T1 tumors treated with Dox, PBS and different types of Dox-loaded magnetic nanoparticles (A). Representative MRI (B) and fluorescent imaging (C) of 4T1 tumor bearing mice 6h after i.v. injection of PS loaded HSA-MNP.

"Clickable" magnetic nanoparticles – a new tool for magnetic hyperthermia

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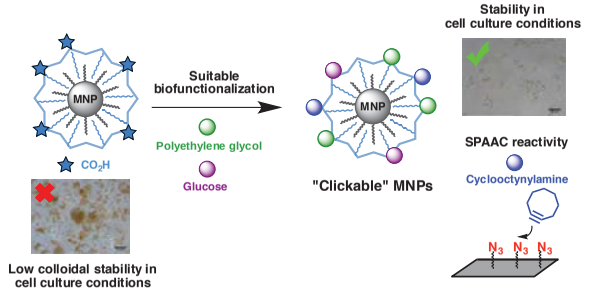
Magnetic hyperthermia (MH), based on the ability of magnetic nanoparticles (MNPs) to generate heat when exposed to alternating magnetic fields (AMF) is a very active area of research, especially for the development of new therapeutic solutions for cancer treatment.[1,2] Recently, our team has initiated a new research line focused on the use of bioorthogonal click chemistry for magnetic hyperthermia studies applied using MNPs covalently attached to cell membranes. Our interest lies in the sub-lethal version of MH, which we believe is a powerful tool to induce a controlled and localized heating of the cell membrane ("hotspots"). This could produce temporal changes in the membrane biophysical properties, which can be used for enhanced delivery of therapeutics.

Bioorthogonal strain-promoted "click" [3 + 2] azide-alkyne cycloaddition (SPAAC) reaction uses the ring strain to activate the alkyne, thus avoiding the use of the cytotoxic Cu(I) catalyst typically employed for standard "click" azide-alkyne cycloadditions (CuAAC).[3] Despite its numerous applications in biology and biochemistry, SPAAC is still relatively new for nanotechnology applications. Herein, we report a simple functionalization protocol to obtain water-soluble MNPs suitable for SPAAC click chemistry.[4] Hydrophobic 12 nm iron oxide MNPs were synthesized following a seed-mediated thermal decomposition methodology and transferred to water by coating with a fluorescent amphiphilic polymer (poly(maleic anhydride-alt-1-octadecene), PMAO-TAMRA).[5] The MNPs were further functionalized step-wise with polyethyleneglycol (PEG) or a glucopyranoside derivative (Glc) to increase colloidal stability and with a cyclooctynylamine derivative. We have tested their reactivity towards azide-functionalized surfaces, demonstrating their potential as bioorthogonal probes. We are currently working on the incorporation of the "clickable" MNPs on more complex substrates (azide-modified lipid bilayers as simplified models of animal cell membranes). Studies regarding the cytotoxicity of the MNPs and their covalent attachment on living cell membranes are also underway.[6]



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References: (1) Q. A. Pankhurst, N. T. K. Thanh, S. K. Jones, J. Dobson, *J. Phys. D – Appl. Phys.*, 42, (2009), 224001. (2) A. Hervault, N. T. K. Thanh, *Nanoscale*, 6, (2014), 11553-11573. (3) J. C. Jewet, C. R. Bertozzi, *Chem. Soc. Rev.*, 2010, *39*, 1272. (4) R. M. Fratila, M. Navascuez, J. Idiago-López, M. Eceiza, J. I. Miranda, J. M. Aizpurua, J. M. de la Fuente, *New J. Chem.*, 2017, *41*, 10835. (5). M. Moros, B. Pelaz, P. Lopez-Larrubia, M. L. García-Martin, V. Grazú, J. M. de la Fuente, *Nanoscale*, 2010, *2*, 1746. (6) J. Idiago-López *et al.*, in preparation.

Magnetic gelfiber based scaffold for theranostic applications

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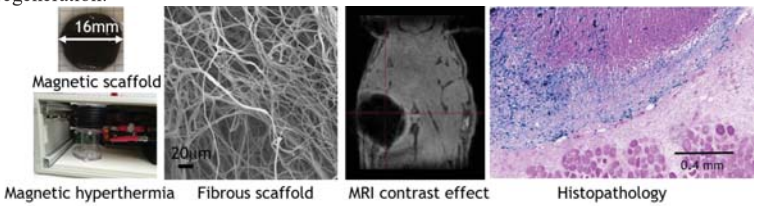
Various polymers are used in medicine for a great variety of applications. With the help of electrospinning technology artificial matrices can be created with a fibre diameter like in the living organism. The two crucial conditions of biomedical usage are biocompatibility and biodegradability. Superparamagnetic nanoparticles can heat the surrounding environment if an alternating magnetic field is applied. Since cancer cells are more sensitive to the temperature increase than healthy cell lines, magnetic hyperthermia is a promising therapeutic method in cancer healing. However, the Magnetic Resonance Imaging (MRI) is also using magnetic nanoparticle to increase the contrast effect between the different tissues. The resulting fibrous mesh amalgamates the unique properties of magnetism and elasticity.

Thus aim of our research is creating and investigating artificial matrices from biocompatible and biodegradable polymers which contain magnetic nanoparticles as a therapeutic and diagnostic agent.

Poly(aspartic acid) could be a good candidate for medical application, which can be created from polysuccinimide by alkaline hydrolyzation. With the help of electrospinning, we created artificial matrixes with a fibre diameter in the nanometer range, loaded with magnetite particles. The morphology of the artificial matrices was examined with Scanning Electron Microscopy and the chemical components with Fourier Transformation Infrared Spectroscopy. The exact iron content was determined. The magnetic hyperthermic and the magnetic resonance contrast enhancing effect was determined with Magnetherm 1.5 and nanoScan PET/MRI (1T) instruments, respectively. Since the particles are superparamagnetic, in an alternating magnetic field the entrapped particles induced an extremely high heat effect in the surrounding area (30°C/5min). The calculated SAR value was higher than 150W6G in case of every samples. For the MRI measurement and the biocompatibility experiments Wistar rats were used and after the termination histopathological study was done. The MRI measurements showed a high contrast enhancement around the implantation area of the membrane after 1 day and 1 week also. The histopathological evaluation showed a normal healing process without any major deviation. Hence in our work magnetic NPs loaded polymer membrane was created, the structure is applicable as a scaffold for tissue engineering or lesion regeneration.



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Invited Talk

Light Robotics and its potential for integrating with magnetic carriers

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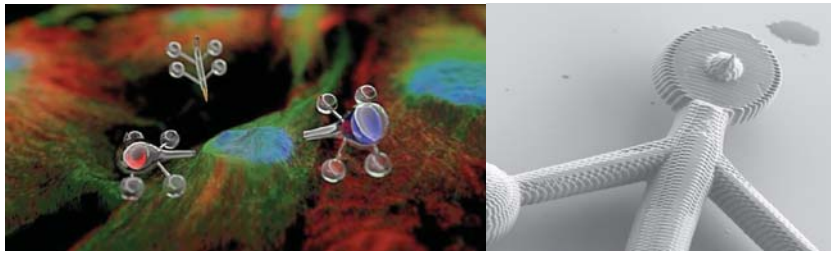
www.PPO.dk www.OptoRobotix.com www.GPCphotonics.com

In our globalized world of R&D there is a constant evolvement of scientific disciplines generating new offspring or sub-disciplines that combine the favorable characteristics from its forerunners. A contemporary example is the merger of biology and photonics producing one such new offspring, Biophotonics, which is harnessing light to study biological materials. Subsequently, we have seen the powerful merger of biophotonics with contemporary nanophotonics into so-called NanoBiophotonics culminating with the 2014 Chemistry Nobel Prize for super-resolution microscopy, now simply coined Nanoscopy. After years of working on light-driven trapping and manipulation, we can see that a confluence of developments is now ripe for the emergence of a new area that can contribute to nanobiophotonics – *Light Robotics* – which combines advances in microfabrication and optical micromanipulation together with intelligent control ideas from robotics, wavefront engineering and computational optics. In the Summer 2017 we published a ca. 500 pages edited Elsevier book volume covering the fundamental aspects needed for Light Robotics including optical trapping systems, microfabrication and microassembly as well as underlying theoretical principles and experimental illustrations for optimizing optical forces and torques for Light Robotics. The Elsevier volume is presenting various new functionalities that are enabled by these new designed light-driven micro-robots in addition to various nano-biophotonics applications demonstrating the unique use of biophysical tools based on light robotic concepts. We have endeavored to make this new discipline accessible to a broad audience from advanced undergraduates and graduate students to practioners and researchers not only in nanobiophotonics and micro- and nanotechnology but also to other areas in optics, mechanical engineering, control and instrumentation engineering and related fields. My talk will try to cast new light on identifying new scientific inspiration on Light Robotics' potential for integrating with magnetic carriers for targeted drug delivery and/or related emerging, interdisciplinary nanobio-applications.



- Glückstad, J., "Sorting particles with light", Nature Materials 3, 9 (2004).
- Papagiakoumou, E., Anselmi, F., Begue, A., Sars, V., Glückstad, J., Isacoff, E., Emiliani, V., Nature Methods 7, 848 (2010).
- Rodrigo, P. , Gammelgaard, L., Bøggild, P., P.-Nielsen, I., Glückstad, J., Opt. Express 13, 6899 (2005)
- Tauro, S., Bañas, A., Palima, D., Glückstad, J., Opt. Express 18, 18217 (2010)
- Glückstad, J., "Sculpting the object", Nature Photonics 5, 7 (2011)
- Glückstad, J. & Palima, D., "Generalized Phase Contrast", Springer Series in Optical Sciences, 315 pages (2009).
- Palima, D., Bañas, A., Vizsnyiczai, G., Kelemen, L., Ormos, P., Glückstad, J.,"Wave-guided Optical Waveguides", Opt. Express 20, 2004 (2012).
- Palima, D., & Glückstad, J.,"Gearing up for optical micro-robotics: synthetic microstructures actuated by optical trapping and optical manipulation", Lasers & Phot. Reviews 17, 478 (2013).
- Wu, C., Palima, D, Novitsky, A; Ding, W; Gao, D; Shukovsky, S; and Glückstad, J.,"Engineering light-matter interaction for emerging optical manipulation applications", Nanophotonics 3, 181 (2014).
- Villangca, M., Bañas, A., Palima, D., and Glückstad, J.,"Dynamic diffraction-limited light-coupling of 3D-maneuvered wave-guided optical waveguides," Opt. Express 22, 17880 (2014).
- Villangca, M., Bañas, A., Palima, D., Glückstad, J., "Generalized phase contrast-enhanced diffractive coupling to light-driven microtools" Opt. Eng. 54, 111308 (2015).
- Villangca, M., Casey, D., Glückstad, J.,"Optically-controlled platforms for single- and sub-cellular transfection and surgery," Biophysical Reviews 7, 379 (2015).
- Villangca, M., Palima, D., Bañas, A., Glückstad, J., "Light-driven micro-tool equipped with a syringe function," Light: Science & Applications, Nature Publ. Group, 5 (9) e16148 (2016).
- A. Bañas and J. Glückstad, "Holo-GPC: Holographic Generalized Phase Contract," Opt. Comm., 392, 190-195 (2017).
- Glückstad, J. & Palima, D., "Light Robotics: structure-mediated nanobiophotonics", Elsevier Science, 482 pages (2017).

Magnetic Microlassos For Single Cell Capture, Manipulation And Cargo Transport

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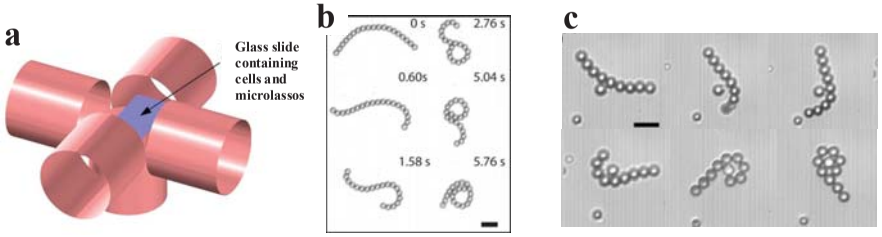
For single cell studies, various cell manipulation technologies have been developed. These methods can be divided into two main categories, contact and non-contact manipulation[1-3]. Several non-contact manipulation methods exist such as optical, ultrasound and electrical field manipulation. These techniques have drawbacks, for instance damaging of the biological samples, lack of precise directional control and shortage in field penetration depth. In this work, we have developed a non-contact cell manipulation method that overcomes all the aforementioned drawbacks. Here we created flexible magnetic microparticle chains that form closed rings or "lassos" in the presence of a programmed external rotating magnetic field. These magnetic rings can be used to grab onto a cell then translate based on a wheel-type mechanism, all without the need for chemical attachment or disengagement. We have shown that magnetic microlassos can translate at velocities up to 20µm/s with precise directional control achieved automatically or manually using a joystick control. In addition, we have demonstrated that once the target destination is reached, captured cell can be easily released upon field removal leaving the magnetic microlasso ready for picking up and manipulating another cell. We believe that with the precise directional control and reversibility properties, magnetic microlassos have great potential in manipulation and cargo transport of cells and biomolecules.



a) External magnetic field applicator setup consisting of 5 air cored solenoids.

b) Video frames show the deformation of a flexible magnetic chain (composed of 4.5um magnetic particles) into a lasso loop under a 2D rotating magnetic field (Scale bar: 12.5µm).

c) Cargo capture with a magnetic microlasso. Video frames show the capture of a white blood cell with a magnetic microlasso controlled with 2D and 3D rotating magnetic fields. (Scale bar: 12.5µm).

d) Cargo carrying magnetic microlassos can be precisely manipulated via joystick control. Figure is created from a movie where the user moves the loaded microlasso in an 'M' trajectory. (Scale bar: 12.5µm).

References: 1. Roman GT, Chen YL, Viberg P, Culbertson AH, Culbertson CT. Single cell manipulation and analysis using microfluidic devices. Anal Bioanal Chem 2007;387:9-12. **2.** Desai JP, Pillarisetti A, Brooks AD. Engineering approaches to biomanipulation. Annu Rev Biomed Eng 2007;9:35-53. **3.** Castillo J, Dimaki M, Svendsen WE. Manipulation of biological samples using micro and nano techniques. Integr Biol 2009;1:30-42.

3D-printing of novel magnetic composites based on magnetic nanoparticles and photopolymers

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Magnetic nanoparticles (MNP) are of great interest in bio- and nanomedicine as they offer numerous promising therapeutic and diagnostic methods being intensively researched. A diagnostic method for the visualization of the spatial distribution of MNP, called magnetic particle imaging (MPI), has recently been introduced. MPI offers a means of direct measurement of MNP with zero background signals from diamagnetic tissue. For MPI-research, long-term stable phantoms with defined geometry and MNP content are necessary to test the resolution, for cross-comparison of MPI scanners or as fiducial markers to verify the spatial position of the body under analysis. A fast and cost-effective way to manufacture phantoms is the technique of generative printing, or commonly called 3D-printing. This additive technique allows manufacturing customized parts with complex shapes out of specific photopolymers solidifying layer by layer under ultraviolet radiation. The aim of our work was to test the feasibility of printing magnetic composites which consist of photopolymers with embedded MNP.

To this end, we developed a protocol for the systematic quality evaluation of 3D-printed magnetic composites. Prior to the actual 3D-printing, essential parameters such as magnetic and chemical properties of basic materials, homogenization procedure, mixture ratio, polymer cross-linking, and long-term stability were characterized and optimized to improve the quality of the resultant mixture. To magnetically characterize the liquid and solidified photopolymers with embedded MNP, we measured the quasistatic and dynamic magnetization behavior by means of dc-magnetometry (QD-MPMS XL, Quantum Design) and Magnetic Particle Spectroscopy (MPS), respectively. To analyze the formability and smallest printable feature of a 3D-printed magnetic composite an appropriate geometry demonstrator was developed (see Fig.) which also considers the orientation of the structures with respect to the printing direction. A homogeneity demonstrator was designed to quantify the settling of MNP during the printing process by MPS.

To demonstrate the developed procedure will present the results of different combinations of MNP (EFH3, FerroTec Corp.; Perimag®, Micromod; Ferucarbotran, Meito Sangyo) and photopolymers (E-Shell 600 and ABS-tough; Envisiontec) and preliminary MPI imaging results of magnetic composites with different geometries.

With the developed procedure, it is possible to advance the characterization of the MPI scanner with defined, long-term stable magnetic composite phantoms. Finally, this opens an elegant way to print complex structures that could resemble body-like parts containing defined amounts of MNP.

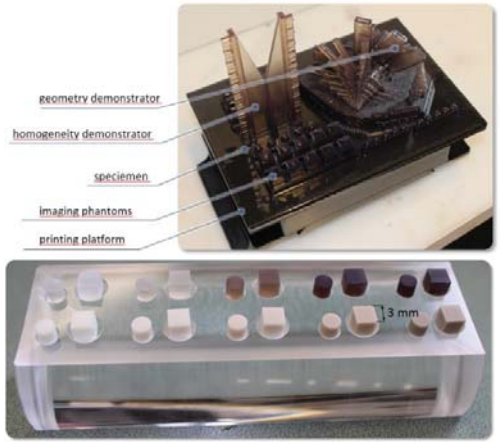

Fig.: 3D-printed demonstrator and phantom platform with different structures to test the quality of the 3D-printed magnetic composites (upper figure). Different combination of MNP (EFH3, Ferrotec Corp.) and photopolymer (E-Shell 600, ABS-tough; EnvisionTec GmbH) at different concentrations and with different geometries (cubes, cylinders).

Electroactive Magnetic Nanoparticles for Electrochemical Signal Amplification under Magnetic Attraction on a Microchip Device

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Magnetic nanoparticles (MNPs) are of particular interest in biomedicine and biotechnologies for bioseparation, hyperthermia, drug delivery and magnetic resonance imaging. For this purpose, their surface must be chemically modified with appropriate molecules or materials to acquire sensing properties and targeting functions. In particular, the introduction of electroactive molecules onto the surface of MNPs is useful for the magnetic control of bioelectrocatalytic processes. It is also a major issue for biosensing as redox property can be used for quantification thanks to the detectable electrochemical signal while magnetism can be used for separation and purification of the analyte from other components after its capture by the MNPs. Ferrocene and its derivatives are good candidates as electroactive molecules due to their electrochemical reversibility, low oxidation potential, stable redox forms and their chemical inertia to oxygen. However, only few studies report on their detailed electrochemical properties when immobilized onto MNPs surface.

In this work, ferrocenecarboxylic acid (Fc) was immobilized onto MNPs. First, carboxy-modified MNPs were functionalized with polyamidoamine dendrimers using carbodiimide coupling chemistry. The amine groups were then used for Fc coupling. The different functionalization steps were followed by colorimetric titration, infrared spectroscopy and zeta potential measurements. Then, electrochemical characterization was performed with classical cell and microchip device. Using electrochemical microchip, it was possible to apply a magnetic field specifically located onto working electrode in order to attract Fc-conjugated MNPs. The influence of this magnetic attraction on Fc electrochemical properties was studied. The diffusion or surface-controlled character of the electrochemical reaction was then compared for free Fc molecules, Fc-conjugated MNPs dispersed in solution and after magnetic attraction onto working electrode surface.

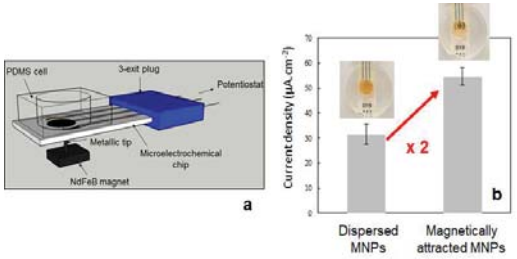

Figure. (a) Electrochemical microchip device and (b) influence of magnetic attraction onto electrochemical signal.

NIL-fabricated multifunctional magnetic nanoparticles as probes for homogeneous label-free biosensing

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We recently introduced a homogeneous and label-free method for the detection of biomarkers directly in the sample solution.[1] The measurement principle is based on optically detecting changes of the dynamics of elongated magnetic nanoprobes in an external rotating magnetic field on analyte molecule binding (see Figure 1). Using chemically synthesized functionalized magnetic core-shell nanorods as probes,[2] we could demonstrate simple mix & measure type detection of the breast cancer biomarker HER2 in spiked serum and saliva samples.[3]

An alternative to the chemical synthesis of nanoparticles as probes for our measurement method is their top-down wafer-scale nanofabrication, which allows superior control over their shape, size distribution and composition. Specifically, the addition of plasmonic properties to the magnetic nanoparticles boosts their optical signal by almost two orders of magnitude, thus leading to enhanced analyte molecule detection limits.[1]

To that end, we are applying nano-imprint lithography (NIL) from flexible stamps,[4] UV-curing and reactive ion etching to produce regular arrays of nanoscale holes within the mrt-resist.[4] Next, we fill these holes by a sputter-deposited multilayer stack comprising Al-doped Zn-oxide (AZO) as sacrificial layer, Au as plasmonic material, Ti-oxide as dielectric spacer and NiFe as magnetic material. Following NIL-resist lift-off, this process results in regular arrays of multifunctional plasmon-magnetic nano-ellipses on their Si-wafer substrate (see Figure 2a), which can be transferred to solution following wet-chemical etching of the AZO-layer (see Figure 2b).

We will present both the biosensing results achieved by chemically synthesized nanoprobes as well as the current status and foreseen stabilization and functionalization routes for the NIL-fabricated nanoprobes.

Acknowledgments: This research has received funding from the European Community's 7th Framework Programme under grant agreement n° NMP4-LA-2010-246479 and by the Federal Ministry of Transport, Innovation and Technology (BMVIT) supported by the Austrian Research and Promotion Agency (project LAMPION, #861414).

Figure 1) Detection principle: Analyte molecule binding is determined optically as a change in the dynamic nanoparticle response to a rotating magnetic field.

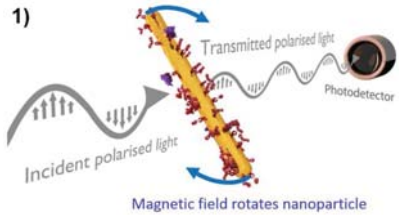
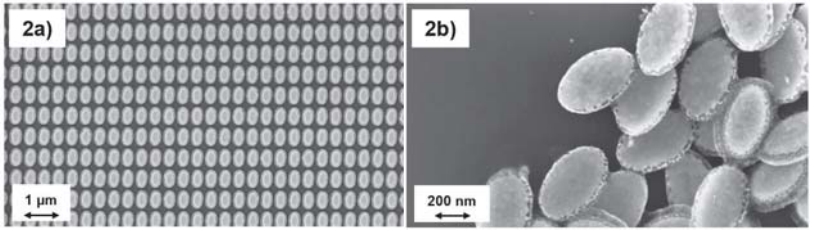


Figure 2) SEM images of NIL-fabricated multifunctional nanoparticles

a) before removal from their Si-wafer substrate.

b) after removal and re-deposition from solution onto a Si-wafer substrate (for SEM imaging).



[1] S. Schrittwieser *et al.*, ACS Nano 6 (2012) 791–801.
[2] S. Schrittwieser *et al.*, ACS Appl. Mater. Interfaces 8 (2016) 8893–8899.
[3] S. Schrittwieser *et al.*, Sci. Rep. 7 (2017) 4752.
[4] M. J. Haslinger *et al.*, Microelectron. Eng. 153 (2016) 66–70.

Structural motifs in Self-Assembling Dipolar Spheres

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The macroscopic properties of magnetic fluids are strongly influenced by changes in shape, sample geometry or temperature. To guide medical applications of these systems and to help develop the bottom-up self-assembly of materials, a detailed knowledge of the constituent units is required.

The structural transitions in suspensions of dipolar spheres are well known: typical chain and ring clusters can be nowadays even observed directly in the experiments[1]. Recently, an exhaustive theoretical classification of possible structures that dipolar spheres can form was proposed in Refs [2-3]. The self-assembly scenario turned out to be much more complex than expected: chains and rings assemble together to form branched structures via the emergence of junctions. Along with linear flexible chains - due to the long-range interactions, which determine a preferred head-to-tail orientation of the dipole moments - four-way junctions (FWJ) become the main ingredient (Figure a): a structure different from the three-way junctions (TWJ), earlier predicted by Tlusty and Safran as the responsible for a possible topological phase transition[4].

In order to avoid the self-assembly's sensitivity to thermal fluctuations and particle polydispersity, we predefine the structural motifs by tethering the magnetic particles in polymer-like supracolloidal structures (SMPs), made by crosslinked magnetic particles[5]. Inspired by self-assembly of non-crosslinked dipolar particles, we focus on four different topologies of the SMPs (chains, rings, Y and X), considering them as the basic unities in the next step of the hierarchical self-assembly.

Indeed, strikingly enough, we find that in any system under study, FWJs (Figure b) seem to be the most probable junction. We both quantify this effect and explain its origin in terms of energy and entropy.

(a) FWJs in dipolar hard spheres

(b) FWJs in SMPs

[1] M. Klokkenburg, R. Dullens, W. Kegel, B. Erné, A. Philipse, *Phys. Rev. Lett* **96** (2006)
[2] M. Ronti, L. Rovigatti, J.M. Tavares, A.O. Ivanov, S.S. Kantorovich and F. Sciortino, *Soft Matter* **13** (2017)
[3] S.S. Kantorovich, A.O. Ivanov, L. Rovigatti, J.M. Tavares and F. Sciortino, *Phys. Chem. Chem. Phys.* **17**, (2015)
[4] T. Tlusty and S. A. Safran, *Science* **290** (2000)
[5] E.S. Pyanzina, E.V. Novak, D.A. Rozhkov, M. Ronti, J. J. Cerdà, T. Sintes, P.A. Sánchez, S.S. Kantorovich, *submitted*

Synthesis and Application of Zinc Ferrite Nanoparticles as Agents for Cancer Thermotherapy

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Magnetic nanoparticles are of current immense scientific and clinical interest due to their application in a number of fields. Among applications in biomedicine, magnetic nanoparticles also find use in thermotherapy where they are utilised to convert one form of energy to heat to either directly induce cell death or sensitise cells for coming chemo- or radio-therapy. Most applications of magnetic nanoparticles heavily depend on the saturation magnetisation of the material with even more complex systems devised to reach such goals. Here we will present how introduction of zinc ions in the ferrite lattice allows for an augmentation in the saturation magnetisation of the nanoparticles due to quenching of antiferromagnetic coupling between the ions in the lattice interstices.[1] We have extensively characterised zinc ferrite nanoparticles' physical and chemical properties and it is shown that we have obtained best-in-class zinc ferrite nanoparticles (smallest size with highest M_S). We have developed a coating procedure to produce stable ferrofluids whose biocompatibility was assessed against iron oxide nanoparticles which are widely considered biocompatible. Our results reveal a cytotoxicity profile which is the same as iron oxide nanoparticles hence zinc ferrites with augmented magnetic properties and same biocompatibility could be the next mainstream magnetic biomaterial of choice.

Magnetic hyperthermia is one way of producing heat with magnetic nanoparticles but it comes with many obstacles such as high concentrations required for effective change in temperature and the low amount reaching the tumour. Photothermia has an inverse relationship to concentration compared to magnetothermia which can tackle the issue of little material reaching the tumour. The SLP values obtained and the absolute change in temperature achieved with photothermia are 7 times higher compared to magnetothermia. Currently, the impact of cell internalization on the efficiency of each modality is evaluated to assess how magnetothermia, which is environment dependent, and photothermia, which is not, compare to each other once they are associated with cells.

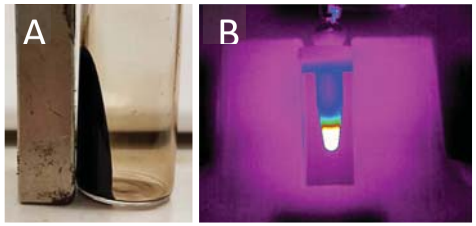

Figure 1: A) Stable ferrofluid of citrate coated $Zn_{0.4}Fe_{2.6}O_4$ nanoparticles B) Magnetophotothermia set up

References

1. Byrne, J. M. *et al.* Biosynthesis of zinc substituted magnetite nanoparticles with enhanced magnetic properties. *Adv. Funct. Mater.* **24,** 2518–2529 (2014).

High Magnetisation, Monodisperse and Water-dispersible CoFe@Pt Core/shell Nanoparticles

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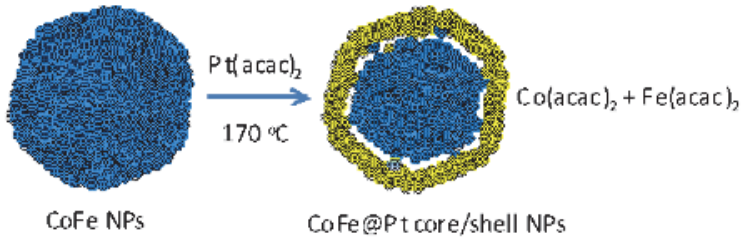
High magnetisation and monodisperse CoFe alloy nanoparticles are desired for a wide range of biomedial applications. However, these CoFe nanoparticles are prone to oxidation, resulting to deterioration of their magnetic properties. In the current work, CoFe alloy nanoparticles were prepared by thermal decomposition of cobalt and iron carbonyls in organic solvent at high temperature. Using seeded growth method, we successfully synthesised chemically stable CoFe@Pt core/shell nanostructures. The obtained core/shell nanoparticles have high saturation magnetisation up to 135 emu/g. The magnetisation value of the core/shell nanoparticles remains 93 emu/g after being exposed in air for 12 weeks. Hydrophobic CoFe@Pt nanoparticles were rendered water-dispersible by encapsulating with poly(maleic anhydride-alt1-octadecene) (PMAO). These nanoparticles were stable in water for at least 3 months and under a wide range of pH from 2 to 11.



Schematic illustration of the formation of CoFe@Pt core/shell nanostructures

Ref: Lu, T. T., Dung, N. T., Nam, P. H., Tung, L. D., Phuc, N. X., **Thanh, N. T. K.*** (2017) High water-dispersible, magnetisation and monodisperse CoFe@Pt core/shell nanoparticle. *Nanoscale*. **9**: 8893–9248.

Revealing dipolar-coupled moment correlations in clusters of superparamagnetic nanoparticles

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For most biomedical applications, understanding the precise influence of dipolar interactions on the magnetic properties of nanoparticle ensembles is of utmost importance. Simulations of core-clusters suggest that also in the superparamagnetic regime (i.e. above the blocking temperature), in which each individual core has a thermally fluctuating moment, the particle moments are directionally correlated due to a dipolar coupling [1]. Few experimental techniques, however, are able to resolve moment correlations on the interparticle length scale and even less are simultaneously capable of taking a snapshot of the internal moment structure faster than superparamagnetic relaxation times.

In this work, we use polarized small-angle neutron scattering (SANS) to obtain information about directional correlations between the moments within clusters of 10-nm iron oxide cores (Figure **Left**) in the superparamagnetic regime. We performed a so-called longitudinal neutron-spin analysis in SANS (POLARIS) [2], through which we detected the purely magnetic scattering cross sections (Figure **Middle**) [3]. By applying a model-independent analysis, based on indirect Fourier transformations (IFTs [4]), we extracted the underlying pair distance distribution functions $P(r)$ of the magnetization vector field. Analysis of the radially averaged 1D intensity verified a preference for antiferromagnetic-like correlations between the particle moments (Figure **Right**) [5]. In this contribution we will present the results of 2D IFTs of the scattering patterns (field- and temperature-dependent), which proves to be a powerful approach to resolve the 3D moment correlations in such nanoparticle clusters.

This work was supported by EU FP7 project NanoMag www.nanomag-project.eu (grant no. 604448). We thank the Institut Laue Langevin for provision of beamtime at the instrument D33.

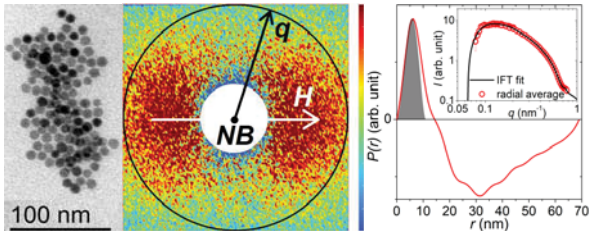

Figure. **Left:** TEM image of a cluster of the 10-nm iron oxide cores. **Middle:** 2D magnetic SANS pattern of the particle powder detected for a field strength of 2 mT (T = 300 K) and covering the momentum transfer q = 0.07 - 0.77 nm^{-1}; the field H was applied perpendicular to the neutron beam (NB). **Right:** Pair distance distribution function $P(r)$ of the magnetization vector field, which was determined by an IFT of the 1D SANS intensity $I(q)$ (azimuthal average of the 2D scattering pattern) [5]. The negative values for r > 10 nm verify a preference for antiferromagnetic-like correlations between the fluctuating particle moments within the clusters.

[1] O. Hovorka et al., *Phys. Rev. B* **89**, 104410 (2014).
[2] D. Honecker et al., *Eur. Phys. J. B* **76**, (2010) 209.
[3] P. Bender et al., *Institut Laue Langevin (ILL)* (2016) DOI: 10.5291/ILL-DATA.5-32-836.
[4] P. Bender et al., *Sci. Rep.***7**, (2017) 45990, P. Bender et al., *J. Phys. Chem. C* **122**, (2018) 3068.
[5] P. Bender et al., *arXiv preprint*, (2018) arXiv: 1803.01747.

Iron deficiencies and structural defects favor magnetic hyperthermia performance of magnetite nanocubes in viscous media

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Magnetic nanoparticle mediated hyperthermia is an emerging therapeutic approach that induces cancer cell death and has succeeded in retreating brain tumor in clinical trials. Anisotropically-shaped magnetic nanoparticles have attracted enormous attention in the last few years owing to their superior magnetic heat dissipation. There has been a significant progress in size and morphology controlled synthesis of iron oxide nanoparticles. A crucial feature of magnetic nanoparticles for intracellular hyperthermia applications is to be minimally affected by high viscosity of the cellular environment. Experimental and theoretical studies have shown that the magnetic loss of magnetic nanoparticles with high magnetic anisotropy is dramatically reduced in viscous media. Thus, the design of suitable magnetic heat mediators with invariable intracellular hyperthermia capabilities is mandatory and yet remains a challenge.

Our study aims to gain critical insights into the evolution of the underlying hysteretic power loss mechanisms of FeO-Fe_3O_4 core-shell (CS) nanocubes upon stepwise phase transformation in order to design viscosity insensitive heat mediators. For this purpose, 18 and 23 nm nanocubes were synthesized, transferred into water, and thermally treated. We have identified that for both sizes the dominant relaxation mechanism switches from Néel in as-prepared CS nanocubes to Brownian in partially oxidized nanocubes up to an eventual presence of both mechanisms in fully treated particles. The 23 nm nanocubes transform to a quasi-magnetite with Fe^{2+} deficiency after 48 h treatment at 80°C, yet containing tiny FeO sub-domains randomly distributed throughout the entire particle. The survival and migration of FeO sub-domains is caused by the high pressure exerted by growing Fe_3O_4 domains. The fully processed 23 nm nanocubes possess a high SAR of 400 W/g_{Fe} at 301 kHz and 24 kA/m field conditions, yet their SAR is exceptionally viscosity insensitive compared to similarly sized iron oxide nanocubes. Our work demonstrates that Fe^{2+} deficiency, FeO sub-domains, and subtle structural defects in magnetite favor the preservation of the SAR in highly viscous media.

Quantitative Measurement of Ligand Exchange on Iron Oxides via Radioanalytical Techniques

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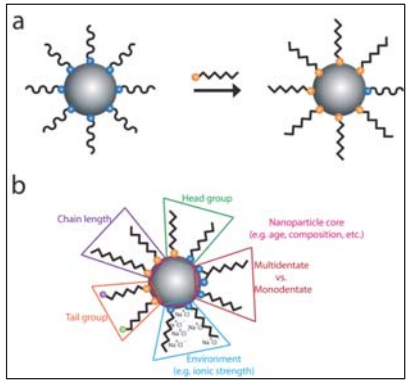
Ligand exchange of hydrophilic molecules on the surface of hydrophobic iron oxide nanoparticles produced via thermal decomposition of chelated iron precursors is a common method for producing aqueous suspensions of particles for biomedical applications. There are many factors which influence ligand exchange including chain length, multidenticity, head group chemistry, and particle aging and oxidation. Despite the wide use, relatively little is understood about the efficiency of ligand exchange on the surface of iron oxide nanoparticles and how much of the hydrophobic ligand is removed. To address this issue, we utilized a radiotracer technique to track the exchange of a radiolabeled ^{14}C-oleic acid ligand with hydrophilic ligands on the surface of magnetite nanoparticles.



(a) Illustration of incomplete ligand exchange. (b) Factors which affect ligand exchange.

Oleic acid coated iron oxide nanoparticles were synthesized via thermal decomposition with trace amounts of ^{14}C-oleic acid on the surface. The particles were modified via ligand exchange with a variety of hydrophilic ligands. The modified particles were measured using liquid scintillation counting (LSC) to determine the activity and ultimately, the total number of ^{14}C-oleic acid chains remaining after exchange. These techniques were used to determine effects of head group chemistry with polymeric ligands and effects of head group chemistry, number of binding groups, and ligand exchange reaction parameters with small molecule ligands. Results revealed catechols displace the most oleic acid during exchange. Furthermore, multidenticity, or multiple binding groups, increases the displacement of the oleic acid.

Davis, K., Cole, B., Ghelardini, M., Powell, B. A., & Mefford, O. T. (2016). *Langmuir*, *32*(51), 13716–13727.
Davis, K., Qi, B., Witmer, M., Kitchens, C. L., Powell, B. A., & Mefford, O. T. (2014). *Langmuir*, *30*(36), 10918–10925.
Davis, K., Vidmar, M., Khasanov, A., Cole, B., Ghelardini, M., Mayer, J., et al. (2018). *Journal of Colloid and Interface Science*, *511*, 374–382.

Synthesis and functionalization of magnetic nanoparticles for remote control of differentiation and oriented growth of neuronal cells

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Neurodegenerative disorders, such as Parkinson's, Alzheimer's or Huntington's diseases are among the most common group of medical conditions in the world, and are expected to surpass cancer by 2040.[1] However no cure exists for such diseases at that time. Cell replacement therapy is among the most promising approach to treat neurodegenerative disorder. In the work presented here, part of the MAGNEURON European project,[2] we used magnetic nanoparticles that are bio-functionalized to trigger neurons' differentiation and growth along the direction of an applied external magnetic gradient. Mature neurons would in term be re-implanted in the patient brain to replace degenerated neurons.

To this goal, we synthesized different types of magnetic nanoparticles. Maghemite (γ-Fe_2O_3) nanoparticles were synthesized by an inverse co-precipitation process [3] and then used to synthesize $\gamma Fe_2O_3@SiO_2$ core-shell nanoparticles (fig. 1-a). The same γ-Fe_2O_3 nanoparticles could also be functionalized with polymers such as poly(ethylene glycol) or poly(acrylic acid) in order to have smaller objects with the same magnetic properties. Finally, in order to study the effect of the shape on the interaction between nanoparticles and cells, magnetic iron oxide nanorods were also synthesized (fig. 1-b). All these particles were optimized in terms of size, charge and magnetization to obtain the optimal balance between colloidal stability and magnetic properties to facilitate intracellular motion.

The magnetic $\gamma Fe_2O_3@SiO_2$ nanoparticles were then functionalized either with a HaloTag ligand or with Green Fluorescent Protein (GFP) by click chemistry, in order to interact specifically with intracellular proteins able to trigger different pathways in the cell. For that purpose, MNPs were then microinjected in the cell (fig.1-c) and showed intra-cellular biased diffusion toward a micro-magnet (fig. 1-f). The magnet can then be used to displace target proteins, attached to the MNPs inside the cell (fig. 1-g,h), and trigger signaling events such as actin polymerization at particular subcellular localizations.[4,5]

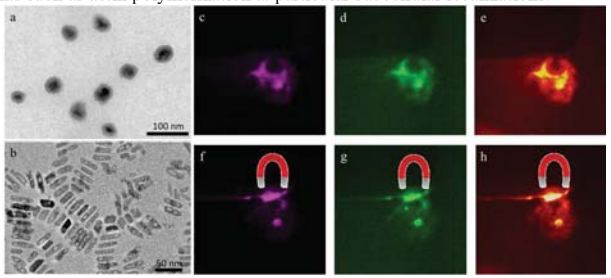

Figure 1. a) TEM image of core-shell MNPs, b) TEM image of magnetic iron oxide nanorods, c-h) fluorescence microscopy image of a cell injected with fluorescent core-shell MNPs functionalized with GFP before (c-d-e) and after (f-g-h) approaching a magnet: c and f show the fluorescence of the core-shell MNPs, d and g show the GFP fluorescence and e and h show the fluorescence of an intracellular protein, intersectin, tagged with an anti-GFP nanobody.

References

[1] World Health Organization, Neurological disorders: public health challenges (2006).

[2] MAGNEURON : H2020 Future and Emerging Technologies (FET) project n°686841, http://magneuron.eu/

[3] Massart R., Preparation of aqueous magnetic liquids in alkaline and acidic media, IEEE Trans. Mag. (1981), 17, 1247–1248

[4] Etoc *et al.*, Magnetogenetic control of protein gradients inside living cells with high spatial and temporal resolution, Nano Lett. (2015), 15, 3487−3494[5] Etoc et al. Subcellular control of Rac-GTPase signalling by magnetogenetic manipulation inside living cells, Nat. Nano (2013) 8, 193-7.

Generation of nano-magnetic hybrid materials by genetic engineering and functionalization of bacterial magnetosomes

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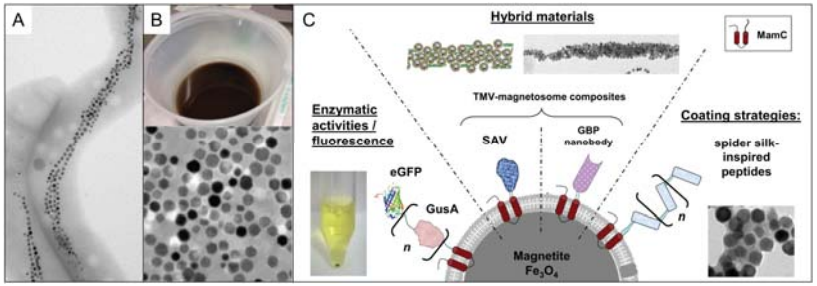
For magnetic orientation, magnetotactic bacteria (MTB) biomineralize magnetosomes, (ferri)magnetic nanoparticles which consist of a monocrystalline magnetite (Fe_3O_4) core enveloped by the magnetosome membrane (MM). Due to their strictly regulated biosynthesis, magnetosomes have a number of unprecedented properties such as high crystallinity, strong magnetization, and uniform shapes and sizes. In addition, both the crystal morphology and the composition of the enveloping MM can be manipulated *in vivo* by genetic means [1]. Because of these unique features, bacterial magnetosomes have the potential to yield biomaterials for use in numerous applications, such as in immobilization of bioactive compounds or magnetic separation.

Until recently, the use of magnetosomes in real-world applications was limited by fastidious, slow growth of magnetotactic bacteria and poor particle yields. However, recent progress in improving cultivation conditions has led to considerably increased magnetosome yields (>50%) and will enable mass production. Moreover, with the generation of overproducer strains, an up to 2.5-fold increased iron accumulation was achieved.

Many real-world applications would benefit from multimodal particles that in addition to their magnetic properties display functional moieties on the surface, like for instance enzymes, receptors, or antibodies. In a systematic stepwise bottom-up approach, we developed a versatile genetic "toolkit" for the generation of (multi)functional magnetic nanoparticles with several 'tailored' properties. Abundant magnetosome membrane proteins were used as anchors for the expression of foreign peptides and enzyme proteins as large hybrid proteins. Particle displayed enzymatic activities could be sequentially increased by expression of enzyme arrays, thereby generating magnetosomes with maximized protein-to-particle ratios (surface coverage up to 90%) [2]. The display of versatile molecular connectors like streptavidin (SAV) or nanobodies allowed specific coupling reactions (for instance with functionalized tobacco mosaic virus particles), which resulted in magnetic biocomposites. Furthermore, we explored different encapsulation strategies. Encapsulation in biocompatible polymers like spider silk-inspired peptides caused the formation of a proteinaceous capsule. In addition, the surface properties could be tuned, and the colloidal stability of the particles was increased [3].

Overall, this illustrates the versatile features of engineered bacterial magnetosomes, with enhanced potential for numerous biomedical or biotechnological applications, such as biosensors or magnetic drug targeting.



Functionalization of bacterial magnetosomes. (A) TEM micrograph of a magnetosome overproducing strain of *Magnetospirillum gryphiswaldense*. Isolated particles (B) were homogenous and free of contaminations. (C) For the generation of multifunctional particles a versatile genetic toolkit was developed, and a set of model particles was created. Expression of *$(gusA)_n$-egfp* arrays resulted in particles that display up to five copies of the enzyme GusA and eGFP as fluorophore, and GusA activities were stepwise increased with the number of GusA monomers. Display of GBP nanobodies or streptavidin (SAV) allowed specific coupling with functionalized TMV particles, thereby generating mesoscopic, strand-like biocomposites. Expression of spider silk-inspired sequences resulted in the generation of an organic/proteinaceous capsule and significantly improved colloidal stability of the particles. Size of particles and proteins not to scale.

[1] Uebe R., Schüler D. *Nat. Rev. Microbiol.* 2016, 14(10):621-37

[2] Mickoleit F., Schüler D. *Adv. Biosyst.* 2018, 2(1):1700109

[3] Mickoleit F., et al. *Biomacromolecules* 2018, 19(3):962-72

Magnetic Molecularly Imprinted Polymers. Synthesis and Applications

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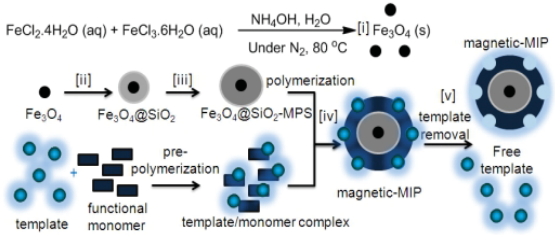
Since the early reports on magnetic separation technology [1], magnetic particles (MPs) have been used as a powerful and versatile preconcentration tool in a variety of analytical and biotechnology applications. This technology has been widely incorporated for researchers in classical methods including PCR and immunoassays, and in emerging technologies such as biosensors and microfluidic devices with application ranging from biomarker detection of infectious diseases, foodborne pathogens, among others. Magnetic particles have been commercially available for many years. The use of MPs greatly improves the performance of the biological reaction by increasing the surface area, enhancing the washing steps and, importantly, minimizing the matrix effect. In addition, MPs can be easily magneto-actuated using permanent magnets. Beside the amazing properties and the huge range of applications, the main drawback of the biologically-modified MPs is their high cost and low stability at harsh conditions. Molecularly Imprinted Polymers (MIPs) are synthetic biomimetic materials mimicking biological receptors [2]. They are highly cross-linked macromolecular structures towards the template which is then extracted after polymerization, originating cavities (binding sites) complementary to the template molecule, acting as plastic antibodies. Although MIPs have in general lower affinity and selectivity compared to the biological counterparts, they show important technological features: i) they can be easily and affordably synthesized on a animal-free large scale procedures, and ii) they show high chemical and mechanical stability, allowing to work in harsh conditions (pH, temperature, solvents). This work addresses the synthesis of magneto-actuated molecularly imprinted polymers (magnetic-MIPs) (Figure 1) in order to merge the outstanding properties of MIPs and MPs [3]. The characterization of the magnetic-MIP is performed by several techniques as SEM, TEM, XRD, FTIR, VSM and BET analysis. Finally, magnetic-MIP is used for preconcentration of the analyte from the complex samples allowing the quantification by the direct electrochemical detection on magneto-actuating electrodes. This novel material was evaluated, among others, in different applications including for the isolation and detection of biotin and biotinylated biomolecules [4], for food pollutants (methyl parathion) and contaminants (histamine) in fish samples as a model and it was also successfully applied for determination of diseases related to hormone (L-thyroxine) giving promising results. This proves the ability of this material to preconcentrate analyte from complex samples and opens the way to incorporate this material in magneto-actuating devices which could be used easily in the field of environmental control, food safety, and medical applications.



References

1. A. Rembaum, R.C.K. Yen, D.H. Kempner, J. Ugelstad, 1982. J. Immunol. Methods 52, 341-351.
2. G. Vlatakis, L.I. Andersson, R. Müller, K. Mosbach, 1993. Nature 361, 645-647.
3. Pividori,M.I. , Sotomayor,M.D.P.T. , European Patent EP15169505.3.(2016).
4. AB Aissa, A Herrera-Chacon, RR Pupin, MDPT Sotomayor, MI Pividori, 2017. Biosensors and Bioelectronics 88, 101-108.
5. S. Lima Moura, L. Martinez Fajardo, L. dos Anjos Cunha, MDPT Sotomayor, , F.B. Bolivar Correto Machado, L.F. Araújo Ferrão, MI Pividori, 2018. Biosensors and Bioelectronics 107, 203-210.

Invited Talk

Assembling magnetic iron oxide cores to produce well-controlled hydrophilic multicore structures

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Through the versatile polyol mediated synthesis, we have assembled magnetite nanocrystals into complex structures, from single-core to multi-core nanoparticles, including nanoflowers and hollow spheres that can give rise to interesting collective magnetic properties considerably different from their equivalent single-core nanoparticles or bulk materials [1, 2]. We demonstrate that the precipitator concentration plays a crucial role in the structure adopted (single-core, nanoflowers or hollow spheres). In addition, while the particle size in the nanoflowers is maintained unchanged, by modification of the recrystallization time, nanoflowers with different core size have been produced (Fig. 1). All samples regardless of their structures show ferrimagnetic behaviour at low temperature but samples with crystal sizes bellow 20 nm display superparamagnetic behaviour at room temperature. The magnetic properties of the nanostructures reflect not only the core size, that justifies the nearly bulk saturation magnetisation values, but also the collective behaviour in the case of the flower-like particles leading to a susceptibility enhancement. We explore factors determining the monodispersity in terms of size and shape and the core assembly, and discuss their implication on the resulting structural, colloidal and magnetic properties.

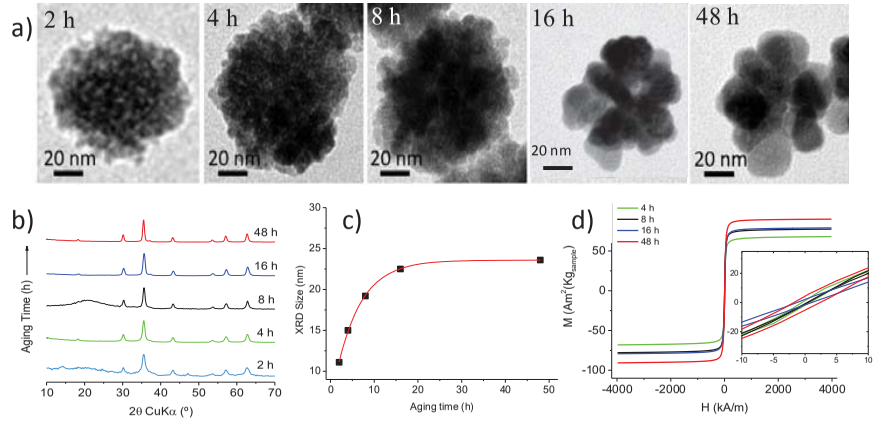

Figure 1: Multicore maghemite nanoparticles prepared by the polyol process at different recrystallization times: a) TEM images, b) X-ray diffraction patterns, c) Crystal size calculated from the X-ray broadening and d) Magnetisation curves at room temperature (inset: magnification of the low field area).

References: 1. H. Gavilán et al., ACS Omega, 2017, 2 (10), pp 7172–7184
2. H. Gavilán, et al, Part. Part. Syst. Charact. 2017, 34, 1700094.

Progress Towards a Magnetic Nanoparticle Imaging System for Immunohistochemistry

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Realizing the promise of personalized medicine in cancer requires accurate quantification of multiple genetic and/or protein expression factors so that the best available cancer treatment can be selected for each patient. Clinical pathology methods for detection of protein in tissue biopsy slides still rely on semi-quantitative and single factor tests or off-site genetic testing that lacks a cellular anatomical context. We have developed a prototype AC magnetic susceptibility nanoparticle imager (NanoPi) with the goal of obtaining quantitative, multiplexed protein expression readouts using antibody-conjugated magnetic nanoparticles (MNPs) on formalin-fixed, paraffin-embedded (FFPE) tissue in clinical pathology.

The basic operating principle of the NanoPi is that an applied AC magnetic field from an array of drive coils will interact with the MNPs due to their magnetic susceptibility and result in altered induced AC currents in an array of pickup coils. A calibration scan of an effective MNP point source yields a sensitivity matrix (a.k.a. imaging model) that can then be used to tomographically reconstruct images of the same MNP material in samples with unknown MNP distribution. The current NanoPi prototype is a functional 2D imaging system with sufficient sensitivity to detect MNPs on FFPE tissue slides at 2-mm resolution.

The NanoPi applies digitally programmable AC magnetic fields of up to 14 mT on a sample above an array of 4 drive coils at frequencies of 20 Hz to 96 kHz. An array of 8 pickup coils is used to sense MNP magnetization harmonics with a digital lock-in amplifier. Samples are placed on a 35-mm petri dish or a 75-mm by 25-mm microscope slide. An xy-stage with a 16-cm by 16-cm range of motion is used for calibration and 2D sample scanning for imaging. The system is sensitive to 1-μg MNP Fe (ferucarbotran) and is effective for detecting bound and unbound MNP. Scanning a 6-cm by 6-cm area with 2-mm resolution takes less than 10 minutes. This report describes our approach to meeting milestones in our development path towards a higher sensitivity, higher resolution system that meets clinical research needs.

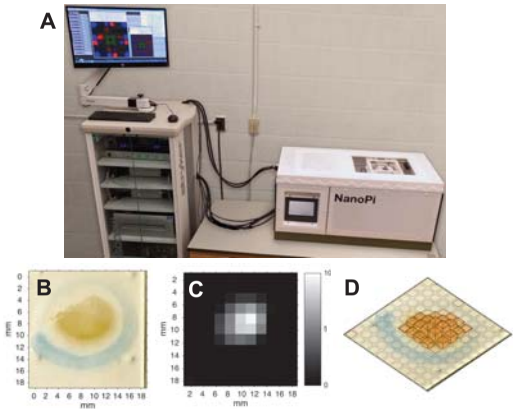

Figure. The NanoPi prototype at Dartmouth. **A)** The NanoPi was designed in the school's CAD-Lab and built in the Thayer School Machine Shop. Sound studio electronics and software were programmed to perform real-time data acquisition, signal processing and image reconstructions. **B)** A 4-μm thick FFPE tissue section is visibly stained brown with MNPs (Ocean Nanotech, IPG-20-01, 20 nm, Protein G). **C)** Image showing a crude estimate of the MNP biodistribution (μg Fe). **D)** Overlay of the NanoPi image on the tissue photograph.

Towards functional magnetic particle imaging of endothelial cells

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The field of biomedical applications using magnetic nanoparticles (MNP) is constantly expanding during the last decades. The usage of MNP for diagnostic purposes showed promising results, e.g. for the visualization of atherosclerotic lesions [1]. To ensure a precise diagnosis, reliable methods to localize and quantify the amount of MNP in different biological environments are crucial. Currently, magnetic resonance imaging (MRI) is used for this purpose in most cases. However, using this imaging technique MNP are imaged as negative contrast and might lead to imaging artifacts. Magnetic particle imaging (MPI) is a young imaging modality capable of determining the spatial distributions of MNP. The combination of static and dynamic magnetic fields generates a specific response based on the magnetic properties of the MNP, which can be used to reconstruct a quantitative 3D image. Furthermore, it has been shown that MPI is capable to distinguish between different particle types and dried MNP [2], a technique termed multi-color MPI.

Previously, we found that the dynamic magnetic MNP response changes depending on their biological environment [3], [4]. Here, we demonstrate that - similar to multi-color MPI - it is possible to generate multiple MPI images out of solely one measurement, weighted for MNP in different environments, by incorporating information about the biological surrounding into the reconstruction process. To this end, we investigated the capability to distinguish MNP in an aqueous solution and a cellular environment. Two individual MPI system functions (SF) were acquired and combined for the image reconstructions. SFs are measured by placing a tiny MNP sample volume at multiple positions inside the field of view (FOV) to obtain reference signals. The first SF was acquired of a 4 μL fluidMAG CT50 (ChemiCell GmbH, Germany) suspension at an iron concentration of 0.317 mol/L, while for the second SF a reference sample containing $1.5 \cdot 10^6$ endothelial cells (EA.hy926) loaded with CT50 (about 20 pg/cell) was used. A phantom consisting of a fluid CT50 sample placed next to a second cell sample (also about $1.5 \cdot 10^6$ cells) was then measured and used for the MPI image reconstruction (Fig 1a). The total data acquisition time was 2 s. Image reconstructions were performed with the Kaczmarz algorithm using 3425 frequency components and 50 iterations.

The reconstructions with the combined SF yield two images, which are sensitive for MNP in an aqueous or a cellular environment, respectively. Therefore, the two samples can clearly be distinguished in both reconstructions based on the same, single measurement (Fig.1 b, c). In conclusion, this shows the high potential of MPI to map the biomedical environments of the MNP in vivo. This opens the way for functional MPI as a powerful diagnostic tool to detect changes on a (sub)cellular scale.

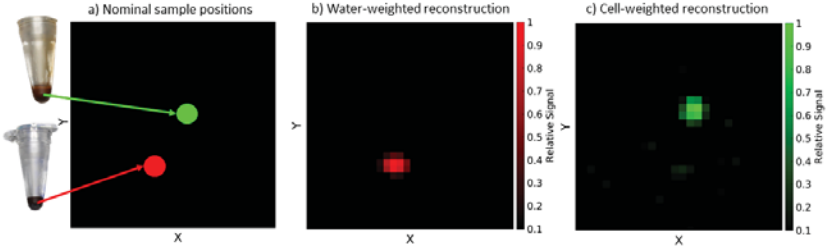

Figure 1: a) Nominal phantom positions of the cell (green) and the liquid sample (red) in the FOV. b,c) Maximum intensity projections along the z-axis of the reconstructed MPI images based on the same measurement. Each image was scaled to the maximum intensity of the individual image.

[1] W. C. Poller *et al.*, *Nano Res.*, vol. 9, no. 11, pp. 3437–3452, Nov. 2016.
[2] J. Rahmer *et al.*, *Phys. Med. Biol.*, vol. 60, no. 5, pp. 1775–1791, 2015.
[3] N. Loewa *et al.*, *IEEE Trans. Magn.*, vol. 49, no. 1, pp. 275–278, Jan. 2013.
[4] N. Löwa *et al.*, *J. Magn. Magn. Mater.*, vol. 427, pp. 133–138, Apr. 2017.

Dynamical monitoring of magnetic markers using quantum diamond magnetometry

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The nitrogen-vacancy (NV) center in diamond crystal has shown great promise as a magnetic field sensor [1]. The method is of particular interest for biological systems, because of its high sensitivity at ambient conditions and the biocompatibility of the diamond carrier.

Tagging of biological samples using magnetic nanoparticles (MNPs) is an important tool for both manipulation and detection of biotargets. NV center based magnetometry has proven capable of resolving single magnetically marked cells [2]. Furthermore the technique allows for scalability to large areas and the analysis of macroscopic sample. This makes the system promising for incorporation in flow cytometry and eventually lab-on-a-chip systems. Meanwhile, the current detection times are in the order of seconds for specially treated diamonds and ranging into hours for untreated samples to obtain high sensitivity.
Here we present a set-up with the option of detecting magnetic particles at high framerates, providing videos of magnetic particles and introducing the opportunity of monitoring motile bacteria or cells in flow.

The magnetic sensing capabilities of the NV centers stem from its energy level structure. The electron spin states are coherently probed using microwaves. The states are optically initialized and read out through laser excitation and monitoring the intensity of the emitted fluorescence, which is sensitive to magnetic fields. The set-up has been characterized, showing a uniform sensitivity of approximately 160 nT/√(Hz)/pixel over a 40 µm x 40 µm field of view working at a frame rate of 60 FPS. Single 2.8 µm beads have been detected and the ability to monitor the movement of beads has been verified for both mechanical movement and varying electric fields.

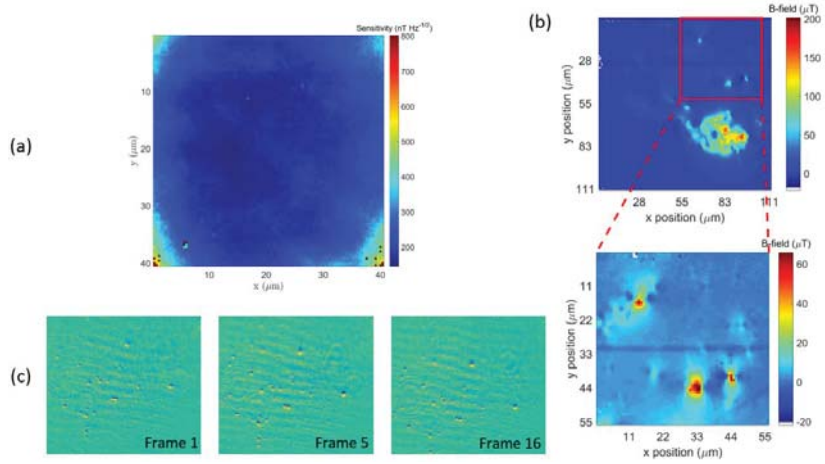

Figure (a) Measurement of the spatial uniformity of the magnetic sensitivity in the optical field of view. (b) Image of magnetically detected beads on the surface of the diamond. The close-up of lone beads and smaller clusters show the expected dipole field distribution. (c) A few frames from dynamical measurements of a number of beads on the diamond surface manually moved with the stage.

[1] L. Rondin, J.-P. Tetienne, T. Hingant, J.-F. Roch, P. Maletisnky and V. Jacques, "Magnetometry with nitrogen-vacancy defects in diamond", *Reports on Progress in Physics* **77** (2014)

[2] D. R. Glenn, K. Lee, H. Park, R. Weissleder, A. Yacoby, M. D. Lukin, H. Lee, R. L. Walsworth and C. B. Connolly, "Single-cell magnetic imaging using a quantum diamond microscope", *Nature methods* **12** (2015)

Avoiding RES retention of magnetic nanoparticles with a dense PEG coating. A biodistribution study by MRI, SPECT, gamma-counting and TEM

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In vitro experiments have shown the great potential of magnetic nanocarriers for multimodal imaging diagnosis[2] and non-invasive therapies, including localized externally-triggered hyperthermia and combined therapies. However, their extensive clinical application is still jeopardized by a fast retention in the reticuloendothelial system (RES) before they can hit their target. Other issue that restrains their potential performance is a slow degradation and excretion that increase their toxicity. In this presentation, we report a promising case in which multicore iron oxide nanoparticles coated with poly(4-vinylpyridine) polyethylenglycol copolymer show low RES retention and high urinary excretion, as confirmed by Single Photon Emission Computerized Tomography, Gamma Counting, Magnetic Resonance Imaging and Electron Microscopy biodistribution studies (Fig. 1). Moreover they show a clear negative contrast in the kidneys that makes them These copolymer beads have high PEG density in their coating that may be responsible for this effect. The average hydrodynamic diameter of the iron oxide/copolymer beads is approximately 20 nm. However, they are nevertheless able to cross the glomerulus wall, which has an effective pore size of approximately 6 nm, which makes them exceptionally useful as magnetic nanocarriers as well as excellent MRI contrast agent for kidneys. Transmission Electron Microscopy inspection of kidney tissue revealed the presence of the beads in proximal tubule cells.

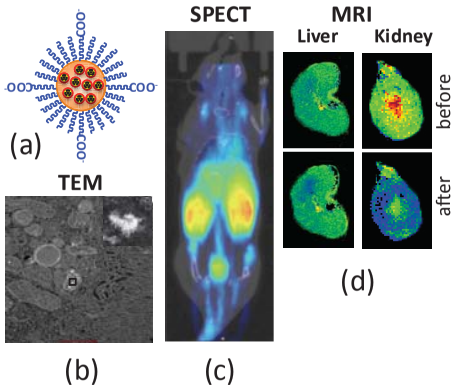

Figure 1. (a) Scheme of IO/Copolymer bead structure. (b) TEM image of a bead inside a proximal tubule cell. (c) SPECT image of a mouse 20 min after intravenous tail injection of IO/Copolymer bead suspension. (d) MRI images of the liver and a kidney of a mouse before and after an intravenous tail injection of a bead suspension.

Quantitative magnetic force microscopy for single magnetic bead characterization

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Many Lab-On-a-Chip applications rely on controlling individual magnetic beads (MBs)[1], however, standard characterization techniques focus on magnetic properties of large amounts of MBs. Here, we characterize individual MBs and the interaction between MBs and nano/micro-scaled[2,3] magnetic structures used in magnetophoretic devices, using custom-made atomic force microscopy (AFM) probes with a MB attached to the apex[4] (e.g. Fig. 1(a) probe with a Dynabeads™ M-270 MB) and variable field AFM (Fig. 1(b)).

Variable field AFM characterization is a three step protocol. First, we measure the deflection of the probe in field (Fig. 1(c)) to derive magnetic properties of the MB, such as coercivity, saturation field, and magnetic moment. Then, we improve the estimation of the magnetic moment of the MB[5], and generate a first approximation of the stray field, by using quantitative magnetic force microscopy (qMFM) to image a reference magnetic sample with the custom-made probes. Finally, we measure the MB's stray field by determining the switching field of out-of-plane magnetization nanostructures when exposed to a bias field plus the MB stray field[2]. Additionally, thermomagnetically patterned hard magnetic films were imaged using the custom-made probes to test how MB's magnetic properties affect the interaction with magnetic nano/microstructures, (e.g. Fig. 1(d) shows strong attraction along magnetic domain's edges and centre).

In this study we compared commercial MBs and custom-made nanoparticles (i.e. magnetite needle-shaped as well as anisometric nanoparticles synthesized using aerial oxidation of iron(II) sulphate and subsequent hydrogen reduction), by measuring their magnetic parameters and interaction with different designs of magnetophoretic films. Thus, providing a simple, direct and reliable method to allow quantifying single MB for Lab-On-a-Chip applications.

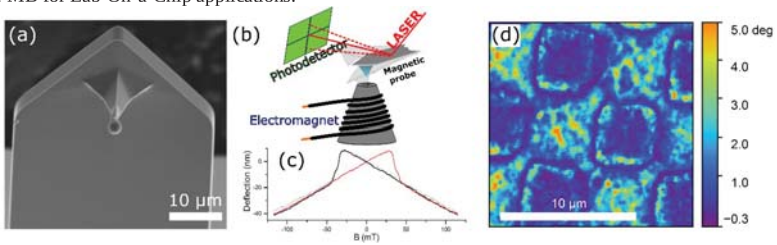

Fig. 1. (a) SEM image of a MB in an AFM probe. (b) Schematics variable field AFM. (c) Probe deflection versus magnetic field. (d) MFM image of a thermomagnetically patterned film obtained using a MB-probe.

Acknowledgements

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References

1. Lim, B., Vavassori, P., Sooryakumar, R. & Kim, C. Nano/micro-scale magnetophoretic devices for biomedical applications. *J. Phys. D. Appl. Phys.* **50,** 33002 (2017).
2. Corte-León, H. *et al.* Magnetic scanning gate microscopy of CoFeB lateral spin valve. *AIP Adv.* **7,** 56808 (2017).
3. Zanini, L. F., Dempsey, N. M., Givord, D., Reyne, G. & Dumas-Bouchiat, F. Autonomous micro-magnet based systems for highly efficient magnetic separation. *Appl. Phys. Lett.* **99,** 232504 (2011).
4. Ponomareva, S. Development and advanced characterization of high performance hard magnetic materials. Materials Science [cond-mat.mtrl-sci]. Université Grenoble Alpes, 2017. English.
5. Panchal, V. *et al.* Calibration of multi-layered probes with low/high magnetic moments. *Sci. Rep.* **7,** 7224 (2017).

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Invited Talk

Magnetic Particle Imaging Emerges into Preclinical Research: Hardware, Nanoparticles, and Animal Models

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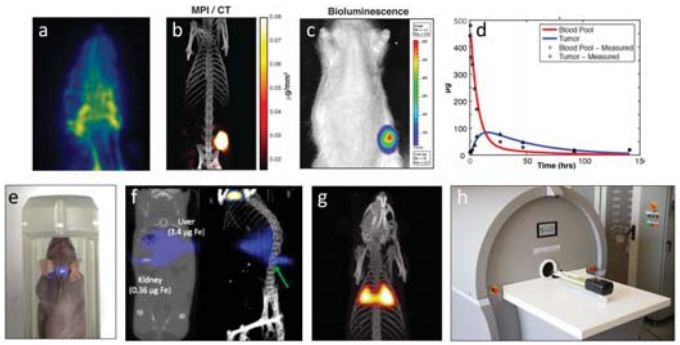
Magnetic Particle Imaging (MPI) is an emerging tracer imaging technique that directly detects superparamagnetic iron oxide (SPIO) nanoparticles with exceptional contrast and high sensitivity at millimeter-scale resolutions.[1] MPI's contrast is similar to nuclear medicine, but without the complex workflow, safety, and half-life limitations of a radioactive tracer. These capabilities help fill in some of the gaps of current technologies and position MPI as complementary to anatomical imaging techniques such as MRI and X-ray/CT.

The magnetic properties of the nanoparticle tracers seen by MPI define the technique's image resolution and sensitivity. This is because the MPI signal arise from the interaction between a magnetic nanoparticle, a strong magnetic field gradient, and a time varying magnetic drive field.[2,3] To improve MPI's spatial resolution, we and others have researched SPIOs with varying core diameters, since Langevin steady-state physics predicts a cubic resolution improvement with increasing SPIO core size. This effort has already produced exciting results, effectively reducing the FWHM resolution by a factor of two and increasing sensitivity an order of magnitude.[4] However, much remains unknown about the physical mechanisms and trade-offs for further improvements.

Our group and others have begun testing MPI on animal models. We have used long circulating PEG coated nanoparticles to image the brain, detect cancers,[5] detect gut bleeds,[6] and visualize traumatic brain injury.[7] MPI's high sensitivity also makes it well suited for cell tracking. For example, we have tracked therapeutic neural stem cells for three months[8] and the biodistribution of mesenchymal stem cells post administration.[9] In the future, we see opportunities for MPI tracking of immune therapies or immune cells to sites of inflammation such as cancer.

After more than a decade in development, MPI has now emerged from the engineering laboratory and into commercial products. This is important as MPI hardware is completely distinct from other magnetic imaging hardware; MPI scans cannot be obtained with an MRI scanner. We now look to the future as our group and others continue to push the limits of the technique as we scale MPI to clinical sizes.



(a) Blood pool image of a rat brain using a PEG coated tracer. **(b)** Breast Cancer (MDA-MB-231) visualization using the EPR effect using the same PEG coated tracer.[5] **(c)** The cancer location detected with MPI matches with bioluminescence. **(d)** MPI is inherently linearly quantitative and can quantitate the total amount of tracer in the tumor and blood pool.[5] **(e)** Tracking macrophages (RAW264.7) in a mouse brain. **(f)** Tracking islet cells implanted under the kidney capsule at day 14 post-transplant.[10] **(g)** Tracking $5x10^6$ mesenchymal immediately after tail vein administration.[9] **(h)** Commercial small animal MPI imager.

References: 1. Gleich, B. & Weizenecker, J. *Nature* **435,** 1214–1217 (2005). 2. Goodwill, P. W. & Conolly, S. M. *IEEE Trans Med Imaging* **29,** 1851–1859 (2010). 3. Rahmer, J., Weizenecker, J., Gleich, B. & Borgert, J. *BMC Med Imaging* **9,** 4 (2009). 4. Kemp, S. J., Matthew Ferguson, R., Khandhar, A. P. & Krishnan, K. M. *RSC Adv* **6,** 77452–77464 (2016). 5. Yu, E. Y. *et al. Nano Lett* **17,** 1648–1654 (2017). 6. Yu, E. Y. *et al. ACS Nano* **11,** 12067–12076 (2017). 7. Orendorff, R. *et al. Phys Med Biol* **62,** 3501–3509 (2017). 8. Zheng, B. *et al. Sci Rep* **5,** 14055 (2015). 9. Zheng, B. *et al. Theranostics* (2015). 10. Wang, P. *et al. Quant. Imaging Med. Surg.* **8,** 114–122 (2018).

Development of MAGpoint: Rapid Isolation, Detection and Targeted Sequencing of Circulating Tumor Cells (CTCs) from Blood Liquid Biopsies at the Point Of Care

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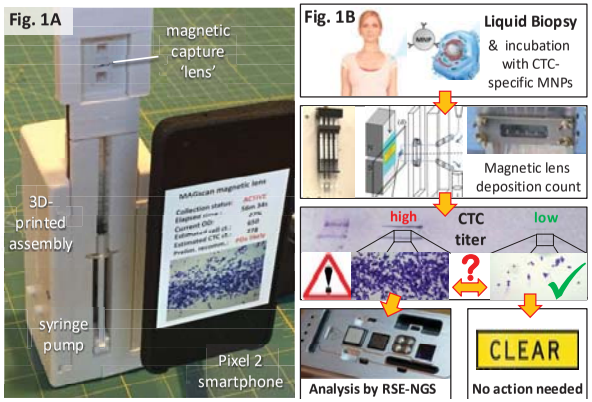
Blood contains various types of cancer-derived materials that allow a detailed molecular genetic analysis: intact circulating tumor cells (CTCs), tumor-derived exosomes and cell-free circulating tumor DNA. They shed from primary or metastatic tumors and - although rare - are thought to be enriched for metastatic precursors, and they can be analyzed to improve the accuracy of prognosis and treatment outcome.

However, the advantage of such analysis is currently restricted by the need for overly complex cellular isolation and CTC processing platforms. According to experts in this field, ultimate therapeutic success will require a high level of integration between real-time diagnostic measurements and targeted interventions.

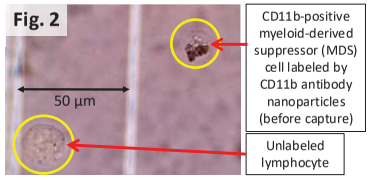
Figure 1A: Our goal is to integrate a magnetic capture 'lens' and the associated fluidics with a smartphone using simple, microscopy-enabled add-on optics and magnetically select CTCs from a 2 5 mL liquid biopsy blood sample through tumor antigen-specific binding, such as EPCAM.

Figure 1B: CTC deposit formation will be quantified and reported in real-time by rapid imaging software. If CTC titers are high, the captured cells are transferred from the deposition slide for optional, cancer-specific gene enrichment by region-specific extraction (RSE) and genetic analysis by next-generation sequencing (NGS).



We have separately demonstrated the highly sensitive collection of tagged cells with the 'magnetic lens' (**Figure 1**), high-resolution imaging of magnetically captured cells using a smartphone (**Figure 2**) and the ability to achieve same-day NGS results from RSE-targeted regions on the MinION platform of Oxford Nanopore.

RSE is the only commercial DNA enrichment technology that can preserve exceptionally long (20-50kb) chromosomal segments during capture. It is therefore particularly well-suited for newer, long-read NGS platforms. The ability to process such long reads is an essential advantage in successfully resolving complex mutations that are typical in metastatic cancer that cannot be resolved by short read capture technologies.



CTC-derived DNA - even from single cells – is readily amplified by commercially available kits (such as REPLI-g) and can then be sequenced at selected regions of interest that are predictive of disease- and patient-specific treatment.

If successful, our 'MAGpoint' system may become a tool for clinicians to quickly (i.e. same day and in their office) determine CTC titers as well as - if indicated - to determine the heterogeneous and dynamic genetic landscape of a patient's metastatic cancer so that the best individualized treatment options can be selected.

Colloidal Stability of Ferrofluids for Magnetic Density Separation

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Colloidal stability in external field is paramount for all applications of ferrofluids. A new example is the recycling of solid materials via magnetic density separation (MDS). We are collaborating with the Urban Mining Corporation,[1] which is building an industrial scale facility [2] to sort various solids based on their magnetic levitation in a ferrofluid, see **Figure a**. The societal objective is to promote a circular economy, avoiding to waste energy and material resources. In MDS technology, degradation of the ferrofluid due to sedimentation of the magnetic nanoparticles must be kept to a minimum.

To guide the development of new ferrofluids that can operate under demanding magnetic conditions, new analytical methods are required that characterize the colloidal stability of relatively concentrated dispersions of magnetic nanoparticles in an external magnetic field gradient. One of the techniques that we are developing involves sensitive time-dependent measurements of the external field produced by a ferrofluid while it is placed on top of a strong permanent magnet; a theoretical analysis of the data allows us to calculate the magnetophoretic sedimentation rate. Another approach uses X-ray transmission [3] to detect time-dependent concentration profiles, confirming the sedimentation deduced from our magnetic measurements, see **Figure b**. Comparison with other characterizations (TEM, VSM, DLS, AUC…) informs on the question whether the magnetic field generates dipolar structures,[4] which would sediment more rapidly than single particles and thus decrease stability.

In our presentation, we will demonstrate the difference in field-dependent colloidal stability of two types of aqueous ferrofluid: a commercial fluid with tiny aggregates and a more stable homemade fluid in which the nanoparticles are dispersed at the single-particle level.

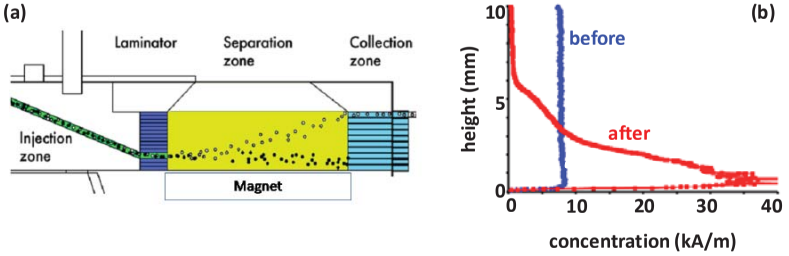

Figure. (a) Industrial approach to magnetic density separation for the recycling of materials; the figure is reproduced from Ref. 1. **(b)** Experimental concentration profile of a commercial ferrofluid before and after prolonged exposure to a magnetic field gradient of 20 T/m. The concentration is expressed in terms of the local saturation magnetization of the ferrofluid.

References

1. www.umincorp.com
2. https://www.youtube.com/watch?v=kVnmflYpkq0
3. https://www.lum-gmbh.com/lumireader-x-ray_en.html
4. Klokkenburg et al., *Phys. Rev. Lett.* 97, 185702 (2006).

Efficient capture of highly diffusive magnetic nanoparticles using micro-magnet arrays

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We have developed hard and soft micro-magnet arrays for a range of bio-medical applications, using both film [1,2,3]) and particle [4] based approaches. The magnetic field profiles produced by the micro-magnet arrays have been characterised by Scanning Hall Probe Microscopy [5], Magnetic Force Microscopy [6] and Magneto-Optic imaging with the aid of indicator films [7]. The forces exerted by micro-magnets on superparamagnetic beads have been quantified using scanning particle microscopy [8,9]. Examples of applications include cell sorting in micro-fluidic channels [10], immunoassays [11], stem cell networking [12] and mechano-transduction studies at embryo [9] and single cell [13] level.
In this work we report on a study of the capture of magnetic nanoparticles (MNP) with a magnetic core of just 12 nm in diameter, using thermomagnetically patterned micro-magnets [2]. Comparison of measured capture kinetics with numerical modelling reveals that a threshold MNP concentration exists below which capture is diffusion-driven and above which it is convectively-driven. This comparison also shows that two-way fluid-particle coupling is responsible for the formation of convective cells, the size of which is governed by the height of the droplet. Our results indicate that for a suspension with a nanoparticle concentration suitable for bioassays (around 0.25 mg.ml^{-1}), all particles can be captured in less than 10 minutes. Since nanoparticles have a significantly higher surface-to-volume ratio than the more widely used microparticles, their efficient capture should contribute to the development of next generation digital microfluidic Lab-on-Chip immunoassays.

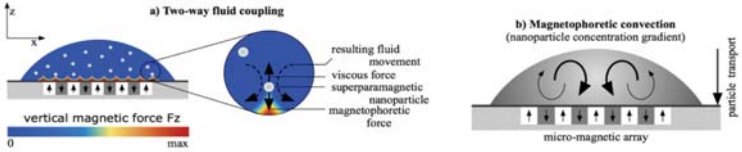

Figure 1: Superparamagnetic nanoparticles are captured locally at the junctions between micro-magnets, accelerating their surrounding fluid. (b) viscous forces then carry distant nanoparticles towards the junctions, where they are in turn trapped. This two-way magnet-viscous coupling leads to magnetophoretic convection. (similar to natural convection) resulting in efficient trapping of highly diffusive nanoparticles.

References

[1] A. Walther, C. Marcoux and B. Desloges, et al., J. Magn. Mag. Mat. 321 (2009) 590
[2] F. Dumas-Bouchiat, L.F. Zanini, M. Kustov, et al., Appl. Phys. Lett. 96 (2010) 102511
[3] D. Le Roy, G . Shaw, R. Haettel et al., Materials Today Communications 6 (2016) 50
[4] N. M. Dempsey, D. Le Roy, et al., Appl. Phys. Lett. 104 (2014) 262401
[5] G. Shaw, R. B. G Kramer, N. M. Dempsey and K. Hasselbach, Rev Sci Inst 87 (2016) 113702
[6] G. Ciuta, F. Dumas-Bouchiat, N. M. Dempsey et al., IEEE Trans Mag 52 (2016) 6500408
[7] R. Grechishkin, M. Kustov, S. Ilyashenko, et al., J. Appl. Phys. 120 (2016) 174502
[8] S. Ponomareva, PhD Thesis, Universite Grenoble Alpes, July 2017
[9] D. Mitrossilis, J.C. Röper, D. Le Roy, B. Driquez, et al, Nat. Comm. 8 (2017) 13883
[10] J. Pivetal, S. Toru, et al., Chemical Sensors and Actuators B 195 (2014) 581
[11] S. Delshadi, G.Blaire, P. Kauffmann, et al., Bioanalysis 9 (2017) 517
[12] V. Zablotskii, A. Dejneka, S. Kubinova et al., PLoS ONE 8(8) (2013) e70416
[13] C. M. Bidan, M. Fratzl, A.Coullomb, Scientific Reports 8 (2018) 1464

Invited Talk

Magnetic Nanocapsules for Brain Repair and Imaging after Stroke

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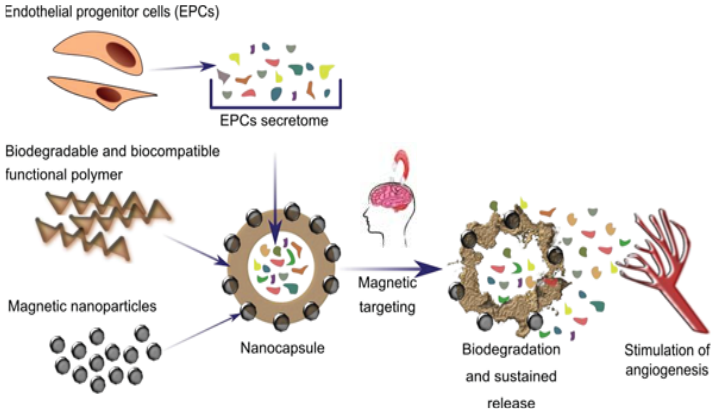
According to the World Health Organization, 15 million persons suffer a stroke worldwide each year. However, the only available treatment is the acute thrombolytic therapy (pharmacological or mechanical) which is being administered to less than 10% of stroke patients due to strict selection criteria. In contrast, neuro-repair treatments could offer the opportunity to include most stroke patients by extending the therapeutic time window.

Novel magnetic nanocapsules could achieve tissue repair in the context of an ischemic event delivering therapeutic growth factors into the injured brain. In the context of the MAGBBRIS project, I will show the results so far demonstrating that that growth factors, secreted by endothelial progenitor cells with proved potential to induce tissue repair, can be encapsulated in magnetic nanocapsules and can be grafted into mouse brains. In the ischemic brain, the capsules will be retained in the vasculature by an external magnetic field. We expect that in the future, our approach will provide an advanced therapy that could be translated to a clinical stage as noninvasive, safe and available to most stroke patients.



Schematic representation of the proposed neurorepair strategy

References:
Carenza et al. Encapsulation of VEGF165 in magnetic PLGA nanocapsules for potential local delivery and bioactivity into human brain endothelial cells. *Journal of Materials Chemistry B*. 2015;3, 2538 & *In vitro* angiogenic performance and *in vivo* brain targeting of magnetized endothelial progenitor cells for neurorepair therapies. *Nanomedicine: NBM. 2014 Jan;10(1):22.*
Rosell et al. Endothelial Progenitor Cells or their Secreted Factors Enhances Angiogenic and Neurogenic Responses after focal Cerebral Ischemia in Mice. *PLoS One.* 2013;8(9):e73244.

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Protein coated magnetic nanoparticles for medical applications

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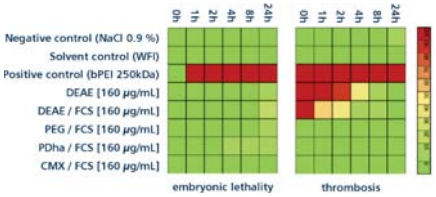
Hybrid magnetic nanoparticles are of major interest for medical applications. Before these promising materials can be applied, cytotoxicity issues as well as interactions with the human body have to be investigated. One of the key factors to address these questions is the investigation of the evolving so-called protein corona, which immediately forms, when magnetic nanoparticles (MNP) are exposed to the blood stream or any other protein source. Therefore, we prepared protein-coated MNP by *in vitro* serum incubation, varying the protein content of the incubation media, and analyzed the evolving corona as well as the interactions of these hybrid particles with living human brain microvascular endothelial cells (HBMEC) and in a hen's egg test on the chick area vasculosa (HET-CAV).

For this, iron oxide MNP with various coatings were incubated in defined mixtures of cell culture medium and fetal calf serum (FCS). Physical properties of the MNP were determined before and after the incubation procedure by means of vibrating sample magnetometry (VSM), thermogravimetric analysis (TGA), transmission electron microscopy (TEM), sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and zeta potential measurements. The effects of the protein coating on the cell viability were investigated for HBMEC by the Presto Blue™ assay and by real time cell analysis (RTCA). By means of flow cytometry of fluorochrome-labelled particles, the precise particle-cell interactions with HBMEC were studied. To assess toxicity in a complex biological system a HET-CAV with systemic injection was performed.



Probability of embryonic lethality or thrombosis for different particle coatings tested in the HET-CAV Model.

Zeta potential measurements, SDS-PAGE and TGA clearly showed the influence of FCS concentration on the formation of the protein corona. A cytotoxicity masking effect of the protein corona was observed for initially toxic MNP coatings. Flow cytometry investigations indicated that the protein coating reduces the particle-cell interactions. Furthermore, HET-CAV shows no cytotoxic effects for FCS-coated MNP with a neutrally or negatively charged surface polymer, whereas FCS coating of MNP with a positively charged surface polymer reduced the tendency to form agglomerates (thrombosis) in the blood stream after systemic injection.

Ongoing investigations focus on corona evolution depending on different parameters like surface charge, temperature and incubation time as well as on *in vitro* and *in vivo* experiments on the biological fate of incubated MNP.

Acknowledgements
This work is supported by Deutsche Forschungsgemeinschaft (DFG) in frame of priority programme 1681 (FKZ: CL202/3-2, DU 1293/4-2, SCHA1640/7-1).

Targeted Magnetic Intra-Lysosomal Hyperthermia produces lysosomal reactive oxygen species and causes Caspase-1 dependent cell death.

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Cancer is a leading cause of death with millions of new people diagnosed with cancer every year. One major difficulty in anti-cancer therapy is the multidrug resistance which appears during treatments. Recently, studies have shown that cancer cells resistant to traditional therapies are sensitive to agents that induce lysosome membrane permeabilization causing lysosomal cell death. To date, lysosomal cell death has been obtained using lysosomotropic agents which could not selectively target lysosomes of tumoral cells. In this context, nanotherapy based on Magnetic Intra-Lysosomal Hyperthermia (MILH) generated by magnetic nanoparticles (MNPs) that are grafted with ligands of receptors overexpressed in tumors appears to be a very promising therapeutic option. Strikingly, in such approach, no perceptible temperature rise in the cell medium occurred during high frequency alternating magnetic field (AMF) exposure. Thus, MILH differs from standard magnetic hyperthermia whereby tumor eradication is achieved with large doses of MNPs which cause a temperature elevation of the whole tumor. As a proof-of-concept, we previously showed that minute amounts of iron oxide MNPs targeting gastrin receptor (CCK2R) are internalized by tumoral cells through a CCK2R-dependent physiological process, accumulated into their lysosomes and killed tumoral cells upon AMF application through lysosomal cell death [1,2]. However, mechanisms whereby MILH induces cell death are still elusive. Herein, we provide evidences that MILH causes cell death through a non-apoptotic signaling pathway. The mechanism of cell death involves temperature elevation at the nanoparticle periphery which enhances the production of reactive oxygen species through the lysosomal Fenton reaction. Subsequently, MILH induces the lipid peroxidation of the lysosome membrane, lysosomal membrane permeabilization and the leakage of lysosomal enzymes into the cytosol, including Cathepsin-B which activates Caspase-1 but not the apoptotic Caspase-3 [3]. These data highlight the clear potential of MILH for the eradication of tumors overexpressing receptors which can be adapted to eradicate all cancer cell types including apoptosis-resistant cancer cells.

References

[1] Sanchez C et al. (2014) Targeting a G-Protein-Coupled Receptor Overexpressed in Endocrine Tumors by Magnetic Nanoparticles To Induce Cell Death. ACS Nano 8(2):1350-63.
[2] Connord V et al. (2015) Real-time Analysis of Magnetic Hyperthermia Experiments on Living Cells under Confocal Microscope. Small 11(20):2437-45.
[3] Clerc P et al (2017) Targeted Magnetic Intra-lysosomal Hyperthermia produces lysosomal reactive oxygen species and causes Caspase-1 dependent cell death. J. Controlled Release (in press).

Superparamagnetic Particle Scaffold for Regenerating Damaged Neural Tissue

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Spinal cord injury affects about half a million people each year and typically leads to paralysis below the injury site. Some treatments are capable of restoring some function, however there currently is no cure for spinal cord injury.

One challenge in achieving complete functional recovery is to develop approaches that encourage directional axonal regeneration that extends through the lesion cavity and reconnects the two severed ends of the spinal cord. Axonal regeneration typically fails because of the formation of inhibitory fibrotic glial scar tissue soon after injury. It is therefore of critical importance to quickly fill in the injury site with a suitable, biocompatible matrix, such as a hydrogel, to stop scar tissue from forming. However this treatment alone does not promote axonal regeneration.

Polymeric fibers with nanoscale or micro scale diameter have successfully been used to stimulate directional axonal regrowth in animal models of spinal cord injury. However the surgical insertion and adjustment of pre-spun, pre-fabricated, 3D-printed or custom-manufactured fiber plugs would be complex, time-consuming and expensive and require an accurate three-dimensional shape determination of the lesion cavity. All of these resources may not be available during the limited time during which reconstructive intervention of spinal cord injury can be successful.

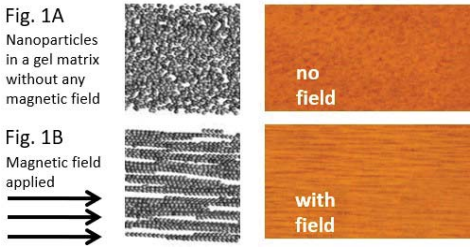
We hope to overcome these problems by providing a method for treating a patient of spinal cord injury with an injectable formulation, called 'Fibermag'.

Magnetic particles in a biocompatible, matrix-forming compound will be injected into the lesion cavity (Fig. 1A) and reorient themselves in response to an externally applied magnetic field.

The result is the formation of extended chains of particles in the orientation of the field lines and the desired axonal regrowth (Fig. 1B).

Fig. 1A
Nanoparticles in a gel matrix without any magnetic field

Fig. 1B
Magnetic field applied



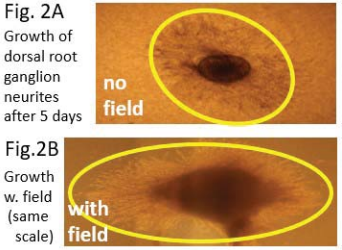
A near-homogeneous magnetic field is applied where the magnetic field lines are aligned in the direction in which the nerve cells are intended to grow and connect. It is possible to create magnetic fields from an external source (using permanent or electromagnets) that do not exhibit any significant field gradient across the treatment area. Externally placed soft magnetic materials can help shape the field in the patient's body into the desired orientation.

We optimized the process of in-situ formation of stable 'Fiberguides' from magnetically aligned nanoparticles in experiments with chicken and rat dorsal root ganglions (DRGs) in the presence of biocompatible nanoparticle formulations and chain crosslinking agents (Fig. 2A).

We successfully demonstrated preferential growth of newly formed neurites in the horizontal orientation of these Fiberguides (Fig. 2B). Fiber studies show that diameters between 500-2000 nm achieve the best neurite outgrowth. Larger particles, due to their larger magnetic moment, are easier to control in a viscous hydrogel matrix in order to achieve the desired orientation of the resulting Fiberguides.

Fig. 2A
Growth of dorsal root ganglion neurites after 5 days

Fig.2B
Growth w. field (same scale)



The Fibermag formulation can be used in conjunction with biomaterials that release neurotropic growth factors and thereby further increase axonal regeneration.

We believe the simplicity of our approach may become a minimally invasive way to use the preciously short window of time during which reconstructive intervention of spinal cord injury can take place before scar tissue formation and other processes irreversibly prevent a successful restoration of spinal cord function.

Magnetogenetic Activation of Small GTPases with MagIcS Nanoparticles Inside Living Cells

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Abstract

Dynamic remote control of cellular functions is still a major challenge in academical and biomedical research. Recently, we developed a technique which we referred to as magnetogenetics. In this proof of concept study, we demonstrated magnetic control of the small Rho-GTPase Rac1 by exploiting magnetic microparticles acting as magnetically controllable carrier of a biological function.[1] However, unbiased and robust application of this approach was hindered by the large size of the used particles. Here, we present latest developments for magnetogenetic activation and probing of GTPases inside living cells. For this purpose, a ferritin-based nanobiomaterial was engineered yielding **Mag**netic **I**ntra**c**ellular **S**tealth nanoparticles (MagIcS NPs).[2] This semi-synthetic NPs of 25nm in diameter were sufficiently small to freely diffuse in the cytoplasm. Most important, MagIcS NPs were not recognized by intracellular machineries based on molecular interactions with the cellular environment, which was a key pre-requisite for unbiased and specific application inside living cells. For loading numerous cells simultaneously with MagIcS nanoparticle approaches were investigated, which were based on (i) scrape loading using glass microbeads, (ii) pore loading by exploiting streptolysin-O and (iii) plasma membrane penetration loading using a protein transduction domain derived from Hepatitis-B virus. With these tools, successful magnetic manipulation of targeted proteins on the surface of the particles in the cytoplasm could be demonstrated. Furthermore, by exploiting re-engineered FRET-biosensors, MagIcS NPs enabled the site-specific and reversible activation of GTPases on a subcellular scale. Overall, magnetogenetic manipulation by exploiting MagIcS nanoparticles provide a novel non-toxic and robust tool for magnetic control of protein activation inside living cells.

[1] Subcellular control of Rac-GTPase Signalling by Magnetogenetic Manipulation Inside Living Cells; F. Etoc, D. Liße, Y. Bellaiche, J. Piehler, M. Coppey, M. Dahan; (2013) *Nature Nanotechnology* **8**, 193-198

[2] Engineered Ferritin for Magnetogenetic Manipulation of Proteins and Organelles Inside Living Cells; D. Liße, C. Monzel, C.Vicario, J. Manzi, I. Maurin, M.Coppey, J. Piehler and M. Dahan; (2017) *Advanced Materials* ***29 (42)***

Differentiation status of stem cells impacts the biotransformations of internalized magnetic nanoparticles: *in situ* investigations using the magnetic imprint

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Magnetic nanoparticles offer exciting possibilities for biomedical applications. More specifically, iron oxide nanoparticles (IONPs) have attracted a lot of attention in terms of biocompatibility for to their iron-based core that can potentially be assimilated by the intrinsic iron metabolism of the organism. However, the *in situ* characterization of their interactions with complex biological systems remains difficult. We recently demonstrated that IONPs' superparamagnetic properties can be used to assess their intracellular fate in a spheroid tissue model[1]. Here, we use this unprecedented methodology to assess the impact of the differentiation status of human mesenchymal stem cells (MSC) on IONPs' transformations.

Human MSC were incubated for 30 min with citrate-coated IONPs (maghemite, γ-Fe_2O_3) in concentrations ranging from 0.1 to 0.4 mM. Labeled MSC were seeded ($2x10^5$ cells/sample) and subjected to chemical-mediated adipogenic, osteogenic, or chondrogenic differentiation. Along 21 days of differentiation, magnetic moments of the samples were determined by vibrating sample magnetometry (VSM), which provides highly sensitive detection of magnetism. Expression of genes involved in iron metabolism (e.g. L-ferritin, H-ferritin) was assessed by quantitative RT-PCR, and transmission electron microscopy (TEM) was achieved to locate and characterize the nanoparticles.

Important differences were evidenced between the differentiation pathways. Under chondrogenesis (Figure, top), a decrease in magnetic iron mass was observed from day 0 to 21 that indicates a progressive degradation of the IONPs. TEM images show light gray nanospots at day 21 attributed to the storage of iron released from the nanoparticles in the ferritin. These observations correlate with the overexpression of the gene coding for the L-subunit of ferritin. Surprisingly, under adipogenesis (Figure, bottom) and osteogenesis, a different pattern is observed. Upon initial decrease in magnetism from day 0 to 3, a significant increase is observed at day 9 that remains significant at day 21. As total iron mass didn't vary from day 0 to 21, this surprising pattern can only be explained by the neo-synthesis of magnetic iron *in cellulo*. TEM images here indicate dark nanospots confined in the endosomes corresponding to magnetic nanoparticles (size and structure), while gene analysis demonstrate the upregulation of the H-subunit of ferritin, which has a ferroxidase activity and might be playing a role in this phenomenon.

The unexpected re-magnetization phenomenon observed here evidences the intrinsic capacities of human MSC to neo-synthesis iron oxide *in situ* upon initial degradation of internalized IONPs. This surprising pattern, observed under specific differentiation pathways only, seems linked to the expression of genes involved in iron metabolism.

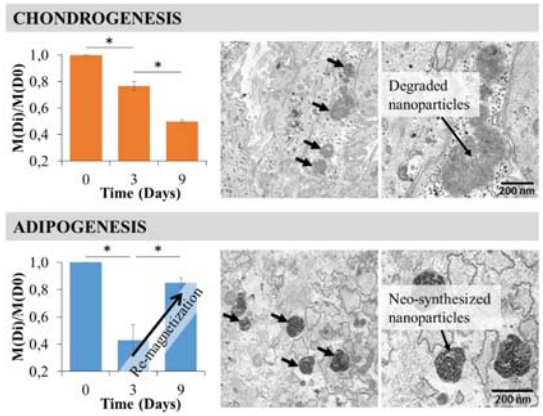

Figure. Evolution of magnetism along mesenchymal stem cell differentiation is assessed by vibrating sample magnetometry (graphs) with M(Di)/M(D0) representing the magnetic moment at a given day i relatively to day 0. Structure of internalized nanoparticles is determined by transmission electron microscopy (images, taken at day 21). Arrows indicate the endosomes, where internalized nanoparticles are present at day 0. Under chondrogenesis, the nanoparticles are progressively degraded as indicated by a loss in magnetism, and the released iron is stored in cytoplasmic and endosomal ferritin seen as light gray nanodots on the images. Under adipogenesis, IONPs are first degraded, indicated by a loss in magnetism from day 0 to 3. Surprisingly, from day 3 to 9, magnetism increases. This re-magnetization phenomenon indicates the neo-synthesis of magnetic nanoparticles *in cellulo*, a processus that seems endosomal as magnetic nanoparticles are observed in the endosomes only at day 21.

Reference

1. Mazuel, F. *et al.* Massive Intracellular Biodegradation of Iron Oxide Nanoparticles Evidenced Magnetically at Single-Endosome and Tissue Levels. *ACS Nano* **10,** 7627–7638 (2016).

Magnetic actuated SPCL scaffolds dopped with iron oxide magnetic nanoparticles as mechano-instructive platforms for tendon tissue engineering

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Tendons are mechano-responsive tissues and their normal function greatly depends on mechano-stimulation mechanisms. Moreover, both TGF-β signaling cascade and mechanical stimulation have been found to activate the Smad2/3 molecules suggesting that stress-activated TGF-β signaling drives tenogenesis [1-3]. In a previous study, we reported that mechano-magnetic forces stimulated the TGF-β signal transduction pathway via Activin A type II (ActRIIA) receptor, inducing the phosphorylation of Smad2/3 in human adipose stem cells (hASCs) and leading to the acquirement of tenogenic phenotype [3].

Thus, in the present study, we assessed the influence of magnetic actuation provided by a vertical oscillating magnetic bioreactor (MICA Biosystems Ltd) in the mechano-responsive TGF-β/Smad2/3 signaling pathway of hASCs laden on 3D magnetic responsive scaffolds, specifically assessing the expression of tendon related genes and proteins.

Aligned magnetic fibrous scaffolds were fabricated by 3D printing made of a blend of starch and polycaprolactone (SPCL) and incorporating iron oxide magnetic nanoparticles (magSPCL) [4]. hASCs were then seeded onto magSPCL and SPCL scaffolds and cultured in αMEM medium (basal) or αMEM supplemented with the TGF-β-like ligand, Activin A (20 ng/mL), under magnetic actuation (1Hz, 1h/every other day). The phosphorylation of Smad2/3 was assessed within 24h upon seeding and 1h after magnetic stimulation, by ELISA assay. Furthermore, the tenogenic commitment of hASCs was assessed by real time RT-PCR, immunocytochemistry and Sirius Red/Fast Green Collagen kit.

Overall, results showed an increment of pSmad2/3 proteins in hASCs laden in magSPCL scaffolds and, after 7 days in culture, collagen proteins increased in magSPCL exposed to magnetic actuation and cultured in basal medium in comparison to SPCL constructs or to magSPCL constructs under static conditions. Additionally, the combination of magnetic field with magnetic responsive scaffolds stimulates the deposition of tenomodulin protein by hASCs, after 14 days in culture.

In summary, magnetic 3D scaffolds seem to influence the mechanosensing response of hASCs towards tenogenic cues, holding therapeutic promise for driving *in vitro* tenogenesis.

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[1] Jones ER, et al. Biochimica et Biophysica Acta 2013; [2] Rothrauff BB, et al. Elsevier 2015; [3] Gonçalves AI, et al. Nanomedicine: NBM 2018; [4] Gonçalves AI, et al. Advanced healthcare materials 2016.

A Systematic Approach to Endotoxin Contamination Assessment of Iron Oxide Nanoparticles for Theranostics applications

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As presented two years ago in Vancouver by Prina-Mello et al. [1], more than 30% of nanotechnologies for biomedical applications fail early preclinical assessment due to undesirable levels of endotoxin. Endotoxin is a component of gram negative bacterial cell walls and efficient contaminants of nanoparticles due to their large surface to volume ratios. The phosphate group and lipid moiety on endotoxins enable it to bind to nanomaterials with cationic and hydrophobic functional groups [2]. Low levels of this contaminant can stimulate the immune system through the upregulation of pro-inflammatory cytokines, which can result in tissue damage, and even induce septic shock. Moreover, nanoparticles have been widely reported to interfere with regulatory accepted assays for its detection [3]. This makes endotoxin a massive hurdle for the clinical translation of nanoparticles [4].

Herein, we describe a systematic approach to getting over interference between iron oxide nanoparticles and these endotoxin detection assays. Endotoxin levels were successfully identified on two lead candidate iron oxide nanoparticles provided by Chemicell GmbH under the European project NoCanTher. After confirming the levels of endotoxin, further assessment was undertaken to establish its source by assessing the levels of endotoxin in the iron oxide nanoparticles core and coating, as well as the water in which it is dispersed. Interference was encountered repeatedly during this process and overcome with various techniques. From these studies, the true source of endotoxin was established, which prevented these once lead nanoparticles from progressing into *in vivo* safety and efficacy studies, saving time, finances and animals.

References:

[1] Prina Mello A., Gobbo O., Volkov Y., Magnetic Nanoparticles Applied to Cancer Theranostics: a Translational Nanomedicine Perspective. Proceedings of the 11th International Conference on the Scientific and Clinical Applications of Magnetic Carriers, Vancouver (Canada), May 2016.

[2] Dobrovolskaia MA. Pre-clinical immunotoxicity studies of nanotechnology-formulated drugs: challenges, considerations and strategy. *Journal of controlled release : official journal of the Controlled Release Society*. 2015;220(0 0):571-583. doi:10.1016/j.jconrel.2015.08.056.

[3] Li, Y., M. Fujita, and D. Boraschi, Endotoxin Contamination in Nanomaterials Leads to the Misinterpretation of Immunosafety Results. Frontiers in Immunology, 2017. 8(472).

[4] Hannon G, Melissa Tutty and Adriele Prina-Mello. 2018. 'Immunotoxicity and Safety. Considerations for Iron Oxide Nanoparticles', Chapter 14, Book title: Clinical Applications of Magnetic Nanoparticles. Editor N. Thanh. ISBN: 9781138051553.

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Invited Talk

Avian magnetoreception: a quantum compass needle

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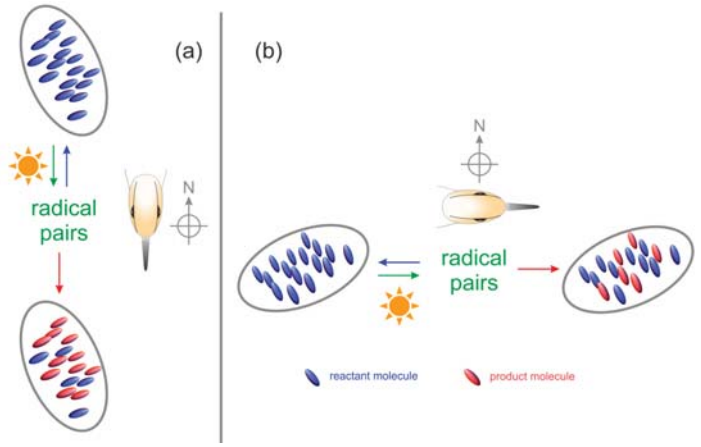
Most physical scientists would probably be sceptical about the suggestion that a chemical reaction could respond to a magnetic field as weak as that of the Earth. After all, the interaction of a molecule with a ~50 μT magnetic field is more than a million times smaller than the thermal energy (k_BT) at physiological temperature. Nevertheless, the kinetics of certain chemical reactions *are* magnetically sensitive. The key molecular species are pairs of transient free radicals whose electron-nuclear spin systems evolve coherently under the influence of internal and external magnetic interactions.

I will discuss the proposal that the coherent quantum spin-dynamics of photo-induced radical pairs in cryptochromes (photo-active proteins) could be the mechanism of the light-dependent magnetic compass sense of migratory birds[1].



The principle of a radical-pair compass. Reactant molecules (*blue*) are photochemically converted into product molecules (*red*). This transformation occurs via radical-pair intermediates, which can either proceed forward to the products (*red arrows*) or return to the reactants (*blue arrows*). The reactants and therefore the radical pairs are aligned relative to one another and oriented within the bird's eye so that they experience a change in the direction of the Earth's magnetic field when the bird moves its head. If this change is to form the basis of a magnetic compass, it must affect the probability that the radical pairs proceed along the red and blue pathways. The figure shows, schematically, the case in which more efficient conversion of reactants to products occurs when the bird's head is (*a*) aligned with the north–south axis than when it is (*b*) aligned with the east–west axis.

Reference

1. The radical-pair mechanism of magnetoreception, P. J. Hore and H. Mouritsen, *Annu. Rev. Biophys.*, **45** (2016) 299-344.

Development of a method to analyse the dynamic magnetic behavior of magnetic nanoparticles during cellular uptake with high temporal resolution

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Magnetic nanoparticles (MNP) are of great interest in bio- and nanomedicine. Among a number of established techniques Magnetic Particle Spectroscopy (MPS) is capable for specific detection of MNP in biological systems and has been proven to be ideally suited to quantify the cellular uptake of MNP. Furthermore, changes in the measured MPS signals of the MNP during cellular uptake were correlated with cellular nanoparticle processing and underline the influence of biochemical components in the extracellular matrix on the magnetism of the MNP [1]. Nevertheless, the main physical and biomolecular origins of these interactions taking place at the initial contact of MNP with the extracellular matrix still need to be discovered.

To investigate the early MNP-cell interaction we used MPS which specifically detects the non-linear magnetic response of MNP exposed to an oscillating magnetic field. With our setup we harnessed the high measurement speed of MPS with a minimum temporal resolution of 40 µs and the high sensitivity ($5{\cdot}10^{-12}$ Am^2). To enable the measurement in the very first seconds of MNP-cell interaction a dedicated sample holder made of quartz glass was developed. The sample holder was additionally equipped with a capillary to transfer the MNP in a cell suspension at the ground of the holder during the running MPS measurement (sample chamber temperature 37°C). The overall sample volume (cell suspension and added MNP) was adjusted so that the potential settling of cells and MNP during the measurement have no impact on the MPS signal. To improve the signal-to-noise ratio the MPS signal was averaged resulting in a final temporal resolution of 4 s. Finally, THP-1 cells were incubated with citrate coated very small iron oxide nanoparticles (VSOP) using the developed setup and procedure. We observed a significant flattening of the MPS spectra directly after injecting the VSOP into the cell suspension and a steady increase during the acquisition time of 760 s (see Fig. blue circles). In contrast, no signal change was observed for MNP injected in the cell medium (phosphate buffered saline, see Fig. grey squares). This rapid MPS signal change was correlated with VSOP binding to the outer cell membrane as visualized by microscopy (TEM).

The results reveal that MPS is a powerful tool to monitor the interaction of MNP with cells. Due to the high temporal resolution of the MPS method it is possible to investigate magnetic changes taking place at the initial contact of MNP with the extracellular matrix. Further activities are underway to investigate different cell types and cell states to estimate the potential of a functional analysis of cells by MPS.

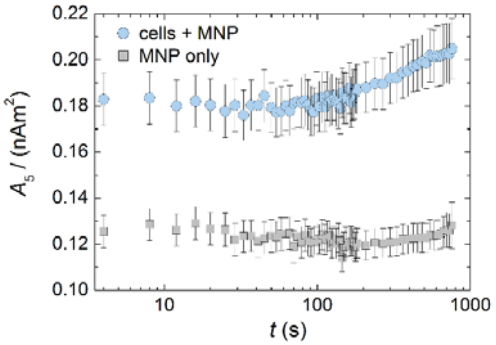

Fig.: Incubation time dependent change of VSOP magnetic behaviour during cellular uptake is expressed by the 5th harmonic of the MPS signal (blue circles). The MPS signal remains unchanged for MNP suspended in cell medium only (grey squares).

References:

[1] Poller WC, Löwa N, Wiekhorst F, Taupitz M, Wagner S, Möller K, Baumann G, Stangl V, Trahms L, Ludwig A. Magnetic Particle Spectroscopy Reveals Dynamic Changes in the Magnetic Behavior of Very Small Superparamagnetic Iron Oxide Nanoparticles During Cellular Uptake and Enables Determination of Cell-Labeling Efficacy. J Biomed Nanotechnol 2016; 12: 337-346.

A Handheld Platform Based on Wash-Free Magnetic Bioassays for the Early Diagnosis of Influenza A Virus

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Early diagnosis of influenza A virus (IAV) is crucial for controlling the infection of possible influenza pandemic. Several kinds of nanotechnologies including silicon nanowires, carbon nanotubes, and gold nanoparticles have been developed to improve the efficiency and sensitivity of IAV detection. However, these methods often require complex instruments, which makes it difficult to realize onsite diagnosis.

Herein, we present a handheld system with wash-free magnetic bioassays to realize the diagnosis of IAV. Superparamagnetic nanoparticles are used as magnetic tags during the detection, which can attach to the target protein on the surface of a giant magnetoresistance (GMR) sensor. The stray field from the magnetic nanoparticles will then be picked up by the sensor, with the magnetic signal proportional to the concentration of the target protein. By mixing the detection antibody, magnetic nanoparticles and the antigen all together at the same time without repeated washing procedures in the traditional bio-functionalization process, we managed to simplify the testing protocol with minimum sacrifice of device performance. The detection limit for IAV nucleoprotein is as low as 0.3 nM. Meanwhile, the validity of detecting unprocessed nasal swab samples from pigs has also been proved with comparable sensitivity to Enzyme linked immunosorbent assay (ELIZA). Furthermore, by integrating the wash-free bioassay with the handheld device, an accurate and efficient point-of-care platform is developed, which is capable of performing daily routine tests that can even be done at home by non-technicians.

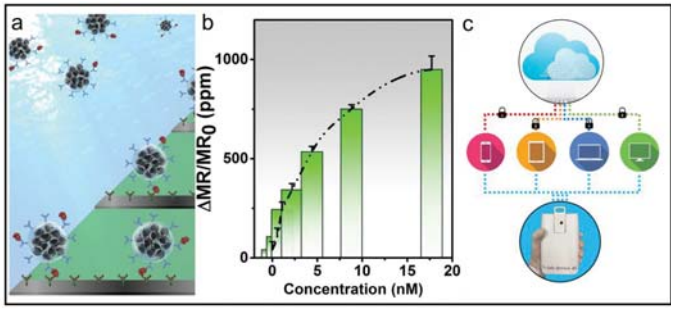

Figure. (a) A schematic view of one-step wash-free magnetic bioassay based on a sandwich assay structure. (b) Averaged sensor signal for different IAV nucleoprotein concentrations. (c) A picture of the handheld device which can connect wirelessly to smartphones, tablets, laptops, and computers.

Magnetic microparticles for new NMR-based force sensing

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The realization that mechanical forces may be as critical to biological systems as chemical cues, has led to the burgeoning new field of mechanobiology. Forces that cells exert on their surroundings, and vice versa, are known to influence everything from cell differentiation to cancer metastasis making measurement of such forces key to advancing the field. However, while forces can sometimes be measured at surfaces or in simple, largely 2-dimensional *in vitro* systems, measurements *in vivo*, or even in simple 3-dimensional opaque *in vivo* mimics, remain a significant challenge.

Here we consider a new application of magnetic microparticles for measuring such mechanical forces. Specifically, we present initial work on new magnetic microparticle complexes that comprise magnetic microdisks embedded in an elastomeric material that undergoes reversible deformation when acted upon by a mechanical force. The deformation of these elastomeric magnetic microstructures leads to a controlled, reversible, change in the magnetic fields as the magnetic components shift with respect to each another. In turn, the changes in these fields, the profiles of which are designed to yield unique spectral shifting signatures detectable via NMR, provide a real-time, radio-frequency measure of the locally induced strain that is compatible with optically inaccessible environments. Combined with knowledge of the Young's modulus and geometry, the strain-based NMR frequency shifts generated by the microparticle structures can be converted into quantitative force measurements. By using elastomers of differing Young's moduli and /or magnetic elements of differing sizes, accessible force ranges can be tailored over several orders of magnitude, potentially providing wireless, NMR-readable, force measurements reaching all the way down to piconewton scales.

Here we discuss how such microparticle systems can be made, detail their function and potential applications, and present our first results using such elastomeric-magnetic microparticle systems for (i) dynamic NMR-based force measurement and (ii) high-resolution magnetic resonance imaging (MRI)-based spatial force mapping derived from an array of magnetic microparticle force sensors (see figure).

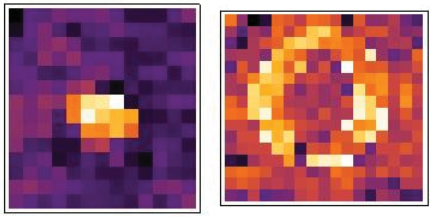

Figure: MRI spatial force mapping using arrays of magnetic microparticle force sensors. Each pixel in each image shows the recorded strain-induced NMR frequency shift generated at that location in an elastomeric sheet embedded with an array of magnetic microdisk particles and subjected to an applied force. Left image shows result for force applied at one point; right image is for force applied in an annular ring. Brighter (darker) shades represent greater (lesser) NMR frequency shifts, which are proportional to the applied force.

Thermal noise magnetometry: a zero-field magnetic nanoparticle measurement method

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In recent years, magnetic nanoparticles have gained a lot of interest due to their appealing properties for biomedical applications. For instance, when exposed to an alternating magnetic field, they generate heat which can be used in the destruction of cancer cells. Furthermore, when equipped with a suitable coating, they can be ideal drug carriers or disease detectors. Finally, the combination of their small sizes, giving them virtually full body access, and a large magnetic moment, enabling noninvasive detection, makes them excellent candidates for use in imaging applications[1]. However, for these applications to work reliably, the nanoparticle properties should be well known and their dynamic behavior should be fully understood.

Typically, magnetic nanoparticles are investigated by measuring their response to externally applied magnetic fields. For example, in magnetorelaxometry[2], the relaxation of the magnetic moment of the nanoparticles is measured after a magnetization phase in an externally applied field. However, such external excitations affect the aggregation state of the particles by e.g. inducing chain formation, and thus influence the measurement results.

We recently demonstrated the feasibility of a new approach[3], in which the noise signal resulting from the thermal switching of the nanoparticles in the absence of any external excitation is measured. With the help of SQUIDs in a magnetically shielded environment, a noise spectrum originating from the nanoparticles has been observed, and the shape of the spectrum was interpreted to estimate the properties of the nanoparticles. Here, we present thermal noise magnetometry results of several magnetic nanoparticle samples, and show the complementarity and similarity to magnetorelaxometry data of the same samples[4] .

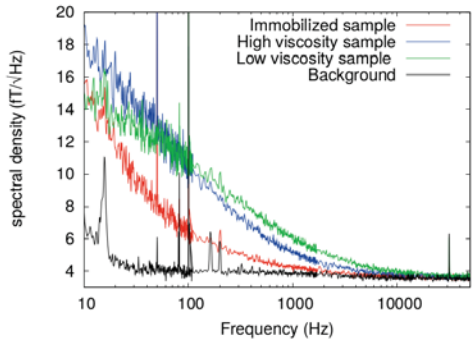

Figure: Thermal magnetic noise spectra of three magnetic nanoparticle samples with the same concentration. The larger the mobility (i.e. lower viscosity), the faster the thermally driven dynamics and the higher the spectral density remains at higher frequencies.

[1] Gleich, B., & Weizenecker, J. *Nature*, *435* (7046), 1214 (2005)
[2] Wiekhorst, et al. *Pharmaceutical research*, *29*(5), 1189-1202 (2012)
[3] J. Leliaert, et al., *Appl. Phys. Lett.*, 107, 222401 (2015).
[4] J. Leliaert*, D. Eberbeck*, et al., *J. Phys. D: Appl. Phys.* 50 (8), 085004 (2017)

Taking advantages of nanomagnetism for detecting biomarkers dispersed in biological fluids

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Magnetic nanoparticles (MNPs) have been widely studied for different diagnosis, therapeutic, and sensing applications. Concerning the latest, transducers based on MNPs functionalised with recognition ligands provide a suitable solution for distinct magnetic detection modalities. Indeed, the potential of MNP-based detection methods relies on: i) the specific interaction between the target biomarker (analyte) and the functionalised MNPs, ii) the variation of the magnetic properties after the MNPs-analyte interaction, iii) the display of the variation of the magnetic properties through distinct physical measurements (i.e. AC susceptibility, [1] magneto-optics, [2] giant magneto-resistance [3], magnetic resonance imaging [4]). Currently, the latest generally take several tens of minutes to be acquired, require relatively high-cost and/or complex instrumentation to be performed, or need exhaustive sample preparation. In overall, all this increases the complexity and cost of detection protocols, but specially the time to get results. Here, we present a novel, quick, and versatile methodology for magnetic detection of biomarkers dispersed in biological fluids (i.e. buffer saline, urine, blood,...), which is based on the variation of the AC hysteresis loops of MNPs after interacting with an analyte (see the figure). Such detectable variations can be measured after short MNP-analyte incubation times (< 15 min), and are associated with the increase of the hydrodynamic volume and/or the magnetic dipolar interactions. The displayer is an AC magnetometer prototype developed in-house that takes just few seconds (< 5s) for measuring the AC hysteresis loop, representing an remarkable time-saving progress with respect to other magnetic detection methods. Among all these suitable features, the most important aspect of our novel method is the fact that the sensitivity of biomarker detection is modulated not only by the analyte-ligand interaction affinity but by different parameters such as nanoparticle size and composition, magnetic field conditions, analyte valence, and the transducer and analyte concentrations. We have performed a set of experimental measurements to complete a successful proof of concept of this novel and versatile magnetic detection methodology, whose potential relies on exploiting the diverse advantages offered by nanomagnetism in order to increase the detection sensitivity. Friendly-use, and fast methodologies for magnetic detection of biomarkers in biological fluids represent a strategic approach for the diagnosis and treatment of diseases, such as cardiovascular or infectious diseases, Alzheimer or cancer.

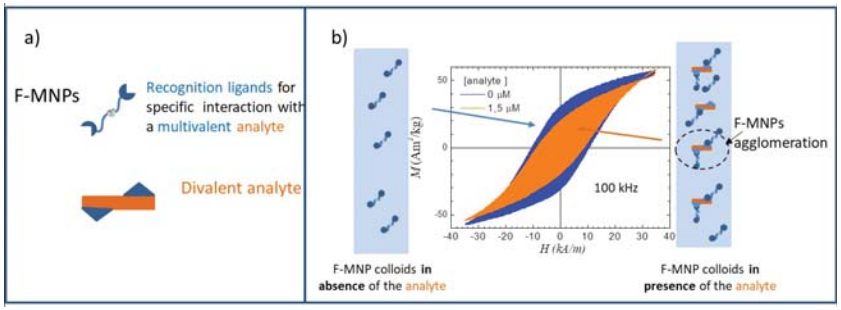

Figure: General scheme of the presented biomarker detection method: a) F-MNPs and multivalente analyte; b) AC hysteresis loops in absence/presence of analyte in the magnetic colloidal dispersion.

[1] A. Fornara et al. *Nanoletters.* **8 (10),** 3423 (2008); [2] A. Mezger et al. *ACS Nano* **9(7),** 7374 (2015)
[3] W.Wang, et al. *Scientific Reports* **4,** 5716 (2014), [4] J.M. Pérez et al. *J. Am.Chem.Soc.* **125(34),**10192 (2003)

Poster Session I - Wednesday, May 23, 2018

	Magnetic Hyperthermia			
1	Asin	Laura	Antitumoral effect of in vivo Magnetic Hyperthermia	Spain
2	Avolio	Matteo	In-gel study of the effect of magnetic nanoparticles immobilization on their heating efficiency for application in Magnetic Fluid Hyperthermia	Italy
3	Gebauer	Benjamin	Magnetic nanoparticle assisted regional deep hyperthermia using a conventional radio wave hyperthermia system	Germany
4	Bilici	Kubra	Investigation of the photothermal therapy potential of small iron oxide nanoparticles over the 730-840 nm spectral region	Turkey
5	Cabrera	David	Influence of the Intracellular Environment on the Dynamical Magnetic Response of Iron Oxide Nanoparticles	England
6	Coïsson	Marco	Hysteresis losses and specific absorption rate measurements in magnetic nanoparticles for hyperthermia applications	Italy
7	Cotin	Geoffrey	Shape matters! The role of shape anisotropy on iron oxide nanoparticles theranostic properties	France
8	Engelmann	Ulrich	Heating of Magnetic Nanoparticles under Gradual Immobilization in Hydrogels	Germany
9	Engelmann	Ulrich	Predicting Size-Dependent Heating Properties of Magnetic Iron-Oxide Nanoparticles from Experiment and Simulation	Germany
10	Fernandez-Gubieda	Maria Luisa	Magnetotactic bacteria as a therasnotic agents	Spain
11	Gong	Tianxing	Development of Magnetic Nanoparticles-Combined Bioceramic Bone Scaffolds for Bone Cancer Treatment	China
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13	Ichiyanagi	Yuko	Functional Magnetic Nanoparticles for Diagnostics and Therapies	Japan
14	Iszály	Zsófi	Functional Magnetic Nanoparticles for Diagnostics and Therapies	Hungary
15	Johansson	Christer	Magnetic particle hyperthermia performance at different core sizes and magnetic interactions	Sweden
16	Kaczmarek	Katarzyna	MAGNETO-ULTRASONIC HEATING WITH NANOPARTICLES	Poland
17	Konopacki	Maciej	Effect of rotating magnetic field on ferromagnetic structures used in hyperthermia	Poland
18	Lemal	Philipp	Exploring the thermal behavior of superparamagnetic nanoparticles at high concentrations	Switzerland
19	Makridis	Antonios	A standardization protocol for accurate and reliable magnetic particle hyperthemia evaluation	Greece
20	Mandal Goswami	Madhuri	Wetchemical synthesis of Fe/Pt nanoparticles: tuning of magnetic properties and biofunctionalization for hyperthermia therapy	India
21	Maniotis	Nikolaos	Modeling the anisotropy effects on the specific loss power of ferromagnetic nanoparticles	Greece
22	Muñoz	David	Checking the efficiency of magnetosomes in magnetic hyperthermia for cancer treatment	Spain
23	Myrovali	Eirini	Regional Focus effect on Magnetic Particle Hyperthermia efficiency	Greece
24	Orsi	Davide	A multi-therapy strategy to treat deep tumors: Fe3O4 - CeF3 - ZnO nanostructures to combine Magnetic Hyperthermia and Self-Lighted	Italy
25	de la Presa	Patricia	AC-magnetometry and calorimetry on Fe3O4 and Fe2O3 nanoparticles	Spain
26	Pieri	Kayla	Enhancing magnetic anisotropy with chained-particle magnetic composites for nanoparticle hyperthermia	United States
27	Riahi	Kalthoum	Evaluation of magnetic nano-perovskites La0.7Sr0.3Mn1-xBxO3 (B=Mo, Ti) synthesized via GNP method for hyperthermia application	Netherlands
28	Shah	Saqlain	Magneto-photothermal effects of pegylated superparamagnetic iron-oxide nanoparticles for multimodal cancer therapy	Pakistan
29	Simsek	Telem	Magnetic and Magnetothermal Properties of Manganese Monoboride (MnB) Nanoparticles	Turkey
30	Stergar	Janja	Mn-Zn ferrite nanoparticles coated with mesoporous silica as core material for magnetically activated release of therapeutic agents	Czech Republic
31	Anilkumar	T S	Dual Targeted Magnetic Photosensitive Liposome for Photothermal/Photodynamic Tumor Therapy	Taiwan
32	Takemura	Yasushi	Enhanced specific loss power from Resovist achieved by aligning magnetic easy axes of nanoparticles for hyperthermia	Japan
33	Rubia-Rodriguez	Irene	In silico prediction of tissue damage and power deposition maps in human models for magnetic hyperthermia treatments	Spain
34	Youhannayee	Maryam	Uptake of iron oxide nanoparticles of different prostate cancer cells	Germany
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35	Beklemisheva	Anna	Ferromagnetic micro-wires arrays for remote regulation of cell migration and focused drug delivery.	Russia

36	Berkov	Dmitry	Permanent magnet system for targeted drug delivery: an optimization methodology	Germany
37	Bernad	Sandor	Drug targeting investigation in the critical region of the arterial bypass graft	Romania
38	Bernad	Sandor	Hemodynamic effects on particle targeting in the arterial bifurcation for a different type of particles	Romania
39	Blümler	Peter	Aggregation and Hydrodynamics of Superparamagnetic particles in Mag-Guider systems	Germany
40	De	Debarati	Synthesis and characterization of different types of manganese ferrite nanoparticles for drug release application	India
41	Gonella	Veronica	Magnetic Drug Targeting: a preliminary numerical model	Austria
42	Idiago López	Javier	Nucleic acid-functionalized iron oxide nanoparticles for controlled drug release using magnetic hyperthermia	Spain
43	Jose	Gils	Hyaluronic Acid Modified Bubble-Generating Liposomes for Magnetically Triggered Targeted Chemotherapeutics	Taiwan
44	Kong	Seong	Cardiac Myocyte targeted Drug Delivery using Magnetically vector nanoparticles	Korea
45	Kotelnikova	Polina	Development of biocompatible nanoparticles modified with magnetite binding protein for cancer theranostics	Russia
46	Kralj	Slavko	Partially hollow nanostructures for magnetically-assisted drug delivery	Slovenia
47	Latha	Subbiah	Chitosan-Polyvinyl alcohol-based pH-sensitive ferrogel beads for magnetically localized mucoadhesive controlled drug delivery in GI tract	India
48	Latha	Subbiah	Preparation and evaluation of magnetoliposomes co-encapsulated with herbal extracts for magnetic targeted therapy of breast cancer	India
49	Ménager	Christine	Magnetic Hyperthermia at the Nanoscale for Remotely Triggered Drug Delivery from Polymeric Nanocarriers	France
50	Mirkasymov	Aziz	Mononuclear phagocyte system blockade, caused by the uptake of magnetic nanoparticles	Russia
51	Ngo	Sin-Ting	PEGylated Nanoparticle-Induced Hypotensive Effects: Hemodynamic Mechanisms	Taiwan
52	Pham	Nguyen	Iron oxide polymer nanorattles as nanocarriers for targeted tumor therapy	Australia
53	Selvamani	Palanisamy	Polyvinyl alcohol-based ferrogel system for magnetic field guided acid triggered delivery of omeprazole	India
54	Shetty	Aishwarya	Development of Fe3O4-CuS nanocomposite modified with Poly-L-Lysine for drug delivery and imaging	India
55	Vlasova	Kseniia	Magnetically responsive liposomes for delivery and remote-controlled release of high-molecular compounds.	Russia
56	Wei	Haoran	Experimental quantification of volume loss rate and flow dynamics due to a magnetically localized fluid region in a laboratory model blood	United States
57	Yu-Jen	Lu	Cetuximab-conjugated thermosensitive magnetic liposome for targeted delivery of Irinotecan in glioma treatment	Taiwan
58	Zahn	Diana	Development of a magnetic field applicator for magnetic nanoparti-cle targeting to the eye	Germany
59	Zahn	Diana	Temperature controlled Camptothecin release from magnetic PLGA microspheres	Germany
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60	Bemetz	Jonas	Detection of Legionella pneumophila in Porous Monolithic Filter Systems by a Miniaturized Nuclear Magnetic Resonance System	Germany
61	Dobroserdova	Alla	The Magnetic Response of Magnetoactive Elastomers via the FORCs Method	Russia
62	Draack	Sebastian	Temperature dependence of harmonic spectra studied by Magnetic Particle Spectroscopy	Germany
63	Elrefai	Ahmed	Magnetic Parameters Evaluation of Magnetic Nanoparticles for Use in Biomedical Applications	Japan
65	Gutierrez	Lucia	Transformations of nanoparticles composition, size and aggregation followed by AC susceptibility	Spain
66	Hillion	Arnaud	Mechanical performance of magnetic nanoparticle assemblies under low frequency rotating field	France
67	Johansson	Christer	Nanorheological studies of xanthan/water solutions using magnetic nanoparticles	Sweden
68	Kai	Hua	GoldMag®-Magnetic Lateral Flow Immunoassay for NT-proBNP Detection	China
69	Löwa	Norbert	Novel platform for the multidimensional analysis of magnetic nanoparticles	Germany
70	Mullerova	Sindy	Magnetic textile solid phase extraction of basic dyes	Czech Republic
71	Radon	Patricia	Laboratory magnetorelaxometry device for characterization of magnetic nanoparticles	Germany
72	Remmer	Hilke	Dynamics of magnetic nanoparticles in Newtonian and viscoelastic media	Germany
74	Schober	Gretchen	Using Multimodal Phosphor Particles to Monitor Radiopharmaceutical Release and Accumulation in vivo	United States
75	Viereck	Thilo	Multi-spectral Magnetic Particle Spectroscopy for the investigation of particle mixtures and particle mobility	Germany
76	Wegemann	Andreas	Automated Synthesis of Magnetic Nanoparticles and Process Monitoring by Nuclear Magnetic Relaxation	Germany
	Nanotechnology			
77	Castellanos-Rubio	Idoia	Inorganic-Polymeric Microdisks Loaded with Ferromagnetic Nanoparticles for Long-Term Cell Control	Spain
78	Chandra	Sudeshna	Surfactant modulated interactions of magnetic nanoparticles with biomolecules: An insight through electrochemistry and surface-enhanced	India
79	Chiriac	Horia	FeCrNbB Ferromagnetic Particles with Shape Anisotropy for Cancer Cell Destruction by Magneto-Mechanical Actuation	Romania
80	Das	Arpita	DNA engineered magnetically tuned cobalt ferrite for hyperthermia application	India
81	Dmitry	Zablotsky	Self-assembly of magnetic particles and rheology of their dispersions studied by numerical simulations	Latvia
82	Emadi	Masoomeh	Synthesis of Magnetic Iron Oxide and Modified Iron Oxide Nanoparticles with Starch and Study of Their Effectiveness in the Separation of	Iran
83	Felder-Flesch	Delphine	Mastering active targeting, alleviating unspecific cellular uptake and tailoring in vivo fate of USPIOs through a dendritic coating	France
84	Gabbasov	Raul	Study of Brownian motion of Magnetic Nanoparticles in Viscous Media by Mössbauer spectroscopy	Russia
85	Gapon	Igor	Ordering of magnetic nanoparticles on surfaces: neutron and x-ray reflectometry data	Russia
86	Garbarino	Francesca	Magnetic microbead sample handling integrated with optomagnetic nanobead detection	Denmark

87	Gdovinova	Veronika	AFM and SANS studied on the interaction of magnetic nanoparticles with lysozyme amyloid fibrils	Slovak Republic
88	Goetzfried	Marisa	Magneto-functional DNA Nanostructures using Ferritin-based Magnetic Nanoparticles	Germany
89	Gorsak	Tanja	The magneto-mechanical effect of barium-hexaferrite nanoplatelets on cancer cells in low-frequency magnetic field	Slovenia
90	Hallali	Nicolas	Experimental setups for the low frequency rotating magnetic field approach in cancer treatment	France
91	Ito	Akira	Magnetic Force-Based Tissue Engineering of Skeletal Muscle	Japan
92	Jiajia	Sun	Simulation of the Magnetophoresis of Magnetic Nanoparticles under Cylindrical Permanent Magnet Using Coupled Particle-Fluid Model	China
93	Kaiser	Martin	Directing the transport of active particles using magnetic nanoparticles in an applied field	Austria
94	Kantorovich	Sofia	Effects of interparticle magnetic correlations on the ferrofluid dynamic response	Austria
95	Knopke	Christian	Magnetic particle labelling of breast cancer cells for immunohistochemistry	United States
96	Köçkar	Hakan	Statistical analyses on optimisation of high saturation magnetisation of iron nanoparticles synthesized from iron oxide by hydrogen reduction	Turkey
97	Lachowicz	Dorota	Biodegradable and Highly Elastic Superparamagnetic Iron Oxide Nanoparticle Composite Hydrogels	Poland
98	Liberti	Paul	Ferro-phasing and Potential Uses	United States
99	Majorosová	Jozefína	Study the stability of protein-magnetic nanoparticle composite	Slovakia
100	Maxim	Veselov	Enzymatic reaction management via Brownian relaxation of magnetic nanoparticles under low-frequency magnetic field	Russia
101	Minina	Elena	Microgels in computer simulations	Austria
102	Mochalova	Elizaveta	Study of Magnetic Particle Interaction with Eukaryotic Cells Using Imaging Flow Cytometry, Scanning Electron Microscopy and MPQ-Cytometry	Russia
103	Novak	Ekaterina	The Influence of External Fields on the Self-Assembly of Magnetic Janus Particles	Russia
104	Petrenko	Viktor	Structure of magnetoferritin solutions and its impact on amyloid aggregates	Russia
105	Prochazkova	Jitka	Magnetically modified electrospun nanotextile and its bioapplication	Czech Republic
106	Pyanzina	Elena	Self-assembly In Magnetic Filament Dispersions: Influence of Internal Parameters	Russia
107	Ramos-Docampo	Miguel	Magnetite-coated polystyrene nanospheres as a potential platform for magnetic guidance applications	Spain
108	Saha	Priyanka	Magnetic, electronic and optical properties of Zn doped Fe3O4 nano-hollow spheres	India
109	Shelat	Ruchita	Detailed toxicity evaluation of bio-functionalized magnetic tracers with reduced signal loss using MPI	India
110	Shi	Zongqian	Investigation on the Self-assembly of Magnetic Nanoparticles under Soft-Magnet Element by Using Discrete Element Method	China
111	Shi	Zongqian	Experimental Analysis of the Magnetophoresis of Magnetic Nanoparticles under Permanent Magnet	China
112	Simchi	Abdolreza	Development of a Superparamagnetic and Luminescence Ferrite@Quantum Dot System for Cellular Imaging	Iran
113	Singh	Pinki	Development of Lumino-Magnetic Iron Oxide Nanoparticles for Multimodal Applications	India
114	Testa-Anta	Martín	Synthetically-driven enhancement of the magnetophoretic mobility of iron oxide nanocrystals	Spain
115	Zhang	Sinong	Genotyping Platform Based on GoldMag Nanoparticles for Clinical Diagnostic Application	China

Poster Session II - Thursday, May 24, 2018

Nanoparticle Synthesis

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117	Arsalani	Soudabeh	NRL-coated magnetic Fe3O4 nanoparticle for MRI contrast enhancement	Brazil
118	Baki	Abdulkader	Development of continuous synthesis of iron oxide nanoparticles as magnetic carriers for biomedical and clinical applications	Germany
119	Balasoiu	Maria	Microstructural and magnetic properties of stable suspensions of CoxFe3-xO4 magnetic nanoparticles for utilization in life sciences	Romania
120	Balasoiu	Maria	Structural studies of Co and Cu-doped ferrihydrite nanoparticles	Russia
121	Begin-Colin	Sylvie	Engineering of magnetic-based nanoplatforms for theranostic	France
122	Chanhom	Padtaraporn	Metalloporphyrins-sensitized titania-silica-iron oxide nanocomposites with high photocatalytic and bactericidal activities under visible light	Thailand
123	Dutz	Silvio	Influence of sterilization and preservation procedures on the integrity of serum protein coated magnetic nanoparticles	Germany
124	Fellows	Benjamin	Extended LaMer Synthesis of Nonstoichiometric Ferrites with Enhanced Magnetic Properties for Magnetic Hyperthermia	United States
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Antitumoral effect of in vivo Magnetic Hyperthermia

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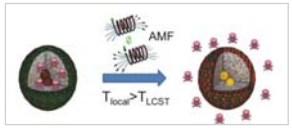
INTRODUCTION

Magnetic Hyperthermia (MH) has been proved as an efficient treatment against tumours but still presents some drawbacks such as the limitations of the alternating magnetic field (AMF) generation sources or the need to achieve high concentrations of Magnetic Nanoparticles (MNPs) in the target tissue. Here, we present two different approaches of in vivo MH treatment with the same tumour model but different heating generation materials, combining one of them with a chemotherapeutic agent release such as doxorubicin. The results show, in both of them, an inhibition in the tumour growth.

MATERIALS AND METHODS

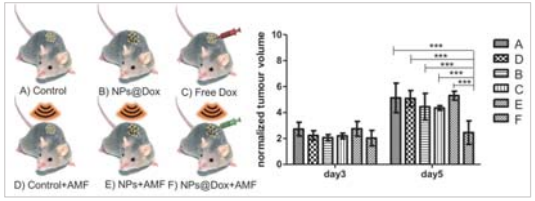
The animal model used consisted of an allograft and heterotopic tumour model, where EL4 murine lymphoma cell line from the strain C57BL/6N was injected subcutaneously in the right flank of immunocompetent C57BL/6 mice. One week after the cell injection the MNPs were administered intratumorally and the same day and two consecutive days MH was performed. Each exposure time was 30 min at 105 kHz and 18 kA·m-1. The first material that was used consisted of iron oxide nanoparticles (IONP) of 12nm synthetized by thermal decomposition, coated with an amphiphilic polymer (PMAO) and functionalize with Glucose to provide stability in biological fluids and to promote cell internalization[1]. The



second type of material consisted of a silica mesoporous matrix that contains IONP and doxorubicin. The silica particles were coated with a thermosensitive polymer with a Lower Critical Solution Temperature (LCST) of 42ºC[2]. Tumour dimensions (length, with and height) and mice weight were daily measured with a Vernier. After the last AMF exposure mice were maintained 3 days or until tumours started to ulcerate. MH treatments were made in group of 5 mice each, and all the needed control groups to validate the treatment were performed.

RESULTS

In both cases, 48h after the last magnetic hyperthermia treatment an inhibitory effect in the tumour growth was observed. Confocal microscopy images of the tumour revealed a good penetration of the material in both approaches. In the case of silica mesoporous material, it is noteworthy that none of the two control, where the effect of the free doxorubicin and the effect of the heat released by the MNPs due to the AMF exposition were analysed as single treatments, showed antitumor effect. However, a synergistic effect of the combination of both treatments was achieved.



(1) Moros, M.; Hernandéz, B.; Garet, E.; Dias, J. T.; Saéz, B.; Grazú, V.; González-Fernández, Á.; Alonso, C.; de la Fuente, J. M. Monosaccharides versus PEG-Functionalized NPs: Influence in the Cellular Uptake. ACS Nano 2012, 6, 1565−1577.

(2) Guisasola, E.; Baeza, A.; Talelli, M.; Arcos, D.; Moros, M.; De La Fuente, J. M.; Vallet- Regí, M. Magnetic-Responsive Release Controlled by Hot Spot Effect. Langmuir 2015, 31, 12777–12782.

In-gel study of the effect of magnetic nanoparticles immobilization on their heating efficiency for application in Magnetic Fluid Hyperthermia

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Magnetic nanoparticles (MNPs) made biocompatible thanks to special coatings and exposed to a not harmful alternating magnetic field (AMF) can be used as agents for Magnetic fluid hyperthermia (MFH), an antitumor technique which aims to damage cancer cells by increasing locally the temperature up to 43°C within the neoplastic region. Superparamagnetic MNPs are generally preferred for MFH, because of their smaller dimensions and minor interactions. Their magnetization is generally oriented parallel or antiparallel to the easy axis determined by the anisotropy, and can switch from one configuration to the other thanks to the reorientation of the spins inside the particle, which happens in a characteristic Néel relaxation time. The entire particle can also rotate in a characteristic Brown relaxation time. These two relaxation times are mainly influenced by the particle size, the magnetic properties and the viscosity of the surrounding medium, and their coupling with the AMF features determines the value of the Specific Absorption Rate (SAR), i.e. the electromagnetic energy absorbed in one second by one gram of material, that needs to be maximized.

Recent studies showed that Brownian rotation cannot take place when performing MFH treatment *in vivo* or *in vitro* because most MNPs are immobilized when injected into the cells, giving rise to lower values of SAR. Despite this evidence, the majority of works published on MFH studies MNPs only in liquid solutions where also the Brownian relaxation takes place. To clarify this topic, this work studies MNPs with features similar to the ones of MNPs already used in clinics, i.e. with suitable dimensions and composition, changing the dispersant media (gels) to approximately simulate human tissues with different viscosities, and therefore giving a systematic and overall overview of how the SAR changes according to all these experimental parameters. The MNPs have been exposed to AMFs with frequencies and amplitudes that meet the main safety criteria. Particularly, we studied three samples of magnetite (Fe_3O_4) MNPs with mean core sizes of 10, 14 and 18 nm at different frequencies (110 to 990 kHz) and intensities (up to 20 mT) of the applied AMF, performing measurements both in aqueous solution and in two agarose gels with different mass fractions (0.5% and 2%): these latter allowed the immobilization of MNPs reproducing an experimental condition similar to the *in vitro* or *in vivo* ones.

Results as the ones reported in figure show that SAR is always relevantly reduced by the immobilization of the MNPs in gel, except for the case of the smallest ones: this outcome is supposed to be transferable to the living tissues of the human body and highlights the need to evaluate the MFH efficiency of MNPs in media other than water. Furthermore, in the case of the 14 nm sample it was possible to estimate the Brown relaxation time of MNPs by fitting the SAR vs H curves in the different dispersant media with known models as the Linear Response Theory (LRT): although the obtained result is only an approximation, this method is considered a new instrument to investigate the physical mechanisms of MFH.

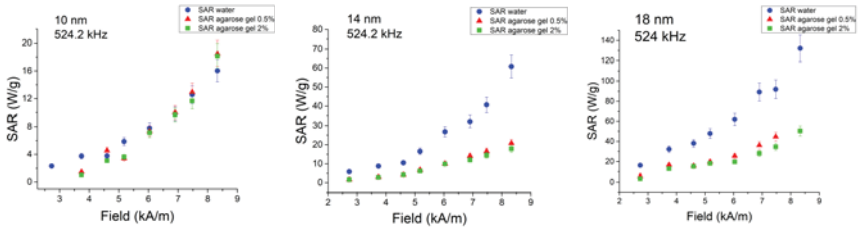

Figure. SAR values as a function of the AMF maximum amplitude at the frequency of 524 kHz for three samples of MNPs with mean diameter of 10, 14 and 18 nm in different media: water and agarose gels with different mass fractions.

Magnetic nanoparticle assisted regional deep hyperthermia using a conventional radio wave hyperthermia system

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Purpose/ Objective:
The latest research has shown that regional deep hyperthermia (DHT) act synergistically with radio- and chemotherapy. During conventional radio wave hyperthermia, the patient is exposed to an electromagnetic field of ~ 100 MHz and due to energy absorption within the body the target region is heated up. Nonetheless, DHT has to deal with challenges such as insufficient temperature distributions and/or hot-spots within the target region, the surface of the patient or organs at risk. The combination of radio wave hyperthermia with magnetic nanoparticles (MNPs) is a promising approach to increase the effectivity of this treatment and thus, to reduce the strength and/or duration of the field exposure. This combination may lead to a reduction of possible field induced side effects and a simplification of the treatment planning.

Materials and Methods:
Measurements were performed with the BSD-2000 3D/MR Microwave Hyperthermia System (Dr. Sennewald Medizintechnik GmbH, Munich, Germany) with the Sigma-30 applicator and an in-house developed water phantom with 3 channels for temperature sensors (Bowman probe, BSD Medical Corporation, Salt Lake City, USA). Two different types of magnetic iron oxide nanoparticles (γ-Fe_2O_3) were added to the in-house developed water phantom: single-core (SCNP) of about 11 nm and multi-core nanoparticles (MCNP) of about 50 nm ± 10 nm, consisting of about 10 nm primary cores. Both particles are coated with polyethylene glycol (PEG) 1000 (Carl Roth GmbH + Co. KG, Germany). The MNPs were stabilized against sedimentation within the phantom by adding of tetramethylammonium hydroxide (TMAH) (Merck KGaA, Darmstadt, Germany). Samples of SCNPs and MCNPs dispersed in deionised water (18 ml) with a concentration of 8 mg/ml or 16 mg/ml were irradiated with different power settings (200 – 300 W) at a frequency of 100 MHz. A simple 4-field setup with a relative power distribution of 100-30-100-30 (100-40-100-40) was used for the measurement. Heating curves were recorded in the Bowman probes and compared to those of measurements of plain water.

Results:
The application of MNP to the target area within the phantom showed an improvement of the temperature rise in the phantom at low power inputs. The SCNP with TMAH were not as stable as the MCNP with TMAH within the phantom. The use of MCNP with a concentration of 8 mg/ml resulted in a smaller temperature increase compared to a concentration of 16 mg/ml. Resulting from this, further measurements were conducted with MCNP dispersions of 16 mg/ml. At this particle concentration the addition of MCNP to the phantom yielded a constant temperature increase of more than 5° C after 30 minutes exposure time. The increase of the water temperature was below 0.5 °C as shown in figure 1.

Conclusion:
Application of MCNP to aid regional deep hyperthermia has proven to be feasible and beneficial for phantom measurements. A lower applied power minimizes possible hot-spots and is able to yield the same results as a conventional treatment. The use of MNPs also enables the application of a simplified treatment plans.

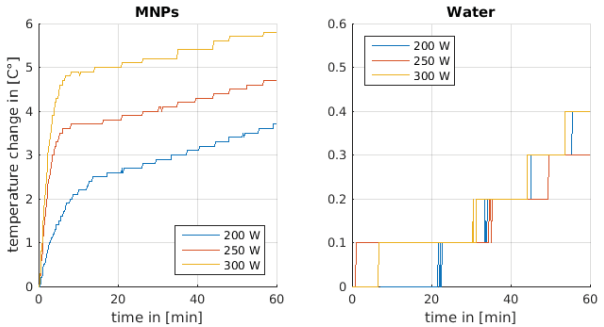

Figure. 1 Heating curves of MNPs versus plain water.

Investigation of the photothermal therapy potential of small iron oxide nanoparticles over the 730-840 nm spectral region

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Photothermal therapy (PTT) emerged as a local and promising therapeutic method in cancer treatment, where irradiation of sensitive nanoparticles with near infrared (NIR) laser creates a local temperature increase (hyperthermia) at the tumor site. Usually gold nanoparticles [1], C-nanotubes [2] and graphene [3] are investigated for PTT. However, there are issues limiting their clinic application such as limited biodegradability and photostability as well as long-term toxicity. Recently, superparamagnetic iron oxide nanoparticles (SPIONs) which are used as contrast agents in MRI and for magnetic hyperthermia have been recognized as PTT agents [4], as well. Some SPION formulations are already in clinical use and magnetic hyperthermia has been investigated for a while but its clinic use may be limited. Hence, PTT potential of SPIONs are very exciting in the field of medicine. PTT, on the otherhand, has still some drawbacks limiting its clinical applications. These include necessity of high particle concentration and high laser power. In the case of SPIONs, although most of the studies relate the photo-thermal heating effect of SPIONs to absorbance, the mechanism underlying this effect is not well understood yet. This study focuses on understanding the factors that affect the photothermal heating and the origin of this phenomenon by using small colloidal poly(acrylic acid) coated SPIONs with 4 nm crystal size and 14 nm hydrodynamic size which are suitable for biomedical applications. The wavelength (between 728 and 838 nm), intensity and power dependence of SPION based photothermal heating is studied and will be discussed. Lastly, PTT potential of these small nanoparticles are demonstrated in vitro on HeLa cells.

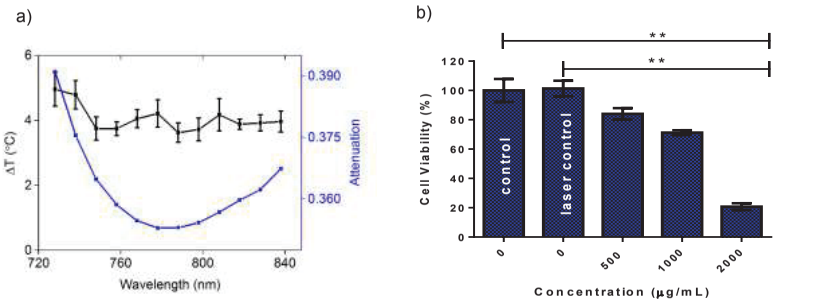

Figure 1: (a) Temperature increase measured for colloidal PAA/SPIONs at different irradiation wavelengths (728-838 nm with 10 nm steps) with 260 mW laser power after 20 min irradiation and (b) effect of photothermal therapy of PAA/SPIONs on HeLa cell viability with different concentrations at 795 nm laser irradiation.

References

1) Cobley, C.M., et al., Gold nanostructures: a class of multifunctional materials for biomedical applications. Chemical Society Reviews, 2011. **40**(1): p. 44-56.

2) Liu, Z., et al., In vivo biodistribution and highly efficient tumour targeting of carbon nanotubes in mice. Nature nanotechnology, 2007. **2**(1): p. 47-52.

3) Yang, K., et al., Graphene in mice: ultrahigh in vivo tumor uptake and efficient photothermal therapy. Nano letters, 2010. **10**(9): p. 3318-3323.

4) Chu, M., et al., Near-infrared laser light mediated cancer therapy by photothermal effect of Fe 3 O 4 magnetic nanoparticles. Biomaterials, 2013. **34**(16): p. 4078-4088

Influence of the intracellular environment on the dynamical magnetic response of iron oxide nanoparticles

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Magnetic nanoparticles exposed to alternating magnetic fields have shown a great potential acting as magnetic hyperthermia mediators[1] for cancer treatment. However, a dramatic and unexplained reduction of the nanoparticle magnetic heating efficiency has been evidenced when nanoparticles are located inside cells or tissues.[2] Recent studies suggest the enhancement of nanoparticle clustering [3] and/or immobilization [4] after interaction with cells as possible causes, although a quantitative description of the influence of biological matrices on the magnetic response of magnetic nanoparticles under AC magnetic fields is still lacking.
Here, we studied the effect of cell internalization on the dynamical magnetic response of iron oxide nanoparticles (IONPs). [5] AC magnetometry and magnetic susceptibility measurements of two magnetic core sizes (11 and 21 nm) underscored significant differences after cell uptake with effects, being more pronounced for larger sizes. Two methodologies have been employed for probing such influence. One methodology allows to experimentally determine the magnetic heat losses of magnetic nanoparticles inside live cells without risking their viability. The second one allows to assess the suitability of the magnetic nanoparticles for *in vitro* hyperthermia studies. Our experimental results -supported by theoretical calculations- reveal that the enhancement of intracellular IONP clustering mainly drives the cell internalization effects on the magnetic losses, rather than intracellular IONP immobilization. Understanding the effects related to the nanoparticle transit into live cells on their magnetic response will allow the design of nanostructures containing magnetic nanoparticles whose dynamical magnetic response will remain invariable in any biological environment, allowing sustained and predictable *in vivo* heating efficiency.

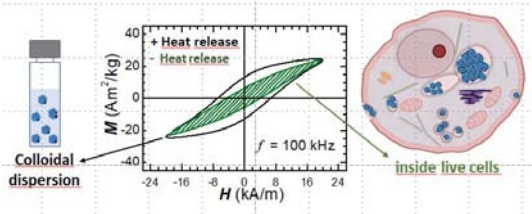

Figure : AC hysteresis loops measured on Iron Oxide Nanoparticles of 21 nm core size dispersed in water (empty area) or inside MCF-7 live cells (patterned area) at f = 100 kHz .

References
[1] E. Perigo et al. Appl. Phys.Rev. **2**, 041302 (2015)
[2] R. Di Corato, et al. *Biomaterials* **35**, 6400, (2014).
[3] M. Levy et al. Nanoscale 3, 4402 (2011); Ovejero et al. Phys. Chem. Chem. Phys. 18, 10954 (2016)
[4] D. Soukup et al. ACS Nano 9, 231 (2015); D. Cabrera et al. *Nanoscale* **9**, 5094 (2017)
[5] D.Cabrera et al. ACS Nano **DOI** 10.1021/acsnano.7b08995

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Hysteresis losses and specific absorption rate measurements in magnetic nanoparticles for hyperthermia applications

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Magnetic hyperthermia exploiting nanoparticles is currently a significant subject of research in the field of tumour treatment, with many open questions concerning the best choice of material, particles size and dose. A major issue consists in the difficulty of obtaining reproducible measurements of the specific absorption rate, i.e. of the amount of heat that is released by the magnetic nanoparticles submitted to an alternating electromagnetic field usually in the range of a few hundreds of kHz and with amplitudes of a few tens of mT.
To address this open issue, the specific absorption rate (SAR) of different families of particles (including magnetite and other Fe oxides, and ferrites with different substituting elements), prepared with different methods and having different sizes and shapes, has been measured using three approaches: static hysteresis loops areas, dynamic hysteresis loops areas and hypertermia of a water solution [1]. For dynamic loops and thermometric measurements, specific experimental setups have been developed, that operate at comparable frequencies (69 kHz and 100 kHz respectively) and rf magnetic field peak values (up to 100 mT). The hyperthermia setup has been fully modelled to provide a direct measurement of the SAR of the magnetic particles by taking into account the heat exchange with the surrounding environment in non-adiabatic conditions and the parasitic heating of the water due to ionic currents.
Dynamic hysteresis loops are shown to provide an accurate determination of the SAR except for superparamagnetic samples, where the boundary with a blocked regime could be crossed in dynamic conditions. Static hysteresis loops consistently underestimate the specific absorption rate but can be used to select the most promising samples.
A means of reliably measuring the SAR of magnetic nanoparticles, within the general subject of metrological traceability in medicine with a specific focus on magnetic hyperthermia, has therefore been developed by exploiting different approaches and by fully modelling the heat exchange processes in a custom-developed hyperthermia setup. The validity of the proposed methods is discussed.

[1] M. Coïsson, G. Barrera, F. Celegato, L. Martino, S.N. Kane, S. Raghuvanshi, F. Vinai, P. Tiberto, BBA General Subjects 1861 (2017) 1545-1558.

Shape matters! The role of shape anisotropy on iron oxide nanoparticles theranostic properties

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In the field of the synthesis and functionalization of inorganic nanoparticles (NPs) for biomedical applications, most researches aim at developing multifunctional theranostic NPs which can both identify disease states and deliver therapy and allow thus following the effect of therapy by imaging. The current challenge for iron oxide based NPs is the design of NPs able to combine in one nano-objects both magnetic hyperthermia (MH) and MRI with the best efficiency in order to reduce the dose injected in the patient. As ultra-small iron oxide NPs are already commercially used as T_2 contrast agent for MRI, the use of MH as a stand-alone or an adjacent therapy for cancer is closer to be a reality in every hospital thanks to the positive results achieved by the clinical trials carried out by *Magforce*^{TM}(Germany) treating glioblastoma. Nonetheless, the need for direct intratumoral injection of large amounts of NPs to achieve a therapeutic effect only points out at the need of improving the available nanomaterials for MH. Different parameters may be varied to increase the effective heat loss of a ferrofluid such as size, shape anisotropy or composition, among others. Shape and aspect ratio may offer interesting possibilities as chain formation has been previously reported to increase heat loss. Furthermore, there is also a need for evaluating the heating efficiency in cellular media as it may be different from that in solution and of course in *in vivo* conditions.

On that basis, plate-like, cubic and octopod shape NPs presenting different aspect ratios were prepared by thermal decomposition of home-made iron stearate, finely characterized, functionalised with dendron ligands to achieve aqueous suspensions and proved suitable for in vivo injection. MH performance was found to be shape-dependent with octopod-shaped NPs exhibiting the highest SAR values of 260 $W.g^{-1}$ (f = 579 kHz, 8 kAm^{-1}, ILP = 7.1 nHm^2kg^{-1}) or 960 $W.g^{-1}$ (f = 796 kHz, 16 kAm^{-1}, ILP = 4.8 nHm^2kg^{-1}). At the same time, their performance in MRI was investigated leading to relaxivity values of 16.9 and 405.5 mM^{-1} s^{-1} for r_1 and r_2, respectively, which was superior to that of commercial products like Resovist®. Cell response was studied as a function of NP concentration and morphology, as well as under MH treatment showing promising results for the anisotropic shapes. The in vivo MRI and MH experiments have allowed evaluating their biodistribution and their therapeutic properties.

The obtained results open the possibility of using these systems as *theranostic* platform thanks to the exhibited performance in hyperthermia and MRI at both in vitro and in vivo levels.

Heating of Magnetic Nanoparticles under Gradual Immobilization in Hydrogels

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The heat generated by magnetic nanoparticles (MNP) forms the basis of magnetic fluid hyperthermia (MFH) tumor therapy and arises from MNP magnetic moments relaxing in an alternating magnetic field. In physiological environments MNP strongly interact with cells, binding to their membranes as well as internalizing inside lysosomes, which alters the MNP magnetic relaxation.

In the present study, we investigate the heating behavior of MNP *in-vitro* for different binding states and compare it to the heating of trapped MNP in dedicated hydrogels of different mesh size, mimicking different immobilization states. We used iron-oxide MNP (mean core size 10 nm) with a biocompatible phospholipid coating, referred to as magnetoliposomes (ML), for *in-vitro* studies, and with citric acid coating (CA-MNP) for studies in hydrogels. All samples were subjected to an AMF (40 kA/m, 270 kHz) for 30 min, and from the recorded time-temperature curve, the specific loss power (SLP) value was calculated. *In-vitro* experiments were performed with L929 cells, which were incubated for 24 h with 225 µg(Fe)/mL ML dispersed in RPMI cell medium. The results of the SLP values were analyzed regarding the internalized ML amount with respect to ML residuals in RMPI medium and compared to fully immobilized ML after freeze-drying (FD): The SLP value of 10.1 % intracellular ML decreased by 20 %, the SLP value of 100 % intracellular ML decreased by 60 % and that of FD-ML decreased by 70 % (Fig 1a). The influence of gradual immobilization of MNP on the heating was investigated by mixing CA-MNP in low-melting agarose and polyacrylamide hydrogels. In agarose and polyacrylamide gels the mean mesh size can be tuned *via* the amount of monomers and cross-linkers, respectively, and in this way the state of MNP immobilization is influenced. SLP values decreased by up to 40 % in agarose gels for mesh sizes smaller than the hydrodynamic size d_H = 20.6 nm. A comparable decrease was observed in polyacrylamide gels (Fig 1b & c). We attribute this drop in SLP values to a gradual immobilization of MNP trapped in the hydrogels, which blocks particle relaxation and therefore decreases heating efficiency. This agrees very well with the results of the *in-vitro* measurements. The relative difference in the SLP drop in agarose and polyacrylamide hydrogels for similar mesh sizes might be explained by their gel-specific microstructures, which influence the MNP freedom of movement. For validation of these results, further investigations of the relaxation behavior of such trapped MNP *via* magnetic particle spectroscopy are currently under progress.

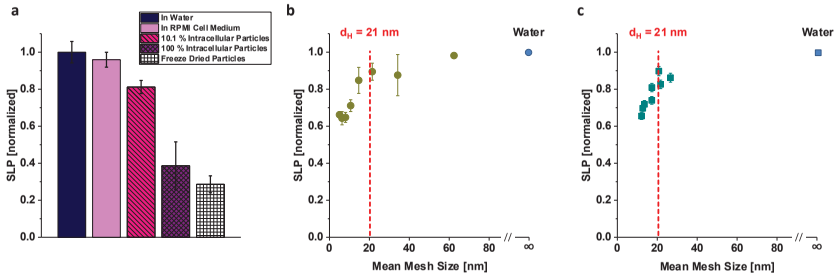

Fig. 1: (a) Normalized SLP values of particles in different environments and binding states. (b) Normalized SLP values of particles immobilized in low-melting agarose gels and (c) polyacrylamide gels for different mesh sizes. All SLP values were normalized to that measured for water. d_H denotes the mean hydrodynamic diameter of the MNP immobilized in gels.

Predicting Size-Dependent Heating Properties of Magnetic Iron-Oxide Nanoparticles from Experiment and Simulation

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In the innovative tumor treatment approach of magnetic fluid hyperthermia (MFH), magnetic nanoparticles (MNP) are accumulated at the tumor site and heated in a time-varying magnetic field to substantially damage the tumor *(1)*. This tumor damage depends mainly on the rate and amount of heat delivered via the MNP locally, which is in turn governed by a multitude of variables including the applied field amplitude and frequency, particle size and size distribution. In this study, we compare measured heating rates of MNP with sizes ranging from 21 nm to 28 nm with those obtained from Monte Carlo simulations of non-equilibrium Langevin dynamics to predict particle sizes and field amplitude /frequency settings for optimized MFH within medically safe tolerances.

We have synthesized monodisperse iron-oxide MNP via thermal decomposition, coated with poly(ethylene glycol) methyl ether amine (mPEG NH_2) as reported in *(2)*. Transmission electron microscopy analysis yielded core sizes (and log-normal distribution width) of 21.9 nm (0.04), 23.1 nm (0.05), 25.3 nm (0.08) and 27.7 nm (0.07). These MNP were subjected to magnetic fields with amplitudes $h_0 = (6...20)$ mT/μ_0^{-1} and frequencies $f = (176...993)$ kHz in a magneTherm hyperthermia device (nanoTherics Ltd., Newcastle under Lyme, UK). From the recorded time-temperature curves we calculated the specific loss power (SLP) as a measure of the heating rate: SLP values increased generally with size and frequency (Fig. 1a), as well as with the field amplitude (not shown here). Monte Carlo based stochastic Langevin equation simulations combining Néel and Brownian rotation relaxation and thermal activation (based on *(3)*) verified this trend (Fig. 1b). Under the assumption of an upper field limitation of f[kHz] · h_0[mT/μ_0^{-1}] ≤ 1758 imposed by medical safety requirements *(4)*, we simulated a heat map based on the parameters obtained from fitting simulation to experiment (Fig. 1c). This map shows maximum SLP values for frequencies $f \sim 100$ kHz (equivalent to $h_0 \sim 17.5$ mT/μ_0^{-1}) at particle sizes of 29 nm and greater. These results can provide a pivotal and integral tool for predicting particle sizes and applied field settings for optimized MFH.

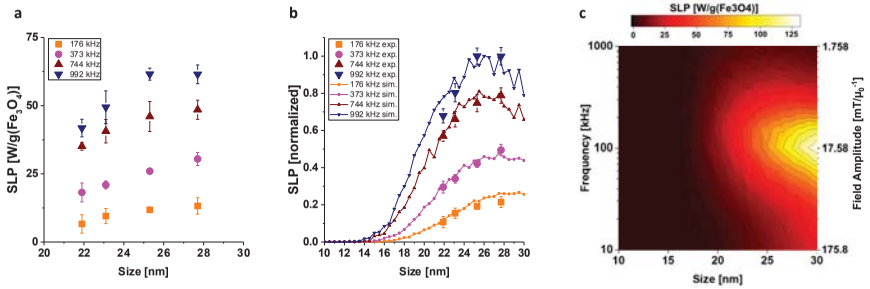

Fig. 1: (a) Experimental size-dependent SLP values for different frequencies measured at 6 mT. (b) Comparison of experimental (cf. (a)) and simulated SLP values calculated for damping coefficient $\alpha = 0.5$ and effective anisotropy constant $K_{eff} = 7{,}000$ J/m³. (c) SLP values calculated from the same simulations for various particle sizes and field amplitude /frequency combinations (s. text).

(1) Kannan M. Krishnan, Fundamentals and applications of magnetic materials, Oxford University Press (2016).
(2) Scott J. Kemp, R. Matthew Ferguson, Amit P. Khandhar, Kannan M. Krishnan, RSC Adv., 6, 77452-77464 (2016).
(3) Carolyn Shasha, Eric Teeman, Kannan M. Krishnan, IEEE Magn. Lett., 8, 1-5 (2017).
(4) Klaus Maier-Hauff et al., Strahlenther. Onkol., 181, 44-44 (2005).

Magnetotactic bacteria as theranostic agents

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Magnetotactic bacteria (MTB) are a diverse group of microorganisms with the ability to orient and migrate along the geomagnetic field due to the presence of a chain of magnetic nanoparticles that behave as a compass needle. In particular, *Magnetospirillum gryphiswaldense* MSR-1 species synthesize cubo-octahedral shape magnetite (Fe_3O_4) nanoparticles with a mean diameter of 45 nm. These nanoparticles are arranged forming a chain of ≈ 20 nanoparticles. In the last years, one of the most interesting approaches for cancer therapy is devising nano-robots capable of targeting and destroying cancer cells. In this work we want to prove the capabilities of *Magnetospirillum gryphiswaldense* bacteria as self-propelled biorobots for cancer treatment, evaluating the magnetic hyperthermia response and the magnetic resonance image (MRI) contrasting efficiency. We compare these results with those found previously in isolated magnetosomes [1].

Figure 1a shows the Specific Absorption Rate, SAR, measured at 300 kHz as a function of the magnetic field. In both cases, bacteria and magnetosomes were dispersed in 2% agarose gel with a magnetite concentration of 0.15 mg/ml. Both samples present high and similar values being the SAR response of the bacteria slightly higher in the high field range reaching 2000 W/g at 48 kA/m. On the other hand, MRI measurements were carried out as a function of magnetite concentrations, in this case, bacteria and isolated magnetosomes were dispersed in water, with an applied field of 1.5 T. Longitudinal relaxation rate, $1/T_1$, values are presented in Figure 1 b and transverse relaxation rate, $1/T_2$, in figure 1c. Obtained results indicate that both magnetosomes and bacteria can work as T_2 contrast agents for MRI, since they give high transverse relaxivity values ($r_2 = 77.0$-95.3 $mM^{-1}s^{-1}$), comparable to those of commercial nanoparticles ($r_2 = 100$ $mM^{-1}s^{-1}$).

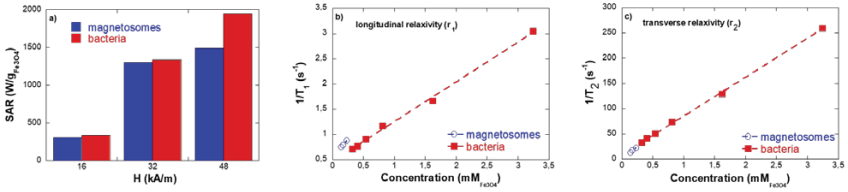

Figure 1. **(a)** SAR values measured at 300 kHz as a function of the applied magnetic field and the MRI longitudinal **(b)** and transverse relaxation rates **(c)** as a function of magnetite concentration for magnetosomes and bacteria dispersed in 2% agar medium (a) and in water (b, c)

References

[1] A. Muela, *et al., J. Phys. Chem. C.* **120** (2016)

Development of Magnetic Nanoparticles-Combined Bioceramic Bone Scaffolds for Bone Cancer Treatment

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Magnetic nanoparticles (MNPs)-mediated hyperthermia has been becoming increasingly impotent to treat cancers due to its less harmful side effects. MNPs transform the electromagnetic energy into the thermal energy and raise the local tissue temperature above 45 °C to destroy tumor cells. This strategy involving MNPs localizes the treatment in the vanity of abnormal tissues and protects healthy organs. Therefore, the combination of MNPs in bone scaffolds can potentially restore cancer-related bone fractures and provide the continuous elimination of tumor cells which cannot be completely removed by the surgical method.

The bone scaffold in this present study is made of calcium phosphate silicate cement (CPSC). CPSC has been proved to possess superior mechanical properties and, upon implantation, encourage osseointegration and osteoinduction. Compared to its ancestor, calcium phosphate cement, who has deficient capacity to restore fractured bones at poorly vascularized sites, CPSC has been incorporated with silicate ions to improve the proliferation and differentiation of osteoblast cells. In addition, the addition of silicate ions significantly increases the mechanical strength of bioceramics, making CPSC better fit for large-sized bone fractures.

The structure of our study is illustrated in the graph below. Bone tissues infected with tumor cells were surgically removed and the fracture region underwent a CT scan to generate the 3D digital model of the fracture. Subsequently, MNPs-combined CPSC bone scaffolds were manufactured by the 3D printing technology and implanted in the fracture region. The high-frequency magnetic fields were applied to induce the hyperthermia on the cancer-infected region to kill residual tumor cells; meanwhile, CPSC scaffolds were resorbed by the body and fractured bones were successfully reconstructed.

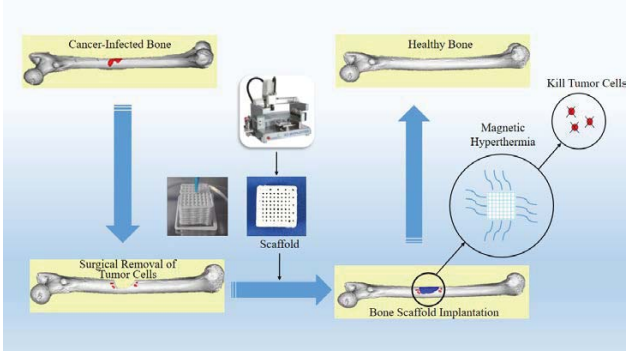

Figure: The schematic drawing of the development of a magnetic nanoparticles-combined bioceramic bone scaffold for bone cancer treatment

A ROADMAP TO THE STANDARDIZATION OF IN VIVO MAGNETIC HYPERTHERMIA

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Despite the fact that magnetic hyperthermia seems to be a promising approach in cancer treatment, the lack of standardization in the treatment conditions and the difficulties measuring the biological effects make it difficult to compare results from different research groups.

33 publications on *in vivo* pre-clinical studies using magnetic hyperthermia have been compared to generate an overview of the current state of the art and trends in this topic. There seems to be an agreement in some of the parameters that affect the final efficacy of the hyperthermia treatment. For example, regarding the composition of the materials, iron oxides are the most popular ones being used in 68% of the studies. More variability has been detected regarding the field amplitude and frequency conditions (Fig. 1). This is a consequence of the heterogeneity of the instrumentation used for hyperthermia applications, as the vast majority of the research papers reported homemade equipment. This variability leads to a plethora of field amplitudes and frequencies resulting in a very broad range of conditions used for the in vivo experiments (Fig. 1).

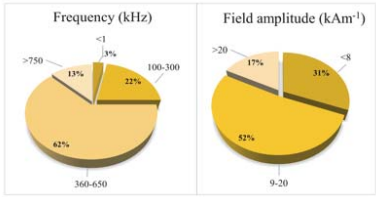

Fig. 1. Analysis of the frequency and field amplitude of the in vivo hyperthermia studies

Among other relevant parameters, high variability has also been observed regarding the length of the hyperthermia treatment, the number of repetitions of the alternating magnetic field exposure or the intervals between them (Fig. 2),

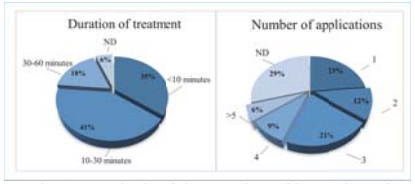

Fig. 2. Analysis of the number of hyperthermia treatments and length of each treatment in our review of in vivo hyperthermia studies

It should also be noted that most of the manuscripts revised for this work did not provide a complete description of the materials and methodology used. This is a consequence of the multitude of parameters that are involved in such complex animal experiments. However, the limited access to these details makes even more complex the comparison between research works.

A bigger effort should be paid by our scientific community to standardize the different relevant parameters having such a complex influence on the final clinical performance of magnetic nanoparticles during in vivo hyperthermia treatments.

Functional Magnetic Nanoparticles for Diagnostics and Therapies

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Magnetic nanoparticles (MNPs) encapsulated with amorphous SiO_2 ranging several nm were prepared by our original wet chemical method. Previously, we reported magnetic, structural and thermal properties. Then, we related our magnetic nanoparticles to biological functions by attaching functional groups. These functional MPs were further introduced into cells. Furthermore, cancer cell selective MNPs were developed. Based on these techniques, we proposed a therapeutic method of magnetic hyperthermia.
Several kinds of ferrite NPs were prepared and AC magnetic measurements were performed in order to improve the heating effect of MNPs for hyperthermia treatment. The relationship between the imaginary part of magnetic susceptibility χ" and the increase in temperature in the AC field was estimated. We have carried out in vitro experiments using cultured human breast cancer cells, and a drastic hyperthermia effect was observed [1].

MR measurements were performed using our particles as one diagnostic method. Our particles showed large T_2 shortening effect and were useful for the material of MRI contrast agents. Signals of the third harmonic components for magnetic particle imaging (MPI) were observed, and CT (X-ray tomography) imaging, mass spectrometric imaging (MSI) [2] are also in progress. We are then proposing development of these magnetic nanoparticles for "theranostics" recently.

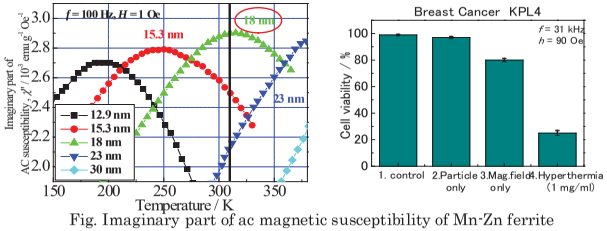

Fig. Imaginary part of ac magnetic susceptibility of Mn-Zn ferrite nanoparticles and cell viability of human breast cancer KPL4.

[1] T. Kondo, K. Mori, M. Hachisu, T. Yamazaki, D. Okamoto, M. Watanabe, K. Gonda, H. Tada, Y. Hamada, M. Takano, N. Ohuchi, and Y. Ichiyanagi, J. Appl. Phys. **117** 17D157 (2015).
[2] Shota Morimoto, Tomoya Ishikawa, Kuminori Hyodo, Takahiro Yamazaki, Shu Taira, Koichi Tsuneyama, and Yuko Ichiyanagi, Surf. Interface Anal. **48** 1127 (2016).

Enhanced and super-localised magnetic hyperthermia

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A possible way to improve the efficiency of magnetic nanoparticle-based hyperthermia for cancer treatment is to chose a proper type of applied field, e.g., a rotating instead of an oscillating one. The latter case is very well studied and there is an increasing interest in the literature to investigate the former although it is still unclear under which circumstances the rotating applied field can be more favourable than the oscillating one. Our goal is to demonstrate that the efficiency of magnetic hyperthermia is drastically increased by the combination of a static and rotating applied fields which has great importance since it is easy to realise in practice. Furthermore, our proposal for the applied field can be used to "super-localise" the heat transfer: in case of an inhomogeneous applied static field, tissues are heated up only where the magnitudes of the static and rotating fields reach a certain required ratio.

https://arxiv.org/abs/1706.07426

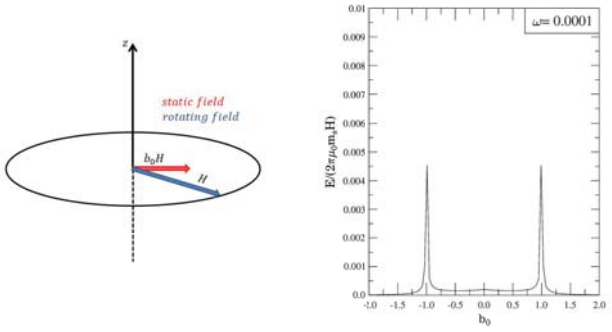

FIG. 1: Energy loss per cycle is evaluated for isotropic nanoparticles for the frequency $\omega = 0.0001/t_0$ with $t_0 = 5 \times 10^{-11} s$ where the applied field is a combination of a rotating and static ones. It has a sharp peak at $|b_0| = 1$, i.e., where the amplitude of the static and the rotating fields are equal to each other.

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Magnetic particle hyperthermia performance at different core sizes and magnetic interactions

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Iron oxide based magnetic nanoparticles (MNPs) can be found in several biomedical applications such as in the areas of diagnosis, actuation, imaging and therapy. One interesting and promising *in-vivo* MNP application is magnetic hyperthermia for cancer therapy. For this application we have earlier showed that the magnetic hyperthermia performance (defined by the ILP value), is crucially dependent on the magnetic core-core interactions in different MNP systems [1]. The ILP should then be lower in multi-core MNP systems than compared to single-core MNP systems since the magnetic interactions increase in a multi-core MNP system. In a multi-core system there is more than one magnetic core positioned close to each other that yields magnetic core-core interactions. To study how the interactions affects ILP, MNP systems with single-core system (only one core per particle) and multi-core MNP systems can be used. In a recent EU project (NanoMag), we have studied ILP values (both experimentally and theoretically) up to 50 different MNP systems (single- and multi-core MNP systems with different core sizes, core packing fractions and number of cores per particle). In figure 1 below, we show experimental ILP values vs mean core diameter of some selected NanoMag MNP samples (single-core MNPs at core sizes below 20 nm and multi-core MNPs with a few numbers of cores per particle, at core sizes above 20 nm). In figure 1 we also show how the experimental ILP correlates with ACS data and a non-interacting ILP model. As can be seen in figure 1 there is a maximum in ILP at around 20 nm. At the maximum in ILP, there is also a maximum in the AC susceptibility (ACS) at constant excitation frequency when the ACS data are normalized to total volume (or mass) of magnetic material. We will further correlate this result with dynamic Monte-Carlo simulations (including core-core interactions).

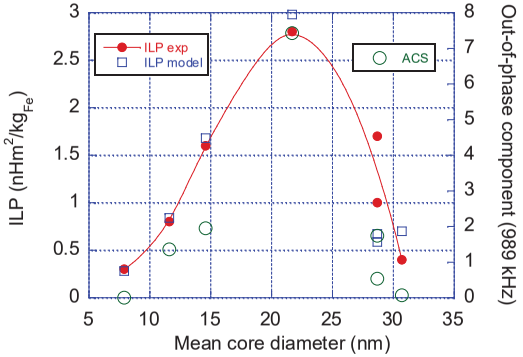

Figure1 (red) Experimental ILP data determined from SAR/ILP analysis (field amplitude 6000 A/m at 989 kHz), (green) ACS data (imaginary component at 989 kHz) normalized to volume magnetic material, (blue) calculated ILP values using a non-interacting ILP model. Used MNP parameters in the analysis are determined from TEM, M vs H and A4F analysis (e.g. core size and core size distribution, saturation magnetization).

[1] C. Blanco-Andujar et al, Nanoscale, 2015, 7, 1768.

MAGNETO-ULTRASONIC HEATING WITH NANOPARTICLES

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In medicine, a controlled increase in temperature up to 41-45°C, that is to temperatures that exceed physiological values of 36.6°C, is called hyperthermia. Hyperthermia is a very promising anti-cancer medical treatment. It induces heat in cancer tissues which leads to their weakening. Weaken cells are therefore more susceptible for other cancer therapies like radiotherapy or chemotherapy.

The temperature increase in tissues can be induced by means of ultrasound waves or alternating magnetic field. The efficiency of ultrasound thermotherapy can be improved by using sonosensitizing materials. Additional scattering caused by these so-called sonosensitizers becomes the source of supplementary ultrasound attenuation which consequently leads to increase in temperature. Good candidates for such material are magnetic nanoparticles. At the same time due to their sensitivity to magnetic field they are source of heat in the magnetic hyperthermia. The novelty of our research is to combine magnetic hyperthermia with focused ultrasound sonication. Synergetic, simultaneous interaction of both thermal methods contributes to a higher temperature increase than either method alone or their sum.

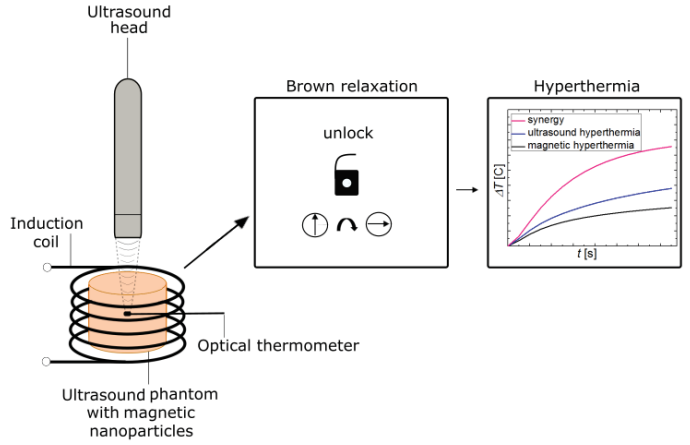

Figure 1. Schematic illustration of synergetic thermal effect achieved by combination of magnetic and focused ultrasound hyperthermia.

The experiments are performed using ultrasound phantoms that mimic biological tissues and their acoustic properties. Additionally those phantoms are doped with magnetic nanoparticles. To investigate the synergetic thermal effect phantom is simultaneously irradiated with the focused ultrasonic wave and the alternating magnetic field. The thermal effect of magnetic hyperthermia will be improved due to ultrasound sonication. Ultrasound impact should activate Brown thermal mechanism responsible for heat generation in magnetic hyperthermia which in the gel phantom is blocked. The preliminary results on hyperthermia of our research group have shown that the synergy of magnetic and ultrasonic heating confirms the theoretical assumptions of additional temperature increase.

Coupling of magnetic and ultrasonic hyperthermia will lead to the possibility of a new, innovative thermal method.

Effect of rotating magnetic field on ferromagnetic structures used in hyperthermia

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The main trend in the magnetic hyperthermia research is focused on the production of biocompatible magnetic material that exhibits the best heating efficiency. Practically, heating of magnetic material with the use of an alternating magnetic field is often used to treat tissues contacting cancer cells. Previous research has established that a rotating magnetic field generates more heat in the magnetic systems with nanoparticles [Bekovic et al., J Magn Magn Mater 355 (2014) 12-17].

In this study, the heating efficiency of magnetically guided nanoparticles has been examined. The effect of graphene oxide (GO), magnetite (Fe_3O_4) and hybrid material (GO-Fe_3O_4) has been studied. The inductive heating property at temperature 42°C of these materials in the rotating magnetic field (RMF) has been evaluated. The temperature changes in the tested probes with the different concentrations of the tested material in PBS medium have been analyzed. These measurements have been realized by means of the dynamic description of the obtained temperature changes. The specific absorption rates related to the mass of nanoparticles (SAR_{NANO}) have been calculated based on these measurements [Peng et al., New J Chem 38 (2014) 2312-2320].

The calculation of SAR is essential for a direct comparison of the heating efficiency form different nanoparticles systems. The obtained results indicated that the application of GO is allowed to enhance the heat transfer process in the tested nanoparticles systems. Figure 1 presents the concentration-dependent SAR_{NANO} values of hybrid material (GO-Fe_3O_4).

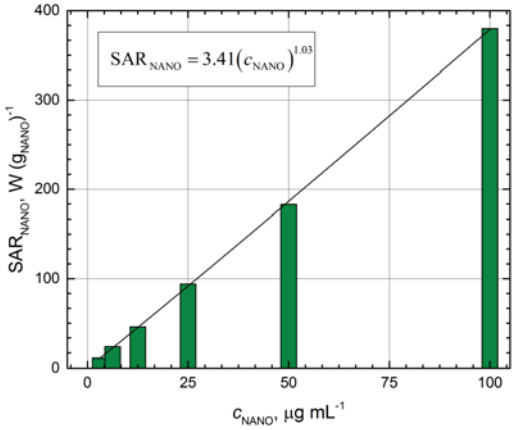

Fig. 1. The concentration-dependent SAR_{NANO} values of hybrid material (GO-Fe_3O_4)

Exploring the thermal behavior of superparamagnetic nanoparticles at high concentrations

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Magnetic nanoparticles (NPs) and their ability to convert magnetic energy into heat are currently explored around the globe for various biomedical applications, with a particular emphasis on hyperthermia treatment [1]. The heating power of these materials is dictated by a myriad of intrinsic (*e.g.* NP size, polydispersity or crystallinity) [2] and external (*e.g.* magnetic field strength or frequency) [3] parameters. To date, very few studies are investigating the effect of particle concentration on thermal behavior of superparamagnetic nanoparticles. A main question is the influence of very high NP concentrations on their heating power.

Today, the applied concentration in a typical hyperthermia treatment in Europe is 112 mg Fe/mL [4], whereas typical concentrations tested in laboratory scenarios range from 0.15 to 20 mg/mL [5]. Therefore, we explore the heating power of different sized magnetic NPs up to this high concentration. To screen the thermal signatures we use fiber optic measurements and a method developed in-house based on Lock-in thermography (LIT), described by Monnier *et al.* [6].

While the lower limits of detection for various sizes of superparamagnetic iron oxide nanoparticles (SPIONs) in an alternating magnetic field (AMF) in LIT have been previously described [7], our objective was to probe the upper detection limit of the LIT system. Therefore, we synthesized magnetic nanoparticles of various sizes by thermal decomposition, up concentrated the samples and analyzed their thermal behavior at different concentrations up to the clinical regime of magnetic hyperthermia, given by the NanoTherm® therapy. Correlation between thermal signature, concentration and particle sizes provide interesting details for future magnetic hyperthermia developments.

References

1. R. A. Bohara et al; “Role of functionalization: strategies to explore potential nano-bio applications of magnetic nanoparticles,” RSC Advances, vol. 6, pp. 4389-44012, 2016.
2. M. Gonzales-Weimuller et al; “Size-dependant heating rates of iron oxide nanoparticles for magnetic fluid hyperthermia,” J. Magn. Magn. Mater., vol. 321, no. 13, pp. 1947–1950, 2009.
3. R. E. E. Rosensweig, “Heating magnetic fluid with alternating magnetic field,” J. Magn. Magn. Mater., vol. 252, pp. 370–374, 2002.
4. K. Maier-Hauff et al; „Efficacy and safety of intratumoral thermotherapy using magnetic iron-oxide nanoparticles combined with external beam radiotherapy on patients with recurrent glioblastoma multiforme,“ J. Neurooncol., vol. 103, pp. 317-324, 2010.
5. A. E. Deatsch, B. A. Evans, “Heating efficiency in magnetic nanoparticle hyperthermia”, J. Magn. Magn. Mater., vol. 354, pp. 163-172, 2014.
6. A. Monnier et al; “A lock-in-based method to look through the thermal signatures of magnetic nanoparticles in liquid, solid and aggregated states,” *Nanoscale*, 2016.
7. Lemal et al; “Lock-in thermography as a rapid and reproducible thermal characterization method for magnetic nanoparticles”, J. Magn. Magn. Mater., vol. 427, no. 4, pp. 206-211, 2017.

A standardization protocol for accurate and reliable magnetic particle hyperthemia evaluation

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The use of biocompatible nanoscale magnetic nanoparticles under the influence of alternating magnetic field to produce heat is a well-known procedure called magnetic particle hyperthermia. In recent years, numerous research groups have extensively examined the heating power of different magnetic nanoparticle systems by studying their specific loss power, while optimal experimental conditions along with notable determination methods of the specific loss power in magnetic hyperthermia have been widely proposed until now. Despite, the remarkable progress in this field, the evaluation process of specific loss power suffers from errors and uncertainties imposed not only by experimental parameters (Figure1: a. particles, b. conditions, c. measurement) but by the estimation methodology (Figure1: d.), as well. In this work, we propose a step by step standardization protocol, starting from definition and recording of potential uncertainty and error sources, occurring in a typical magnetic hyperthermia sequence concluding to an error minimalization of Specific Loss Power values. The error of each specific parameter is estimated and translated to ultimate heating efficiency evaluation. The specific loss power parameters and their associated uncertainties analysis presented in this work serve as a significant guide for performing an accurate, reliable magnetic particle hyperthermia experiment and evaluation.

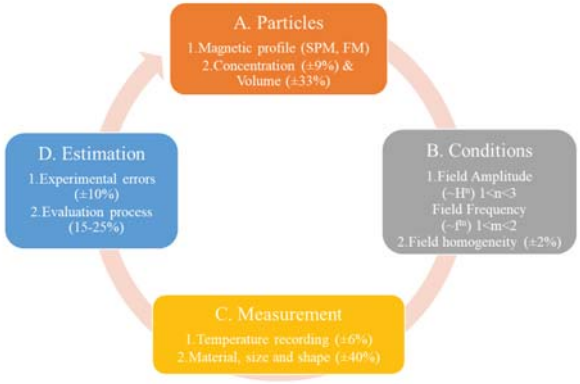

Figure1: Parameters affecting magnetic heating efficiency evaluation via Specific Loss Power Index (SLP index variations due to specific parameters appear in brackets).

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Wetchemical synthesis of Fe/Pt nanoparticles: tuning of magnetic properties and biofunctionalization for hyperthermia therapy

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Abstract:

This work reports on a new approach of synthesis of FePt nanoparticles in aqueous medium using cetyl trimethylammonium bromide (CTAB) surfactant as capping agent and we also have changed the sizes of the particles to tune the magnetic properties by changing the micelles concentrations. Here we see above CMC particles are of uniform size with uniform shape but below CMC particles poly-dispersed in size. Above CMC particles formed are of smaller size compared to the size prepared in below CMC. The magnetic properties of these particles are studied and the effect of particle size on magnetic property was also investigated. The ordering parameter of the particles after annealing at 550 ^{o}C with the variation of particle size was also studied. The particles are prepared in aqueous medium keeping the aim into mind to use the particles for biological purposes like magnetic hyperthermia therapy, fluorescence cell imaging etc. How heating abilities of the particles under AC magnetic field are changed with change of size is also checked as our aim is to use the particles for hyperthermia therapy in future. The studies on cyto-toxicity and fluorescence imaging after bio-functionalization are also performed on these materials.

Key Words: Micelles, FePt, Nanoparticles, fct phase, Hyperthermia therapy

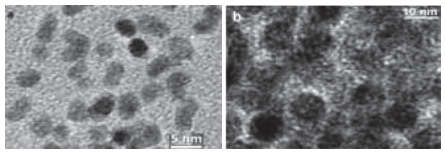

Figure 1: Two different particles of about 5nm(a) and 10 nm(b) prepared in two different micelles concentrations

Modeling the anisotropy effects on the specific loss power of ferromagnetic nanoparticles

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Up to date, ferromagnetic nanoparticles are competing performance magnetic particle hyperthermia agents. To evaluate their heating efficiency, it is a prerequisite to calculate their energy potential, i.e. the area of their hysteresis loop under alternating magnetic field. This work presents a theoretical combinatory study of the magnetic and thermal properties of ferromagnetic nanoparticles focusing on their magnetic anisotropy dependencies. Numerical simulations calculate the hysteresis loop area via Monte Carlo technique by employing Stoner-Wohlfarth model, valid at the ferromagnetic regime. The figure of merit of magnetic particle hyperthermia, is the specific loss power index which may be derived from the hysteresis loop area. To assess the validity of our simulations, we compare our findings with analytical models, providing information about the magnetization and energy behavior of a system of ferromagnetic nanoparticles exposed to variable magnetic fields (0-100 mT). [1], [2] Our results show that, first the hysteresis loop area, and second the specific loss power index, increase with the anisotropy values. Figure 1a shows the Monte Carlo hysteresis loops, at room temperature for a typical hyperthermia field (i.e. 30 mT) and a typical range of anisotropy (K) values from 2 to 11 kJ/m^3. With a finite element method (COMSOL Multiphysics), we perform heat transfer simulations to calculate the temporal distributions of temperature, of an aqueous dispersion of magnetic nanoparticles, for an AC field (30 mT, 765 kHz) and variable K values (Figure 1b). As input, we consider the energy dissipated, as heat, by the magnetic nanoparticles to their environment by the Monte Carlo hysteresis loop area (Figure 1a). Our estimations are found in excellent agreement with experimental measurements, for a 40 nm powder magnetite nanoparticles' sample (shaded region: experimental hysteresis loop in Figure 1a) and its 1mg/mL solution (dashed line: experimental hyperthermia curve) for the anisotropy value of K=9 kJ/m^3, a typical value for magnetite nanoparticles. Consequently, the heating response of a ferromagnetic particle hyperthermia agent, may be safely predicted and optimized with respect to its magnetic anisotropy.

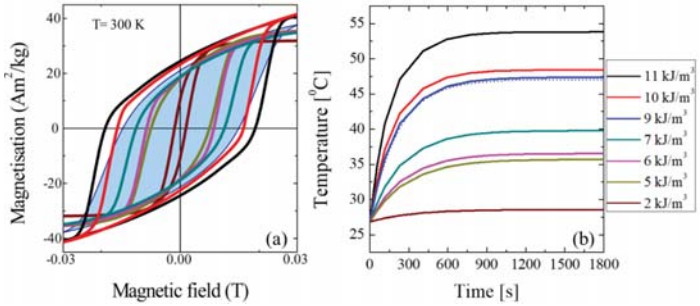

Figure 1. Anisotropy effect on the hysteresis area (a) and on heating response (b). Excellent agreement with experimental sequences of a magnetite nanoparticle sample (hysteresis loop as shaded region in (a) and hyperthermia curve as dashed line in (b)) for K=9 kJ/m^3.

[1] J. Carrey et.al. J. Appl. Phys. 109, 083921 (2011).
[2] M. Respaud, J. Appl. Phys. 86, 556 (1999).

Checking the efficiency of magnetosomes in magnetic hyperthermia for cancer treatment

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Magnetosomes are magnetic nanoparticles synthesized by magnetotactic bacteria, a group of aquatic prokaryotes with magnetic response. These microorganisms are able to align and navigate along the Earth´s magnetic field lines due to the presence of magnetosomes, magnetite nanocrystals typically arranged in one or more chains inside the cells [1].

We work with *Magnetospirillum gryphiswaldense* MSR-1, which produces cuboctahedral magnetosomes of 45 ± 6 nm in size. They are monodomain particles, with uniform size and morphology and thermally stable magnetic moment. In addition, they show high biocompatibility due to the presence of a surrounding membrane of proteins and lipids. These remarkable properties make them suitable for biomedical applications such as magnetic hyperthermia for cancer treatment [2].

In magnetic hyperthermia, the treatment makes use of the heat-releasing power of magnetic nanoparticles when exposed to an alternating magnetic field (AMF), reaching moderately high temperatures to produce cell death. In the present work, we analyze the heating efficiency or specific absorption rate (SAR) of the magnetosomes, and determine that the optimal working conditions for the AMF, within the safety criterion imposed by Hergt *et al*., (H.f<5x10^9 Am^{-1}s^{-1}) [3], are a frequency *f*=150 kHz, and an amplitude, H=25 kA/m [4]. Under these conditions, the magnetosomes show SAR values around 900 W/g.

Moreover, we analyze the effect of the magnetosomes on cell culture assays in a magnetic hyperthermia treatment model working under the conditions described above. For that purpose we work with two different cell lines: ANA-1 murine macrophages and A549 lung adenocarcinomic cells; incubating them with 30µgmL^{-1} of magnetosomes and checking the cell viability 2h and 24h after the treatment. The results clearly indicate that the magnetic hyperthermia treatment works (Figure 1a), due to a joint action of AMF effect and the intrinsic cytotoxic effect of the magnetosomes. In this regard, both cell lines show a reduction of the cell proliferation because of the internalized nanoparticles (Figure 1b).

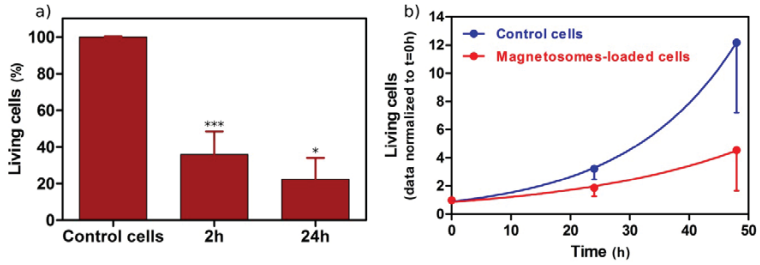

Figure 1. a) Effect of magnetic hyperthermia 2h and 24h after the application of the treatment. b) Cytotoxic effect in terms of long-term inhibition of cell growth (error bars = SD).

[1] L, Yan, *et al., Microbiol Res.* **167** (2012)
[2] E. Alphandéry, *Front. Bioeng. Biotechnol.* **2** (2014)
[3] A. Muela, *et al., J. Phys. Chem. C.* **120** (2016)
[4] R. Hergt, *et al*., *J. Phys.: Condens. Matter.* **38** (2006)

Regional Focus effect on Magnetic Particle Hyperthermia efficiency

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A typical problem of the application of hyperthermia is the difficulty to locate the heat without damaging potentially healthy surrounding tissues. In this work, we propose, a simple upgrade, of a magnetic particle hyperthermia device, which localizes the heat at very small tunable regions with the combination of a static (DC) and an alternating (AC) field. Thus, the main objective of this work is to examine the targeted heating induced by magnetic nanoparticles within agarose gel, which mimics human tissue. The quantifiable measure of heating efficiency is expressed by the Specific Loss Power (SLP) index, expressed in W/g.

Our setup consists of two permanent NdFeB magnets (B_r~1.2T), placed opposite to each other, to create a DC magnetic field configuration as illustrated in Figure 1a. The workspace is a 6×6 cm^2 square, as shown in Figure 1a. We estimated numerically the DC magnetic flux density B using Comsol Multiphysics 3.5a. The colored scale bar in Figure 1a corresponds to the magnetic flux density, ranging from 0 to -25, expressed in decibels (dB), by evaluating the base-10 logarithm of B/B_r. Thus, the workspace may be split to 9 regions according to the visualized geometry of the Field Free Region (FFR) and an appropriate AC field may be jointly applied. The two shaded cycles in Figure 1a show the area of the measuring sample and AC coil with red and blue color respectively. Inside the FFR (bluish colors in Figure 1a), where the DC field is virtually zero, nanoparticles should freely follow the AC field, preserving their initial heating efficiency expressed at absence of DC field. On the contrary, outside the FFR (yellow and green color in Figure 1a), nanoparticles pre-orient with the DC field, thus, the effect of AC field is hindered leading to reduced heating response i.e. smaller SLP values. To evaluate experimentally, this argument, a test sample, a typical high performance, 40 nm, magnetite nanoparticle solution stabilized in agarose matrix, was tested to variable DC fields jointly with a typical hyperthermia AC field (375 kHz, 50 or 60 mT). Our results, show that the coexistence of DC and AC fields in a magnetic particle hyperthermia sequence, practically leaves intact SLP values within FFR, while outside this region SLP value reduction down to 60% is observed. The combination of AC and DC fields may be a feasible method to optimize and regionally focus magnetic particle hyperthermia effect to specific malignant sites.

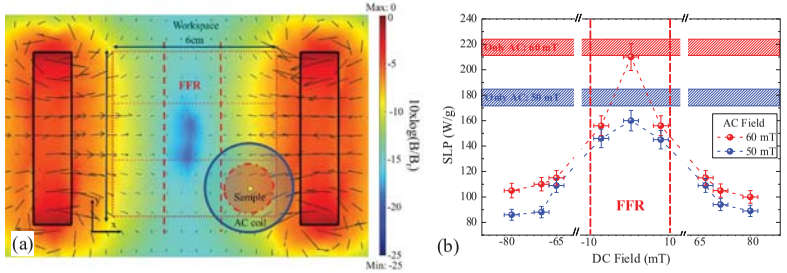

Figure1: a) Magnetic field mapping of a two permanent NdFeB magnets' setup. The colored map corresponds to the magnetic flux density (B) decrease, with respect to B_r, and measured in dB. The black arrows depict the direction of **B** normalized to Br. Experiments are performed in 9 different points within the 6x6 cm^2 square workspace. The two cycles show the area of the measuring sample and AC coil with red and blue color respectively b) SLP values with respect to DC field spatial variations for simultaneous application of a magnetic particle hyperthermia cycle (AC field: 375 kHz, 50 or 60 mT).

Acknowledgements: This work has been supported by the General Secretariat of Research and Technology (GSRT) and the Hellenic Foundation for Research and Innovation (HFRI).

A multi-therapy strategy to treat deep tumors: Fe_3O_4-CeF_3-ZnO nanostructures to combine Magnetic Hyperthermia and Self-Lighted Photodynamic Therapy

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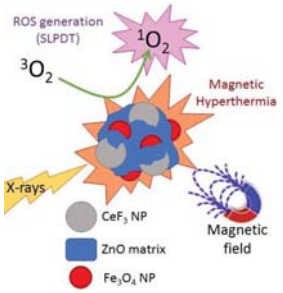
The onset of resistance to chemotherapy drugs and radiotherapy, is a severe challenge that can be tackled using multi-modal strategies. A single nanostructure designed to perform multiple therapies at once at the site of the tumor could allow a significant reduction of doses and of systemic side effects [1]. Our research focuses on the treatment of deep solid tumors by the combined application of Magnetic Hyperthermia (MHT) and of the X-ray triggered generation of reactive oxygen species (ROS), a therapy known as Self-Lighted Photodynamic Therapy (SLPDT).



In the already established Photodynamic Therapy (PDT), a photosensitizing agent is activated by UV or visible light to generate ROS, such as singlet oxygen ($^1O_2$); this induces oxidative stress in cancer cells with consequent DNA damage, apoptosis or necrosis. The applicability of PDT is limited to superficial tumors by the short penetration depth of light. SLPDT extends PDT to deep tumors by nanostructures that act as localized light sources when activated by highly penetrating radiation, typically 6MeV X-rays used in radiotherapy. The nanostructure transfers the absorbed energy to its photosensitizing constituent, which generates ROS leading to localized oxidative stress [2].

We already developed a nanostructure for SLPDT made of a nano-sized matrix of the photo-sensitizing material ZnO, that embeds scintillating CeF_3 nanoparticles [3]. The SLPDT efficiency has been proved on human adenocarcinoma cells (A549). Irradiation with low doses (< 2Gy, 6MeV) of X-rays from a radiotherapy source triggers ROS and singlet oxygen generation; this reduces the viability of cancer cells and blocks the cellular cycle before mitosis [4].

We are now extending its range of action by adding MHT functionality by means of magnetite superparamagnetic nanoparticles. MHT performances of the pristine magnetite nanoparticles are well characterized [5]; the MHT efficiency of the multi-material nanostructure are currently being tested. Finally, we plan to test in vitro the combined efficiency of sequential sessions of SLPDT and MHT on cellular lines of deep solid tumors (e.g. A549 cells).

[1] Kemp et al. Advanced Drug Delivery Reviews 98, 1 pp 3-18 (2016)
[2] Fan et al. Chemical Society Review, 45 pp.6488 (2016)
[3] Rimoldi et al., Journal of Materials Science, Materials in Medicine 27,10, 159 (2016)
[4] Orsi et al., in preparation (2017)
[5] Campanini et. Al., Nanoscale 7, pp. 7717 (2015)

AC-magnetometry and calorimetry on Fe_3O_4 and γ-Fe_2O_3 nanoparticles

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The magnetic hyperthermia has been largely studied in the recent years, mostly as a potential weapon in the fight against cancer. Many physical and chemical parameters take part in the processes of heating and the main purpose of this work is to compare different samples to investigate the relationship between heating efficiencies and physical properties such as nanoparticle size, anisotropy field and magnetic interactions. To achieve this purpose, a systematic study of different Fe_3O_4 and γ-Fe_2O_3 nanoparticles has been done. dc- and ac-magnetometry and ac-calorimetry measurements have been carried on particles with sizes ranging from 8 to 35 nm. ac-magnetometry at fields as high as 60 mT reveals, through the susceptibility enhancement, how the applied field induces chain ordering in large particles. The chain formation is inhibited in the smaller one due to the competition between magnetic and thermal energy. Therefore, chain formation will depend not only on particle size and their magnetic properties, but also on temperature.

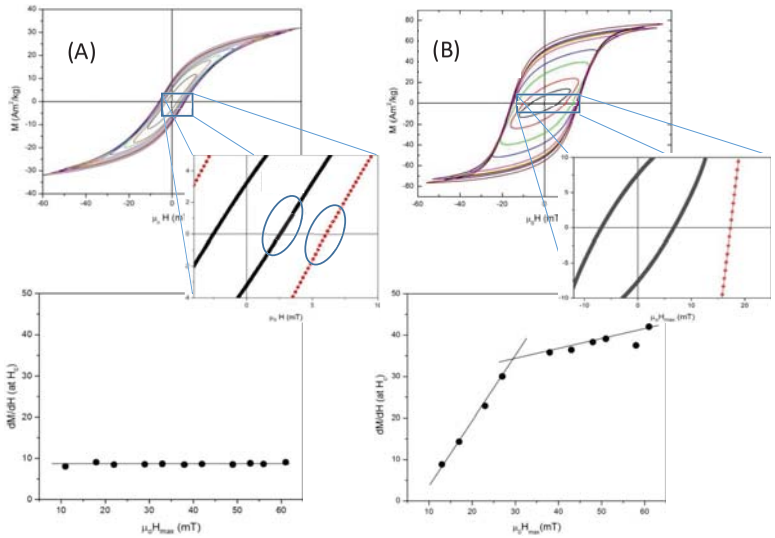

Figure: χ calculated at Hc with increasing ac-field in (A) 12 nm and (B) 35 nm nanoparticles.

Acknowledgements

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Enhancing magnetic anisotropy with chained-particle magnetic composites for nanoparticle hyperthermia

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Magnetic nanoparticle hyperthermia continues to show great promise for *in vivo* therapeutics and magnetically-stimulated drug delivery, and researchers continue to design advanced nanoparticle products capable of faster heating or enhanced targeting. However, it is becoming increasingly apparent that interparticle magnetic interactions in concentrated ensembles may dominate the individual attributes of a nanoparticle. In *in vivo* application, it is particularly difficult to control local particle concentrations, and this leads to diminished or unpredictable heating. In previous work, we have shown that encapsulating magnetite nanoparticles (8 nm) within a siloxane microsphere matrix (0.3 – 30 μm) yields fine control over the nanoparticle microenvironment, which may be tuned *a priori* for maximal heating.

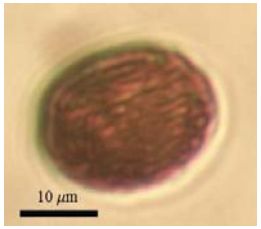

Figure 1. Optical image (10x) showing chaining within a large composite microsphere.

In the present study, we demonstrate an additional degree of control over the nanoparticle microenvironment by chaining the nanoparticles with an applied magnetic field. In the presence of a 300 G field, the material arranges itself into chained structures (Fig 1) which enhance the local magnetic anisotropy of the material. The material is subsequently cross-linked to lock in the chained geometry. Using torque magnetometry, we are able to indirectly measure the anisotropy induced by the chaining (Fig 2a) in bulk materials. Furthermore, we show that this anisotropy results in enhanced SAR in a high-frequency magnetic field (Fig 2b). The correlation between torque magnetometry and SAR suggests that magnetometry may be a useful tool in evaluating the efficacy of anisotropic nanoparticle ensembles.

Using these techniques, we have demonstrated nanoparticle chaining in bulk materials and in magnetic microspheres (0.3 – 30 μm). Torque measurements below were taken on bulk materials (~0.5 mL) using a torque magnetometer built in-house to measure torques from 0.001 to 0.8 mNm. Hyperthermia measurements taken on the same samples indicate that bulk materials heat faster when nanoparticles are chained and aligned parallel to the RF magnetic field.

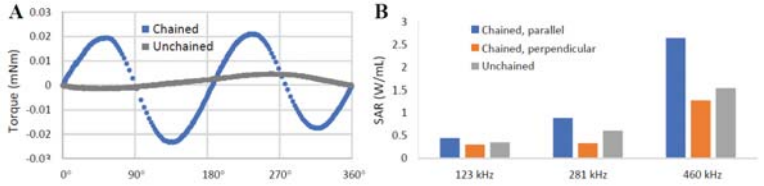

Figure 2 A) Measured torques in chained and unchained samples measured in a 1000 G field shows evidence of chain formation within the composite. **B)** Specific Absorption Rate (SAR) of sample indicates the increased heating of chained composite when in parallel alignment with the RF magnetic field in comparison to perpendicular to the field and samples with unchained particles.

Evaluation of magnetic nano-perovskites $La_{0.7}Sr_{0.3}Mn_{1-x}B_xO_3$ (B=Mo, Ti) synthesized via GNP method for hyperthermia application

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This current work deals with the study of the magnetic and biomedical characterization of magnetic nanoparticles for hyperthermia applications of $La_{0.7}Sr_{0.3}Mn_{1-x}B_xO_3$ (B=Mo, Ti) nanoparticles prepared through an aqueous combustion process (Glycine Nitrate Process, GNP).

We succeed to synthesize particles with a mean crystallite size about 18 nm. The incorporation of Ti^{4+} and Mo^{6+} in Mn-site induced a structural transition from rhombohedral structure with R-3 C space group of $La_{0.7}Sr_{0.3}MnO_3$ compound to orthorhombic one with Pnma space group of $La_{0.7}Sr_{0.3}Mn_{0.95}Mo_{0.05}O_3$ and $La_{0.7}Sr_{0.3}Mn_{0.95}Ti_{0.05}O_3$ compounds. The mean size of grain is distributed in the range of 82–90 nm. All samples exhibit a classical behavior from ferromagnetic (FM) to paramagnetic (PM) transition as the temperature increases. A decrease in Curie temperature (T_C) has been remarked from 84 °C to 54°C and 82°C for $La_{0.7}Sr_{0.3}MnO_3$,$La_{0.7}Sr_{0.3}Mn_{0.95}Ti_{0.05}O_3$ and $La_{0.7}Sr_{0.3}Mn_{0.95}Mo_{0.05}O_3$ respectively. The magnetic heating characteristics in AC field were measured in alternating magnetic fields of 23.89 mT at a fixed frequency of 518.7 kHz. Moreover, the intrinsic loss power (ILP) has been calculated from SAR values. The different nanoparticles with a high ILP may be useful for the insitu hyperthermia treatment of cancer.

Magneto-photothermal effects of pegylated superparamagnetic iron-oxide nanoparticles for multimodal cancer therapy

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Iron-oxide nanoparticles have a fascinating feature that they respond to the electromagnetic radiations of the visible spectrum. Unfortunately this aspect of these nanoparticles has not been properly investigated. Photothermal therapy and magnetic hyperthermia have been combined in this study to form a single bimodal cancer therapy system. The iron-oxide nanoparticles were synthesized using co-precipitation method. The particle size was tuned into the superparamagnetic regime and they were coated with PEG to achieve the optimum colloidal stability. HeLa cells were investigated for cytotoxicity and cellular viability using proteome apoptosis kit and MTT assay. Magnetic hyperthermia was performed under an alternating field of 170 Oe and 375 kHz. A laser diode was used to study the photothermal properties operating at 808 nm which is near infrared regime. The procedures were carried out in combination as well as separately and the combined therapy showed remarkable results in treating the cancer cells. *In-vivo* studies are under progress.

Magnetic and Magnetothermal Properties of Manganese Monoboride (MnB) Nanoparticles

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Magnetic nanoparticles exhibiting soft magnetic behaviour are promising candidates for biomedical applications such as imaging, therapeutic or theranostic agents. Due to their high chemical stability and saturation magnetization some transition metal boride nanoparticles attracts the attention of the researchers recently. Among the transition metal borides, manganese monoboride is the most suitable alloy for magnetic hyperthermia applications with the highest saturation magnetization. This work reports the structural, magnetic and magnetothermal properties of manganese monoboride (MnB) nanoparticles.

The nanoparticles were fabricated by surfactant-assisted ball milling of arc melted bulk MnB ingot. Milling experiments were performed in the presence of heptane and organic liquids oleic acid (OA) and oleylamine (OY) with controlled weight ratios. Planetary type ball mill and zirconia vial and balls were used in order to hinder the magnetic impurities. The liquid medium composed of heptane and surfactants was used to prevent cold welding and reduce particle size which provides an easy and reliable way to obtain nanoparticles with narrow size distribution. The magnetothermal response of MnB nanoparticles having average crystallite sizes of 20 nm to 10 nm was measured in AC magnetic field with strengths of 3.19x10^4 A/m at a frequency of 302 kHz. The specific absorption rate (SAR) of the MnB nanoparticles with 7.7 nm average crystalline size was measured as 97.5 W/g which is much higher than those of iron oxides and several transition metal ferrites measured under similar conditions.

Mn-Zn ferrite nanoparticles coated with mesoporous silica as core material for magnetically activated release of therapeutic agents

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The nanoparticles for magnetically activated release of drugs are designed as complex architectures, in which magnetic nanocrystallites can be covered by a mesoporous shell that enables loading with drugs and functionalization with secondary thermoresponsive or thermosensitive coating. The present contribution considers Mn-Zn ferrite nanoparticles encapsulated into mesoporous silica as potential core material for theranostic systems that combine the activated release and MRI tracking.

Hydrothermal synthesis was employed to prepare three samples of Mn-Zn ferrite particles of the spinel structure ($Fd\bar{3}m$) and the mean size of crystallites of ~11 nm, whose composition was accurately determined by XRF to $Mn_{0.62}Zn_{0.41}Fe_{1.97}O_4$, $Mn_{0.70}Zn_{0.31}Fe_{1.99}O_4$, and $Mn_{0.82}Zn_{0.21}Fe_{1.97}O_4$. SQUID magnetometry confirmed ferrimagnetic ordering with magnetization of 103–106 Am^2/kg at 5 K and 52–66 Am^2/kg at 300 K. Bare nanoparticles dispersed in glycerol were chracterized by magnetic heating experiments in AC fields of various frequencies in the range of 54–968 kHz and field amplitudes up to 18 mT at 54 kHz and 8 mT at 968 kHz. The heating efficiency of bare $Mn_{0.62}Zn_{0.41}Fe_{1.97}O_4$ particles, selected for the subsequent study, reached a relatively high values of *SAR* = 50 W/g(Mn+Fe) at 485 kHz and 11.5 mT.

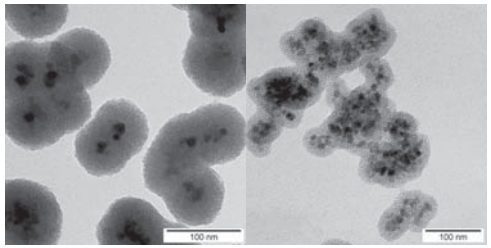

Fig. 1. TEM images of $Mn_{0.62}Zn_{0.41}Fe_{1.97}O_4$ nanoparticles coated with mesoporous silica of different porosity.

The coated particles were subjected to the evaluation of the heating efficiency in AC fields (see Fig. 2) to determine the actual heating efficiency of the particles in a colloidally stable aqueous suspension.

Finally, preliminary experiments on the loading the particles with paracetamol as a model drug were performed, followed by spectrophotometric determination of the drug.

The $Mn_{0.62}Zn_{0.41}Fe_{1.97}O_4$ ferrite nanoparticles were encapsulated into mesoporous silica of different porosity (see Fig. 1), which resulted by controlled growth of the shell in the presence of cetyltrimethylammonium bromide at distinct concentrations. The residual surfactant was carefully removed by an ion-exchange procedure.

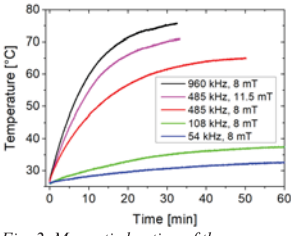

Fig. 2. Magnetic heating of the aqueous suspension of $Mn_{0.62}Zn_{0.41}Fe_{1.97}O_4$ nanoparticles coated with mesoporous silica with the concentration of 1.9 mg(ferrite)/mL in different AC fields.

Dual Targeted Magnetic Photosensitive Liposome for Photothermal/Photodynamic Tumor Therapy

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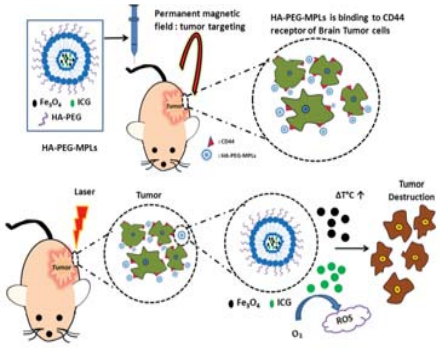
The goal of current research is to evaluate the potential of magnetic photosensitive liposomes (MPLs) prepared by entrapping photosensitizer indocyanine green (ICG) and Fe_3O_4 magnetic nanoparticles (MNPs) in polyethylene glycol-hyaluronic acid (PEG-HA)-coated liposomes as a nano-vehicle for dual-targeted (magnetic and ligand) and dual mode (photothermal/photodynamic) cancer therapy. Using solvent evaporation/hydration technique, MPLs were prepared from 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC), dimethyldioctadecylammonium bromide (DDAB) and cholesterol (CH) by encapsulating ICG and citric acid-coated MNPs (CMNPs) in cationic liposomes. HA was conjugated to MPLs by self-assembly of HA-PEG on liposome surface through ionic interactions of negatively charged HA and positively charged lipid DDAB (HA-PEG-MPLs). The prepared liposomes were characterized for physico-chemical properties by dynamic light scattering, X-ray diffraction, Fourier-transform infrared spectroscopy and thermogravimetric analysis. The photothermal heating efficiency of MPLs was confirmed by irradiation with 808 nm laser at 2 W/cm^2 and thermal images captured with an infrared thermal imaging camera. The in vitro cell culture experiments with human glioblastoma cells (U-87MG) showed excellent biocompatibility of HA-PEG-MPLs at 37 ^oC while short-term exposure to 808 nm laser induced photothermal/photodynamic cancer cell killing effects due to ICG and MNPs in HA-PEG-MPLs. Xenograft tumor model of subcutaneously implanted U87 cells in nude mice could also confirm the photothermal effects and anti-tumor efficacy of HA-PEG-MPLs in vivo after short-term exposure to 808 nm laser.



HA-PEG-MPLs for dual targeted photothermal/photodynamic therapy.

Enhanced specific loss power from Resovist achieved by aligning magnetic easy axes of nanoparticles for hyperthermia

Guannan Shi[1], Ryoji Takeda[1], Kizuku Nishimoto[1], Suko Bagus Trisnanto[1], Tsutomu Yamada[1], Satoshi Ota[2], Yasushi Takemura[1*]

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We experimentally derived the specific loss powers (SLPs) of magnetic nanoparticles (MNPs) under various hyperthermia conditions. SLP was enhanced by orienting the magnetic easy axes of nanoparticles. We also quantified the maximum SLPs expected from iron oxide nanoparticles including Resovist®.

We demonstrated that SLPs of MNPs could be precisely calculated based on dynamic hysteresis measurements [1]. The advantage of this evaluation method is that the applied magnetic field intensity and frequency can be widely varied for various fluids, solids, and intracellular samples [2] —which otherwise could not be achieved using caloric analysis by measuring self-heating temperatures of MNPs.

Two solid samples were prepared from Resovist®: One containing MNPs fixed with epoxy bond and solidified in the absence of a magnetic field and the other containing MNPs fixed with epoxy bond and solidified in a direct current (DC) magnetic field of 575 kA/m applied by an electromagnet for 8 h. Thus, the magnetic easy axes of the nanoparticles were randomly oriented and aligned in the first and second samples, respectively [3, 4].

Figure 1 shows the DC and alternating current (AC) hysteresis curves of solid Resovist® showing an aligned easy axis. The magnetic field was applied either parallel or perpendicular to the nanoparticle orientation. The area enclosed by the AC hysteresis curve was larger when the AC field was applied parallel to the nanoparticle orientation, indicating a larger increase in hyperthermia temperature. This feature originated from the magnetic anisotropy energy of nanoparticles and agreed well with our simulation results. Figure 2 shows the SLP of Resovist® experimentally derived from AC hysteresis curves measured under various hyperthermia conditions. Liquid samples usually show higher SLPs than solid ones in the range below approximately 500 kHz because of the delay in Brownian relaxation [5]. The SLP of the solid sample showing an aligned easy axis measured under an AC field applied parallel to the axis was 3 times higher than that of the liquid sample, which is significant because intracellular MNPs are almost nonrotatable and exhibit magnetic properties similar to those of fixed MNPs [2]. We discussed how to achieve enhanced SLP and how to maximize the increase in hyperthermia temperature. This result is essentially different from those obtained in hyperthermia experiments conducted using an applied AC magnetic field superimposed by a DC one, which reduces the SLP. The SLPs of superparamagnetic 4-nm-diameter γ-Fe_2O_3 and ferromagnetic 20–30-nm-diameter Fe_3O_4 MNPs were also presented.

Acknowledgement: This work was partially supported by JSPS KAKENHI 15H05764, 17H03275, and 17K14693.

[1] Ota *et al.*, *Nanomater.*, **6**, 170 (2016).
[2] Ota *et al.*, *J. Nanomater.* **2015**, 9 (2015).
[3] Yoshida *et al.*, *JMMM* **427**, 162 (2017).
[4] Takeda *et al.*, *J. Mag. Soc. Jpn.*, **42**, 55 (2018).
[5] Ota *et al.*, *APEX* **10**, 085001 (2017).

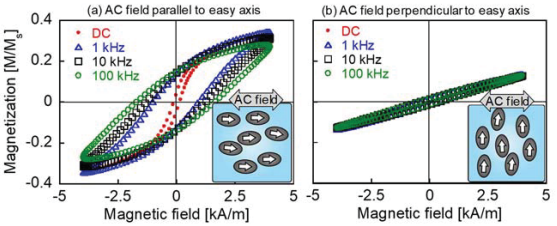

Fig. 1 DC and AC hysteresis curves of solid Resovist®. Magnetic field was applied (a) parallel or (b) perpendicular (b) to nanoparticle orientation.

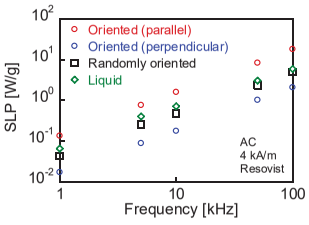

Fig. 2 Frequency dependence of SLP of Resovist®.

In silico prediction of tissue damage and power deposition maps in human models for magnetic hyperthermia treatments

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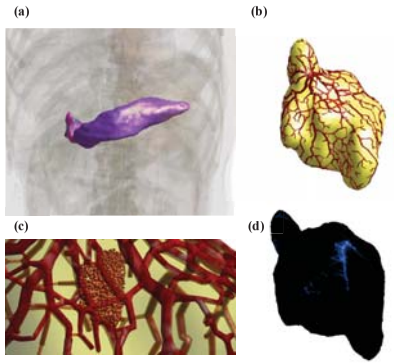
Computer simulations (in silico) have helped in vitro and in vivo experiments to develop new therapies in medicine, reducing time and costs to produce the tests. In the particular case of healthcare, in silico trials can predict the effectiveness of a treatment and optimize it to get the best benefit/risk ratio. In turn, this improvement will allow for adjusting the treatment to each patient following into high-performance, precision therapies. Magnetic hyperthermia is one of the nanotechnology-driven cancer treatments benefiting from computer simulations as the prime component of its planning platform [1]. When addressing localised tumours, magnetic hyperthermia relies on the intratumoral injection of magnetic nanoparticles that are excited by an external alternating magnetic field. The nanoparticles then release heat through hysteresis losses, eventually killing tumour cells.

Magnetic hyperthermia has already been trialled in clinical settings as coadjuvant to chemotherapy and radiotherapy to successfully treat several types of tumours [2-3]. The specialised instrumental development is moving forward, and commercial systems are being deployed throughout Europe and USA [4]; nevertheless, research on safety, dosimetry, and treatment planning does not progress to the same pace. The therapy has to be adapted to each patient and tumour as well as to the evolution of the tumour along the process.

Due to the complexity in programming the therapy plan because of the many variables to take into account, we intend to develop a software platform that allows the clinicians a semi-automatic treatment planning by evaluating the dosimetry values (SAR) and the tissue damage (CEM43). The core element is a virtual 3D model of the tumour and its vascularization obtained from diagnostic images of the patient to treat, along with other devices that may guarantee a safe therapy. Previous works have shown the relevance of some of these gadgets on superficial hyperthermia treatments to avoid overheating the skin [5]. In this work we present the results obtained regarding the SAR and CEM43 distributions generated upon heating inner tissues depending on the characteristics of the tumour and the role of external cooling devices on those distributions.



Figures: (a) Human 3D model with pancreas and a pancreatic tumour highlighted. **(b)** 3D model of a pancreatic tumour with its vascularization. **(c)** 3D model of the magnetic nanoparticles injected inside the tumour model. **(d)** SAR distribution map over the tumour surface.

[1] "Fundamentals and advances in magnetic hyperthermia", E. Périgo, G. Hemery, O. Sandre, D. Ortega, E. Garaio, F. Plazaola and F. J. Teran Applied Physics Reviews **2** (2015) 041302
[2] "Clinical hyperthermia of prostate cancer using magnetic nanoparticles: presentation of a new interstitial technique", M. Johannsen et al., Int. J. Hyperthermia **21**, 637 (2005); "Morbidity and Quality of Life During Thermotherapy Using Magnetic Nanoparticles in Locally Recurrent Prostate Cancer: Results of a Prospective Phase I Trial", M. Johannsen et al., Int. J. Hyperthermia **23**, 315 (2007); "Magnetic hyperthermia", D. Ortega and Q. A. Pankhurst, in Nanoscience: Vol. **1**: Nanostructures through Chemistry (2013, RSC) p. 60
[3] http://www.nocanther-project.eu. This NoCanTher project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 685795.
[4] www.magforce.de
[5] "Development of a guideline for the water bolus temperature in superficial hyperthermia" M. L. Van der Gaag et al., Int. J. Hyperthermia **22**, 8 (2006); "Study of water bolus effect on SAR penetration depth and effective field size for local hyperthermia" M. A. Ebrahimi-Ganjeh et al., Progress in Electromagnetics research, **66** (2006).

Uptake of iron oxide nanoparticles of different prostate cancer cells

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In this project, the cellular uptake of magnetite nanoparticles (MNP) covered by N-(2-aminoethyl)-3 aminopropyltrimetoxysilane (Aminosilane - APTES) was examined by transmission electron microscopy (TEM) and flow cytometry (FC). Magnetite nanoparticles were synthesized by co-precipitation of aqueous solution of ferric chloride and ferrous sulfate iron salts with ammonium hydroxide as a base and functionalized by APTES to increase the viability and affinity of the particles to the cells. Prior to the uptake, the structural and magnetic properties of these particles were characterized by TEM, X-ray diffraction (XRD), Fourier transformed infrared spectroscopy (FTIR) and vibrating sample magnetometer (VSM).

To study the cellular uptake *in vitro*, two prostate cell lines were investigated: PC3 as a cancerous cell line and BPH1 as a benign epithelial cell line. Cells were cultured up to 70% confluency. Under the same condition and confluency, both cell lines were incubated for 24h with different concentrations of APTES-MNP (100, 200 and 500 µg/ml and one untreated sample as a control). TEM and FC analyses were subsequently carried out to monitor cellular uptake of APTES-MNP. FC data revealed an increase in cell granularity following treatment with high concentration of the particles. PC3 cancer cells did uptake more particles than BPH1 benign cells. The results from FC and TEM analyses demonstrate magnetite nanoparticles have higher internalization into PC3 cells than BPH1 cells. The effect can be regarded to surface functionalization of nanoparticles to PC3 cells as a malignancy prostate cell than BPH1 as a benign cell. The approach may help to optimize the efficiency of hyperthermia for prostate cancer.

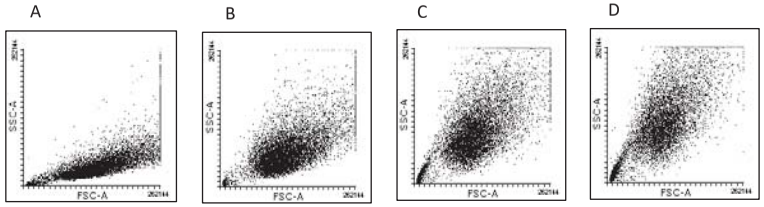

Fig. 1: Cytogram of SSC and FSC indicate increasing of SSC and decreasing of FSC by incubation of PC3 cells with different concentration. Panel A to D shows treatment of PC3 cell control, 100, 200 and 500 µg/ml respectively.

Ferromagnetic Micro-wires Arrays for Remote Regulation of Cell Migration and Focused Drug Delivery

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Magnetic micro-wires of appropriate design have attractive features making them useful to a variety of bio-medical applications. The magnetic micro-wires could be implanted within a targeted area of tissue or blood vessel to retain either bio-functionalized magnetic nanoparticles (MNPs) or magnetically labeled stem cells circulating in blood. The spatiotemporal precision of generating magnetic fields from magnetized micro-wire arrays may offer a compelling interface for magnetophoretic devices to control the migration of living cells and targeted drag delivery. We have studied the possible trap of diamagnetic cells with a dual micro-wire dipole system producing a unique magnetic field energy profile (see Fig. 1a) and also forced diffusion of magnetic nanoparticles suspended in a carrier liquid under the influence of a magnetic field gradient within a magnetic micro-wires array. The distributions of the MNPs concentration along the direction of the wire magnetization is shown in Fig. 1b (approach to the stationary regime).

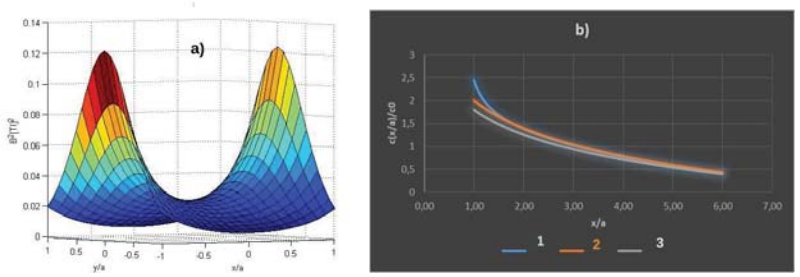

Fig.1. In (a): calculated magnetic induction (B^2) spatial distribution from a pair of diametrically magnetized micro-wires along the x-direction; and in (b): concentration profiles $c(x/a)/c_0$ along the x-axis in the vicinity of stationary regime (c_0 is the initial concentration of MNPs and a is the micro-wire diameter) . The concentration plots are given for different values of the parameter $\gamma = \frac{\mu_0 V \chi M^2}{2kT}$, (line 1- γ=1, line2- γ=0.01, line 3- γ=0,01) where V is the particle volume, M is the wire magnetization, χ is the magnetic susceptibility, kT is the thermal energy.

Thus, an inhomogeneous magnetic field causes a redistribution of a suspension of magnetic nanoparticles. This magnetic-field forced diffusion lead to MNPs focusing in the areas with the highest value of magnetic gradient. Such a magnetic gradient can also direct stem cell spreading [1], which accompanies tissue repair and wound healing. Our findings provide a minimally invasive magnetic method for remote manipulation of cell migration and precise drug delivery, offering a valuable tool for other cell-based therapies [2].

Reference:

[1] V. Zablotskii, O. Lunov, S. Kubinova, T. Polyakova, E. Sykova, A. Dejneka. J. Phys. D, (2016) 49 (49), 493003.

[2] M. Rubio Ayala, T. Syrovets, S. Hafner, V. Zablotskii, A. Dejneka, Th. Simmet. Biomaterials. (2018) 163, 174

Permanent magnet system for targeted drug delivery: an optimization methodology

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Magnetic nanoparticles are widely employed in modern biotechnology with numerous applications including hyperthermia, magnetic resonance imaging and genetic engineering. One of the most prominent approaches in medical applications is the use of ferrofluid particles as drug carriers. Such a particle comprises a magnetic core covered by a biologically active component. Significant efforts in this approach should be devoted to the development of magnetic system which produces magnetic drag force with the maximal magnitude and prescribed orientation.

This talk presents a new optimization method for permanent magnet systems, where the placement of magnets in close vicinity of the targeted area is complicated or even impossible. This situation occurs in the case of a human eye: many deceases require drug delivery directly to the retina. If a non-invasive treatment is aimed, than the movement of nanoparticles through the whole volume of the vitreous body has to be controlled by the external magnetic field only.

In our approach, the system to be optimized consists of a large number of magnets positioned on a prescribed grid. The magnetization orientations of these magnets are determined according to the optimization criteria, which include both the maximization of the magnetic field gradient and its desired direction in an *extended area* (vitreous body in our case). As a consequence, the magnetic drag force, which is proportional to the magnetic field gradient, provides a controllable movement of nanoparticles in the desired direction. The presented methodology is applicable for any class of biological objects, where magnetic targeted drug delivery is required.

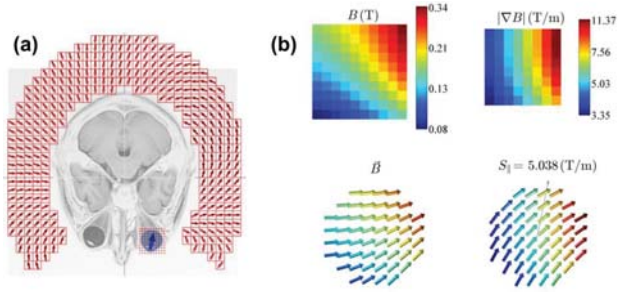

Fig. 1 (a) A system of Nd-Fe-B permanent magnets optimized with respect to their magnetization directions in order to provide the desirable magnetic field configuration. **(b)** Corresponding magnetic field and its gradient in the vitreous body of a human eye.

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Drug targeting investigation in the critical region of the arterial bypass graft

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After surgical intervention, arterial bypass graft remodeling is a complex process involving many biological and hemodynamic factors. Different methods have explored in literature for vascular delivery of anti-proliferative drugs to reconstructed bypass graft anastomosis region. Our experimental set-up simulates the capturing of the FMP's from the flow flux and the building of particle deposits onto the anastomosis wall of the bypass graft (Figure). Accumulation of FMP during magnetic targeting is the result of the action of the magnetic forces from the applied magnetic field and the hydrodynamic drag forces from the bloodstream. The 50 ml syringe was used to inject a sample of the FMP suspension into the experimental setup. Suspension injection performed at the constant flow rate, and the exit section of the syringe velocity have the same rate of the working fluid through the bypass graft. Taking into account that the distance between FMPs injection point (inlet section of the graft) and the bypass anastomosis is small practically after the time of 1s the FMPs particle starts to deposit on the tube wall. It is evident from Figure (I) that the particles with large radius 10μm (in our case) are captured easily in the targeted region.

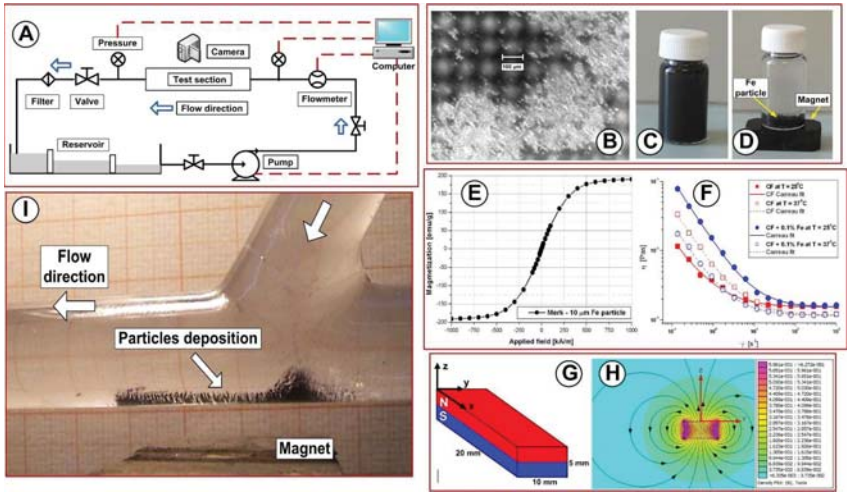

Figure: (A) Experimental setup used for bypass graft particle targeting. **(B)** Ferromagnetic particles (FMP's) morphology. To evaluate the magnetic particles targeting in the bypass anastomotic region, FMP's were utilized for quantification of the particle deposition. The particles were multi-domain soft iron particles with 10μm in size (Merck KGaA, Darmstadt, Germany) and density of ρ=7.87 g/cm^3, which are used to model the magnetic carriers and to provide easy flow visualization. **(C)** Diluted suspension of multidomain iron particles in a 60% glycerin and 40% water mixture (carrier fluid). **(D)** Separation of the magnetic particle in the presence of the magnetic field. **(E)** Magnetization curve for CI particles was measured using a vibrating sample magnetometer VSM 880 at room temperature. **(F)** Rheological and magnetorheological investigations of the carrier fluid with an Anton Paar Physica MCR 300 rheometer. **(G)** The magnetic field is generated using a NdFeB permanent magnet. **(H)** Magnet position was determinate using a numerical simulation of the two-dimensional field intensity profile of magnetic fields in different sections of the used NdFeB permanent magnet (2D Finite Element Method Magnetics program – FEMM 3.4. **(I)** FMP's retention in the critical bypass graft anastomosis region where intimal hyperplasia (IH) developed after surgical intervention.

Hemodynamic effects on particle targeting in the arterial bifurcation for a different type of particles

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Drug therapy of vascular disease has proposed for the inhibition of angiogenesis in atherosclerotic plaques and the prevention of restenosis applied in special anatomical locations (e.g., vascular bifurcations). To improve the duration and local efficiency of drug release, a combination of MNPs and external magnetic fields is very promising. In this work, we investigated if it is possible to accumulate MNPs at the lesion-specific location of the arterial bifurcation using a permanent external magnet. By the SYNTAX study (SYNergy between PCI with TAXUS™ and Cardiac Surgery) we investigated the particle targeting for the Type D lesion (Type D: Stenosis involving the main vessel and ostium of the side branch, Figure A+B). We also compare the effects of the hemodynamics on the accumulations in the targeted site of the different type of MNPs. The investigation results show that the accumulated particles in the main artery are higher than that on the branch tube.

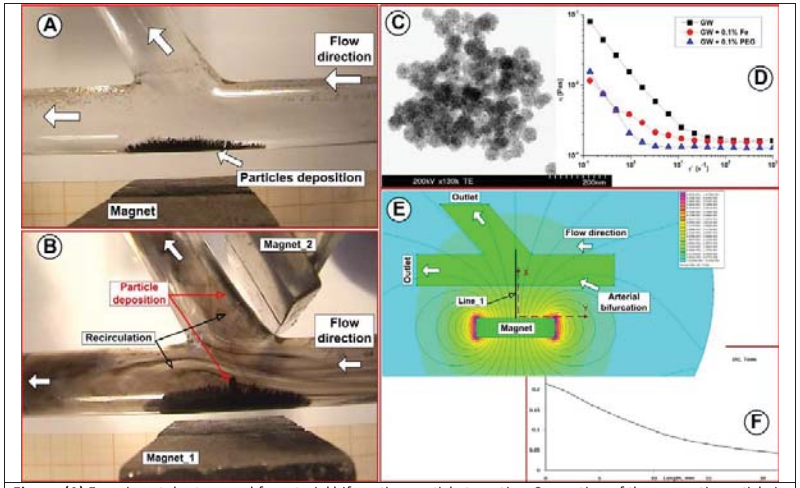

Figure: (A) Experimental setup used for arterial bifurcation particle targeting. Separation of the magnetic particle in the presence of the magnetic field. A sliding device is used to precisely position the permanent magnet at the retention area. **(B)** Bifurcation flow profile in the presence of the particle accumulations. **(C)** Morphology of the prepared PEG-coated NPs (TEM images of NPs). **(D)**. Rheological investigations of the different used suspensions ("carrier fluid" = 60% glycerin and 40% water mixture – GW; GW + 0.1% Fe (diluted suspension of multidomain iron particles); GW + 0.1% PEG (hydrophilic polymers, polyethylene oxides – PEG, coated NPs)). **(E)** Magnet position was determinate using a numerical simulation of the two-dimensional field intensity profile of magnetic fields in different sections of the used NdFeB permanent magnet (2D Finite Element Method Magnetics program – FEMM 3.4. **(F)** . Magnetic field intensity along the Line 1 used for precise positioning of the magnet to the obtained different concentration of the targeted NPs.

Aggregation and Hydrodynamics of Superparamagnetic particles in Mag-Guider systems

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Recently we developed a permanent magnet system to guide and image super-paramagnetic particles (SPP) by simple rotation of Halbach cylinders [1]. This instrument was nicknamed Mag-Guider (**Ma**gnetic **Guid**e and Imag**er**). The velocity of various SPPs was studied with this system. Although the size of the SPPs varied from some 10 nm to several µm this was not reflected in the measured velocities, which were all several magnitudes bigger than calculated for the single particles. This behavior is explained by the formation of long chains and lentil shaped agglomerates of the particles due to strong and homogeneous magnetic field in the device (cf. Fig. 1). This field orients and magnetizes the SPPs so that they form long chains. Beyond a critical length of such chains particles can not only attach at the poles of such chains but also at their sides, causing lentil shaped forms (cf. Fig. 2).
The experimental data were reproduced by simulations and an analytical hydrodynamics model is suggested and compared with the measurements.

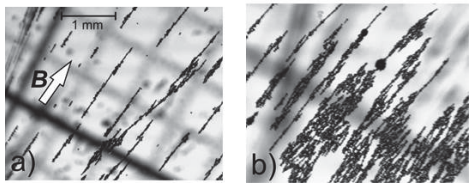

Figure 1: Micrographs of SP-iron oxide particles with an average diameter of 30 µm in the Mag-Guider system. (a) Initially the particles from long chains or strings. (b) A few seconds later lateral growth sets in resulting in carpet- or raft-like structures.

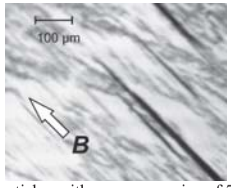

Figure 2: Micrographs of cobalt ferrite particles with an average size of 70 nm at higher resolution showing big clusters with a needle or lentil shape.

Reference

[1] O. Baun, P. Blümler, J. Magn. Magn. Mater. **439** (2017) 294-304

Synthesis and characterization of different types of Manganese Ferrite Nanoparticles for drug release application

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Abstract: Therapeutic and diagnostic uses of magnetic nanoparticles (MNPS) are receiving immense interest in various branches of medical science because of their high surface to volume ratio and high porosity, novel magnetic properties etc. But optical properties are also very important features to study for their biological applications. We have shown here a new protocol where manganese ferrite (MF) shows novels fluorescence properties after functionalization with tartarate molecules. Different types of MF nanoparticles such as solid and hollow were synthesized which is made water soluble. The particles were analysed by X-ray diffraction (XRD) and transmission electron microscope (TEM). Fluorescent property of these particles was observed after functionalization of these particles with different organic molecules such as sodium tartarate, oleic acid, folic acid. In this study it was observed that the MF NPs behave like a fluorescent molecule after functionalized with sodium tartarate in the excitation wavelength of 440 nm and 550 nm although the MF NPs and sodium tartarate individually have no fluorescent property in the same excitation range. This fluorescent property is very stable and intense for future use in cell imaging technique. Drug DOX is loaded in these particles and drug release study is done under different stimuli like AC magnetic field and pH which indicate that these particles will be useful for cell probing for its fluorescence properties and drug release.

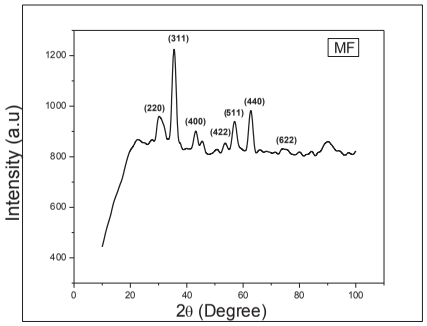

Figure1

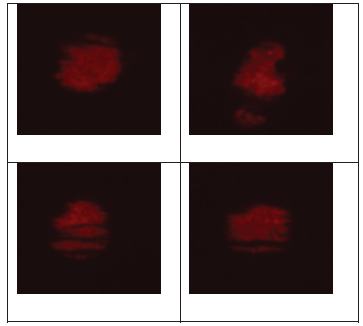

Figure2

Figure1: XRD of MF NPs and Figure2: Fluorescence image of sodium tartarate functionalized MF NPs.

Magnetic Drug Targeting: a preliminary numerical model

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Introduction: Magnetic Drug Targeting (**MDT**) has been recently proposed as an innovative medical technique towards cancer treatment to increase the selectivity and the efficiency of the delivery of drugs [1]; the process of guiding Magnetic Nanoparticles (**MNPs**) to the target site, through an external magnetic field control, needs to be modelled mathematically to effectively plan patient-specific therapies. In this work, a preliminary model of blood-flow mediated **MNPs** transport under the influence of an external magnetic field is presented. This model will be further developed to take into account the interaction between magnetic nanocarriers and biological tissues.

Methods: The geometry of a human descending aorta was reconstructed from CT data (https://www.vmtk.org/). A computational fluid dynamic (**CFD**) model of the advection-diffusion equation was then developed using COMSOL Multiphysics® to track the path of a diluted species concentration, with properties typical of an injectable **MNPs** solution, in the blood flow. The fluid domain is connected to a porous media domain, representing a simplified tumour tissue, through an interface which is defined on the vessel wall; this interface (highlighted in blue in **Fig.1.A**) has typical properties of a human arterial wall. An external permanent magnet with a radius of 5 mm and a height of 2.5 mm is positioned close to the tumour site and modelled with a cylindrical shape (**Fig.1.A**); it produces a magnetic field intensity of about $-700\ \frac{kA}{m}$ in the direction perpendicular to the vessel centreline. As a first modelling approach, the space between the fluid dynamic model and the permanent magnet is assumed to be filled by air. Furthermore, the behaviour of **MNPs** with different diameters was investigated: values of 1 nm and 100 nm were tested as limit cases.

Results and Discussion: Preliminary results suggest that for **MNPs** diameter ranging from 1 nm to 100 nm, mass transport is mainly governed by advection with a small diffusive contribution (**Fig.1.B**). Moreover, the magnetic field has a noticeable influence on **MNPs** behaviour, especially regarding the concentration profile and the final quantity that enters the tissue. As it can be seen in **Fig.1.C**, the magnetic field lines play a role in the distribution pattern of the diluted species concentration; the field vectors show the direction towards which **MNPs** are attracted.

Conclusion: The discussed preliminary results show that the coupling between the advection-driven mass transport and the diffusion-driven mass transport needs to be further investigated in detail [2], especially concerning the additional contribution introduced by the external magnetic field, in terms of magnetic driving force. It is planned to determine the permanent magnet position and orientation which increases the accumulation of magnetic carriers at the targeted site. In addition, the porous media model of the tumour tissue needs to be further refined to be more representative; in this respect, patient-specific tumour geometries and experimental data will be included in the computational framework.

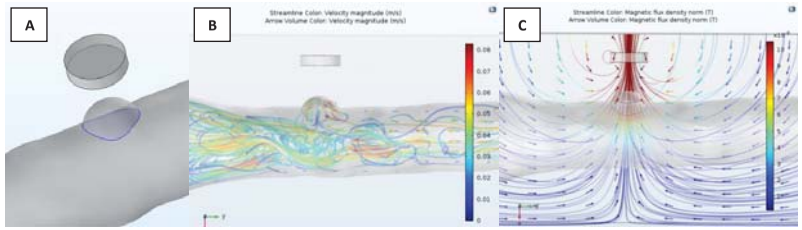

Fig.1: **(A)** Interface defined on the vessel wall; **(B)** Velocity field in the fluid domain; **(C)** Magnetic field lines.

[1] A. Nacev et al., *J. Magn. Magn. Mater.* 323, pp. 651–668, 2011.
[2] M. W. Dewhirst et al., *Nat. Rev. Cancer* 17, pp. 738–750, 2017.

Nucleic acid-functionalized iron oxide nanoparticles for controlled drug release using magnetic hyperthermia

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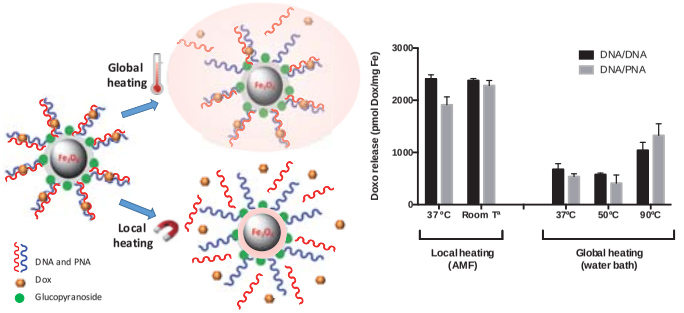
The use of nanoparticles to deliver drugs to tumours represents an attractive clinical therapy in comparison with traditional chemotherapy.[1] Herein we describe an innovative controlled release system based on nucleic and peptide nucleic acid-engineered magnetic nanoparticles (MNPs). DNA and its pseudo-peptide backbone derivative (PNA)-functionalized nanoparticles present two main advantages: (i) nucleic acids can be denatured with heat at a specific temperature and (ii) a multifunctional system can be created with the incorporation of different molecules (antibodies, tumoral markers, drugs, etc.) linked to complementary oligonucleotide strands.[2] Our aim is to release a drug in the desired target by combining the heating properties of MNPs under an alternating magnetic field (AMF) with the nucleic acid denaturation effect. 12 nm iron oxide MNPs were synthesized and transferred to water by coating with an amphiphilic polymer – poly (maleic anhydride-alt-1-octadecene), PMAO following the protocol optimized in our group.[3] The MNPs were further functionalized step-wise with 4-aminophenyl ß-D-glucopyranoside (to increase colloidal stability) and two different complementary DNA systems: DNA/DNA and DNA/PNA (1/1 ratio). The antitumoral drug doxorubicin (Dox) was intercalated between the double strands of both systems and the intercalation was quantified by fluorescence spectroscopy. The *in situ* release of the drug from both systems was evaluated after the application of global (water bath) and local heating (AMF, 829 kHz, 252 Gauss, 30 min). In the first case, temperatures of 37°C, 50°C and 90°C were tested and in the second case, magnetic hyperthermia was applied at constant physiological temperature (37°C) and at room temperature. The results showed that there is a higher release of drug in the case of local heating induced by the AMF, indicating a high temperature increase in the vicinity of the MNPs and the subsequent nucleic acid denaturation.[4] All of this brings to light an important advantage for future hyperthermia treatments as less quantity of nanoparticles and weaker magnetic fields will be required. The internalization profiles, as well as the cytotoxicity of the MNPs were also tested in different cell lines. We are currently investigating the *in vitro* magnetic hyperthermia-mediated drug delivery and cell death induction using these MNP systems.



References: (1) Jabir, N. R. et al., *Int. J. Nanomed.*, **7**, 4391, 2012. (2) Dias, J. T. et al., *Angew. Chem. Int. Ed.*, **52**, 11526-11529, 2013. (3) Moros, M., et al., *Nanoscale*, **2**, 1746-1755, 2010. (4) Herrero, V. et al., *in preparation*.

Hyaluronic Acid Modified Bubble-Generating Liposomes for Magnetically Triggered Targeted Chemotherapeutics

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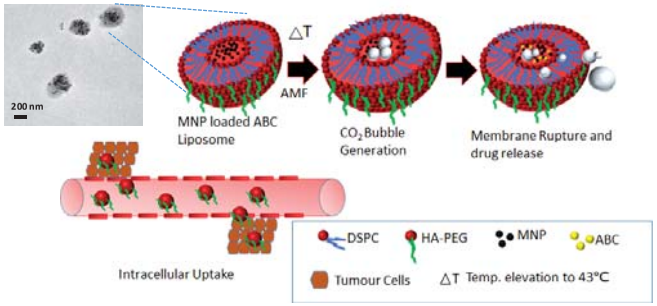
The combination of liposomes and magnetic nanoparticles (MNPs) is a promising approach for cancer therapy due to its capability to release higher percentage of drug at the diseased area. Contemporaneously, bubble-generating liposomes are of great interest in the field of cancer therapy, due to its triggered drug release properties and its ability to rupture cancer cells through cavitation induction. The objective of the present work is to synthesize an efficient and cost-effective magnetically triggered, targeted drug delivery system for cancer therapy. Iron oxide MNPs-loaded bubble-generating liposomes modified by hyaluronic acid (HA) were synthesized by the lipid film hydration technique. The resultant particles were found to be spherical in shape with an average size ranging from 150 to 250 nm. The physico-chemical properties of the particles were characterized through FT-IR, NMR, XRD, TEM, Cryo-TEM and DSC. In vitro drug release studies were carried out to evaluate the triggered drug release induced by CO_2 bubbles generated in the liposomes under an alternating magnetic field (AMF), which induced localized heat generation by MNPs to decompose ammonium bicarbonate (ABC) co-entrapped in the liposomes. In vitro cell culture studies using human glioblastoma cells (U87) were performed to evaluate the targeting efficiencies and anti-tumor efficacy of the prepared liposomes. The confocal microscopic analysis confirmed the enhanced intracellular uptake of HA-modified liposomes by U87 though binding of HA to the overexpressed CD44 receptors on U87 surface, which facilitated internalization of liposomes by endocytosis. Thus, we could conclude the effective drug release from liposomes by AMF guided bubble generation, followed by triggered release of chemotherapeutics for anti-tumor efficacy.



Schematics of magnetically triggered drug release from bubble-generating liposome.

Cardiac Myocyte targeted Drug Delivery using Magnetically vector nanoparticles

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With the continuous technological advancements being made in the medical field every day, the ability to improve drug delivery uptake in cardiac research is a prominent topic of discussion. Nanoparticles provide the opportunity to improve the efficiency off drug therapy while minimizing chemotherapy side effects through controllably releasing the encapsulated drug at the target site. Mono-disperse Fe_3O_4 nanoparticles/polystyrene composite nanospheres with a large volume fraction of trapped magnetite and fluorophores were used in an *in vivo* experiment. In this study, magnetic nanoparticle feasibility of delivering to the heart by external magnetic field was successfully achieved. Mono-disperse Fe_3O_4 nanoparticles dramatically localized in the heart myocardium by magnetic field guidance, and increased the quantity of delivery to the cardiac myocytes in comparison to nanoparticles that did not have a magnetic field applied.

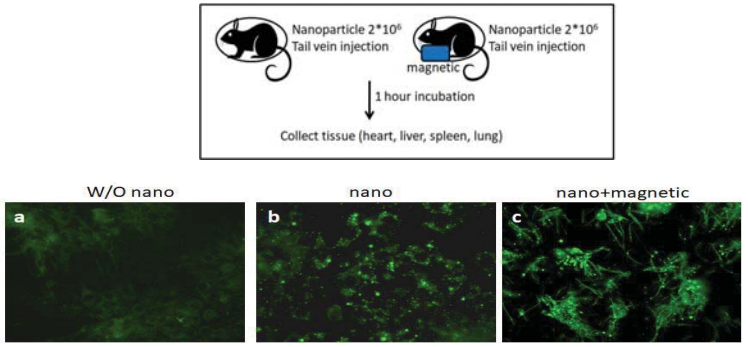

Figure. Fluorescence microscopy image demonstrating cellular internalization of the Fe_3O_4-loaded nanoparticles into neonatal cardiac myocyte by magnetic field guidance

Development of biocompatible nanoparticles modified with magnetite binding protein for cancer theranostics

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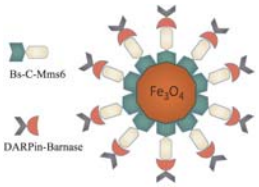
Engineering of nanostructures which combine diagnostic and therapeutic functions can help fight cancer without injuring healthy cells. However, such constructions transition to clinical practice is often hampered with complicated chemical conjugation of functional components (antibodies, toxins, etc.) to nanostructures. Moreover, biocompatibility and stability under physiological conditions of most of them is severe problem during the development of functionally active structures for cancer therapy and diagnostics (and combination of the approaches, namely, theranostics).

Facile modification of nanostructures with functional components can be achieved via self-assembly of components using non-covalent protein interaction of two proteins of bacterial origin, namely Barnase and Barstar (K_{aff} = 10^{14} M^{-1}). By means of newly developed recombinant fusion protein of Barstar and Mms6, namely Bs-C-Mms6, it is possible stabilize and modify magnetic nanoparticles without using any toxic polymer during synthesis or chemical conjugation. Magnetotactic bacteria orient in the Earth's magnetic field due to specialized organelles named magnetosomes that contain membrane-enveloped single crystalline magnetite. Mms6 is a protein that plays a crucial role in magnetite crystals biomineralization and magnetosomes formation in *Magnetospirillum magneticum* (strain AMB-1). Barstar is a natural inhibitor of ribonuclease Barnase. These proteins are stable, soluble and accessible for genetic fusion, forming the basis of module protein assembling.

We developed a new recombinant protein Bs-C-Mms6 that was expressed in *E. coli* [strain BL21(DE3)]. It was shown that in a wide range of concentrations the protein does not impact on cell viability. Measurement of the enzymatic activity of Barnase after incubation with different concentrations of Bs-C-Mms6 showed that Bs-C-Mms6 inhibit Barnase activity demonstrating the retention of the functionality of Barstar in the fusion protein. The specificity and selectivity of the Barnase*Bs-C-Mms6 protein complex formation was also shown on the surface of eukaryotic cells.



Magnetite nanoparticles were incubated with Bs-C-Mms6 and Barstar as a control. Only Bs-C-Mms6 stabilized magnetite particles under physiological conditions and non-significantly increased the hydrodynamic size of particles in comparison with the control samples. Besides, the biocompatibility of Bs-C-Mms6-coated particles was demonstrated with the MTT-test on different eukaryotic cell lines. Finally, the assembly of the whole structure with magnetic nanoparticles DARPin-Barnase and Bs-C-Mms6 was demonstrated, both in solution and on the surface of cells by flow cytometry and fluorescence spectroscopy.

In this work we developed a novel system for magnetic nanoparticles modification without any chemical conjugation with retention of functional activity of component being attached and demonstrated the potential of the system for cancer cell targeting. Such method of modification could be successfully used for development of magnetic nanostructures-based theranostic agents.

The work was supported by the Russian Science Foundation (grant № 17-74-20146).

Partially hollow nanostructures for magnetically-assisted drug delivery

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Magneto-responsive materials based on superparamagnetic iron oxide nanoparticles (SPIONs) attract special attention in biomedical field, since they enable magnetic drug targeting i.e. the remote control over the distribution and accumulation of the nanocarrier in the body. However, preparation of effective and robust magnetically responsive nanocarriers still represents a great scientific challenge, due to physical limit of individual SPIONs, namely the too small magnetic force acting on individual nanoparticle when exposed to magnetic field gradient, resulting in their ineffective spatial guidance (Kralj S. *et al*. Curr. Med. Chem., 2017, 24, 454-469). The solution to this shortcoming could be assembly of numerous individual SPIONs into SPION clusters, which exert good magnetic responsiveness, due to the increased volume of magnetic phase. In well-defined magnetic fields the SPION clusters can form various hierarchical nanostructures (i.e., nanobundles, nanochains) (Kralj and Makovec, ACS Nano, 2015, 9, 9700-9707), having a great potential for the preparation of innovative magneto-responsive nanodelivery systems (Figure).

Partially hollow magnetic nanostructures are prepared by selective partial dissolution of iron oxides in the core of silica-coated nanoclusters or nanochains in acidic conditions. To achieve colloidal stability of prepared partially hollow nanostructures in physiological environment, their surface was additionally modified with functional alkoxy-silanes and hydrophilic polymers. Results of preliminary experiments showed promising drug-loading efficiency.

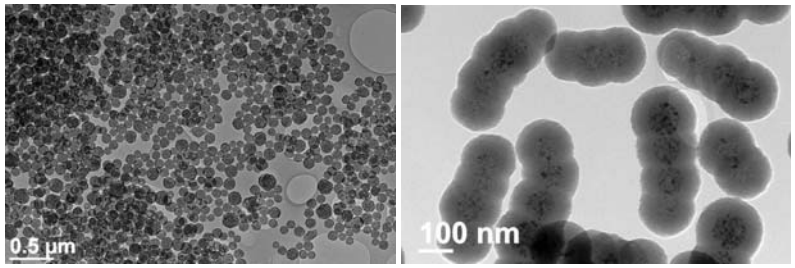

Figure. TEM images of magnetic, partially hollow silica nanospheres (left) and partially hollow nanochains (right).

Acknowledgements:

The authors acknowledge financial support of the Slovenian Research Agency through the projects “Nanotheranostics based on magneto-responsive materials” (J1-7302) and “Tunnelling nanotubes for innovative urinary bladder cancer treatments” (J3-7494).

Chitosan-Polyvinyl alcohol-based pH-sensitive ferrogel beads for magnetically localized mucoadhesive controlled drug delivery in GI tract

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Stimuli responsive biomaterials offer a unique advantage over traditional local drug delivery systems in that the drug elution rate can be controllably increased in response to either internal or external stimuli or both to combat developing symptomology in order to maintain high local elution levels for efficient disease therapy. There is an expanding curiosity in stimuli-responsive hydrogels to achieve controlled and self-regulated drug delivery. Stimuli responsive polymer hydrogels with their ability to swell/de-swell under varying pH conditions present themselves as a potential candidate for controlled drug delivery. In this study, chitosan-polyvinyl alcohol-based pH sensitive ferrogel beads have been formulated using solution mixing method. Briefly, iron oxide nanoparticles were dispersed in aqueous poly vinyl alcohol solution incorporated with ranitidine (A) and then mixed with chitosan solution prepared in glacial acetic acid (B). Ferrogel beads were prepared by injecting the above dispersions mixed together in a medium consists of 0.1 M NaOH prepared in aqueous ethanol solution maintained under constant stirring at room temperature. The beads formed therein was subsequently exposed to low temperature thermal cycling for cross linking yielded chitosan-polyvinyl alcohol-based drug loaded pH sensitive ferrogel beads. The developed ferrogel beads were characterized for its physiochemical, pharmaceutical, magnetic targeting and mucoadhesive properties. Preliminary results confirmed that the developed beads are biodegradable, biocompatible and promising in controlling the release of incorporated drug in response to the internal stimuli i.e., pH of the GI tract and to the external stimuli i.e., magnetic field which aids the localized drug delivery, thus enhances bioavailability at the desired sites of action, which can enable them to bypass physiological or pathological obstacles and also to achieve enhanced therapeutic efficacy.

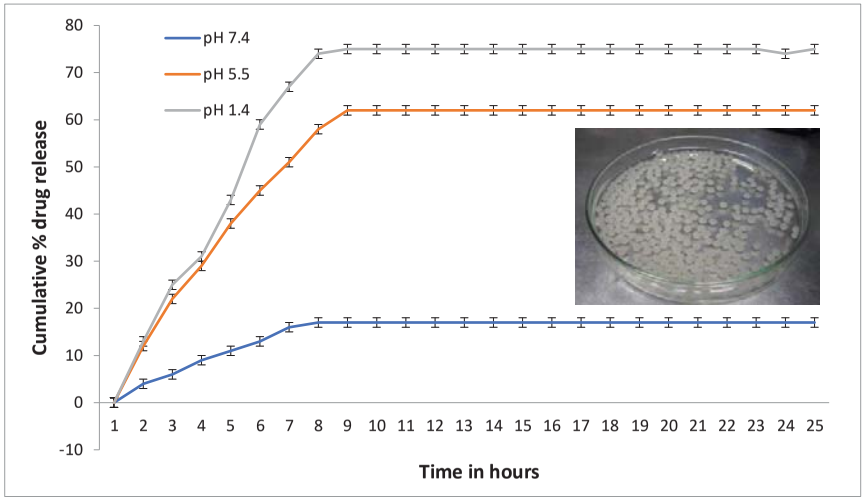

Fig 1. Cumulative ranitidine release profile from chitosan-polyvinyl alcohol-based pH-sensitive ferrogel beads in simulated gastric buffer medium maintained at various pH confirms pH sensitive drug release

Preparation and evaluation of magnetoliposomes co-encapsulated with herbal extracts for magnetic targeted therapy of breast cancer

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Liposomes are microscopic phospholipid vesicles with a bilayer membrane structure described as an ideal drug carrier system that protect and transport loaded compounds to the site of action. Liposomes can overcome many of the problems associated with other drug carrier systems, such as those involving solubility, pharmacokinetics, *in-vivo* stability and toxicity. Magneto-sensitive liposomes allow concentration of the liposomes in the desired area of the patient's organs by applying external magnetic field, often augmented by magnetic agglomeration. By enhancing local drug concentration, an improved therapeutic outcome may be achieved while off-target side effects in healthy tissues may be prevented. The present study involves the preparation of magnetic liposomes designed to act as herbal anticancer drug carriers, which can be effectively delivered to solid tumors via intravenous administration. Magnetic liposomes co-encapsulated with crude herbal extracts [Traditional Indian Medicinal Plants believed to possess anticancer activity viz., *Saccharum spontaneum* (Fam: Poaceae) and *Andrographis echioides* (Fam: Acanthaceae), *Wrightia tinctoria* (Fam: Apocynaceae); *Asystasia gangetica* (Fam: Acanthaceae) *Desmotachia bipinata* (Fam: Poaceae)] were prepared by the ether as well as ethanol injection method. The developed magnetoliposomes were evaluated for their physiochemical, pharmaceutical and magnetic properties through a series of assays is presented herewith. Our preliminary results suggest that this new treatment approach is biocompatible, biodegradable, non-toxic and non-immunogenic which involves intravenous administration of magnetic liposomes and restriction of magnetoliposomes through application of external magnetic field that might be effectively used for breast cancer therapy.

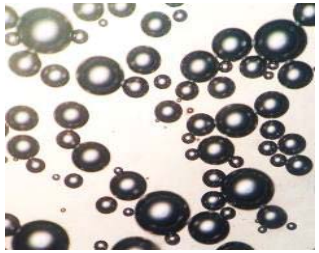

Fig. 1. Optical microscopic image of prepared magnetoliposomes containing crude herbal extracts

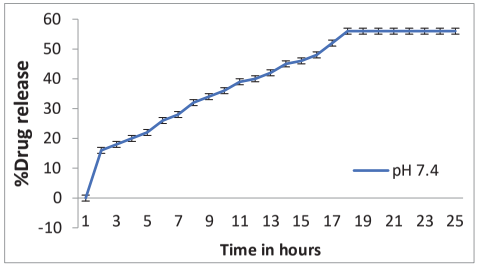

Fig 2. Cumulative drug release profile from magnetoliposomes buffer medium maintained at pH 7.4

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Magnetic Hyperthermia at the Nanoscale for Remotely Triggered Drug Delivery from Polymeric Nanocarriers

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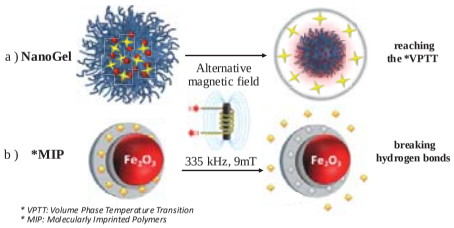

Schematic illustration of a) MagNanoGels and b) Molecularly Imprinted Polymers (MIP) releasing doxorubicin (DOX) under an alternative magnetic field (AMF)

Heat generated by magnetic nanoparticles when they are submitted to an alternative magnetic field is called magnetic hyperthermia. This magnetic hyperthermia can act at the macroscopic scale by increasing the global temperature of their environment or at the nanoscale by changing the chemical properties of a material.
We will focus the presentation on two examples based on the use of magnetic nanoparticles (MNP) as hot spots and how they can modify the properties of thermoresponsive polymers, such as nanogels, or of molecularly imprinted polymers (MIP). We demonstrate that this local heat can be used for the controlled release of doxorubicin.
In the first example, biocompatible and thermo-responsive nanogels, based on oligo (ethylene glycol) methacrylate monomers (OEGMAs) and methacrylic acid co-monomer (MAA) were prepared by conventional precipitation radical co-polymerization in water and post-assembled by complexation with MNP (Scheme 1a). These magnetic nanogels, labeled MagNanoGels, with a hydrodynamic diameter from 328 to 460 nm, as a function of MNPs content, have a swelling-deswelling behavior at their volume phase temperature transition (VPTT) around 47 °C in a physiological medium (pH 7.5), which is above the human body temperature (37 °C). Applying an alternative magnetic field (335 kHz, 9 mT) increased twice the release of DOX, while no macroscopic heating was recorded. This enhanced drug release is due to the polymer shrinking under local heating, as illustrated by the MagNanoGels size decrease under AMF. In cancer cells, not only the DOX-MagNanoGels internalize DOX more efficiently than free DOX, but also DOX intracellular release can be remotely triggered under AMF, in athermal conditions, thus enhancing DOX cytotoxicity. (1)
In the second example, we designed a new magnetic doxorubicin delivery system, noted MNP@DOX-MIP, by growing molecularly imprinted polymers from an individual iron oxide nanoparticle surface via an acrylic acid (AA) monomer used as the polymerization initiator anchored on the surface of MNP and doxorubicin as the template (Scheme 1b). The DOX release of such functional MNPs was investigated in *in vitro* and in cells under AMF excitation. The cumulative drug release *in vitro* reaches 60% under AMF compared to 15% without AMF in the same conditions, due to the breaking of hydrogen bonds between DOX and MIP. More interestingly, after internalization in cells, Fe_2O_3@DOX-MIP nanoparticles did not induce cancer cell death demonstrating that when bonded to the MIP (and thus to the nanoparticles), DOX is inactive. By contrast, after AMF application cancer cell viability was affected, with a cell viability reduced to 60% after 1 h30 min treatment. It is very important to emphasize that this AMF-induced cancer cell death was achieved under athermal conditions. Indeed, during the AMF exposure, the cell medium was maintained at 37 °C.(2)

(1) Cazares-Cortes, E.; Espinosa, A.; Guigner, J.-M.; Michel, A.; Griffete, N.; Wilhelm, C.; Ménager, C. Doxorubicin Intracellular Remote Release from Biocompatible Oligo(Ethylene Glycol) Methyl Ether Methacrylate-Based Magnetic Nanogels Triggered by Magnetic Hyperthermia. *ACS Appl. Mater. Interfaces* **2017**, *9* (31), 25775–25788.
(2) Griffete, N.; Fresnais, J.; Espinosa, A.; Wilhelm, C.; Bée, A.; Ménager, C. Design of Magnetic Molecularly Imprinted Polymer Nanoparticles for Controlled Release of Doxorubicin under an Alternative Magnetic Field in Athermal Conditions. *Nanoscale* **2015**, *7* (45), 18891–18896.

Mononuclear phagocyte system blockade, caused by the uptake of magnetic nanoparticles

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Due to their unique properties magnetic nanoparticles (MNPs) find new applications in medicine as contrast and therapeutic agents. However, during intravenous administration many MNPs are rapidly eliminated from the bloodstream by the mononuclear phagocyte system (MPS), and that greatly reduce the effectiveness of therapy.

In this work method of MPS blockade was investigated in terms of it's ability to prolong the circulation of MNPs in the bloodstream. MPS blockade is the phenomenon of reducing macrophage activity after they have eliminated previously introduced high doses of nontoxic nanoparticles. We studied blockade efficiency depending on particles' physical and chemical properties. For that purpose we synthesized a library of silica MNPs of different sizes using hydrolysis of tetraethoxysilane. To obtain magnetic silica particles, superparamagnetic iron oxide nanoparticles were used as centers of nucleation in Stober process. Also, magnetic particles with different polymer coatings were used for MPS blockade.

High doses of silica particles were administered intravenously to partially block MPS before injection of MNPs, and their circulation time in the bloodstream was measured (Figure). Real time noninvasive MNP quantity detection was carried out based on their nonlinear magnetization in response to an applied alternating magnetic field generated at two frequencies (MPQ-technique) [1].

It was shown that MPS blockade leads to increasing the circulation time of particles by more than 20 times, with efficiency increasing with the particles' size and depending on the coating type.

The method described in this work in combination with the obtained results can be used for significant increase in the efficiency of nanosized agents *in vivo*.

The work was supported by the Russian Science Foundation (grant № 17-74-20146)

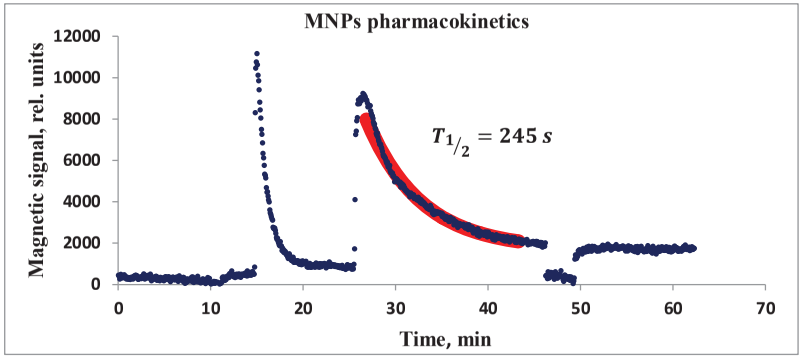

Figure. The first peak and the exponential fall correspond to the elimination of blocking nanoparticles. The second is the elimination of secondly administered dose of tracing particles.

Literature

[1] - *Nikitin M.P., Torno M., Chen H., Rosengart A., Nikitin P.I.* Quantitative real-time in vivo detection of magnetic nanoparticles by their nonlinear magnetization // *Journal of applied Physics. 2008. 103*(7), 07A304.

PEGylated Nanoparticle-Induced Hypotensive Effects: Hemodynamic Mechanisms

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Magnetic nanoparticles (MNPs) are potential drug carriers in target drug delivery, and polyethylene glycol (PEG) is a common surface modulator used to increase the half-life of nanoparticles in circulation. Although nanocomposites may interact with components of blood and vessel in circulation, our previous studies demonstrated that dextran-coated MNPs did not alter mean arterial pressure (MAP) or iliac blood flow in rats. In the current study, we asked whether PEGylated MNPs (PEG-MNPs; nanomag®-D, micromod) with dextran coating may alter hemodynamics *in vivo*. In anesthetized Sprague Dawley rats, MAP was recorded by a direct cannulation of carotid artery, and drugs were administered *via* a jugular vein catheter; renal blood flow (RBF) and abdominal blood flow (ABF) was measured by ultrasound flowmetry. Administration of PEG-MNPs with dextran coating (250 nm) reduced MAP by 31 ± 5 mmHg, which was recovered to 50% of the basal levels by 18 ± 4 min (t_{τ}; n=7), respectively. In addition, RBF decreased by 6.2 ± 0.9 ml/min from a basal level of 10.8 ± 1.1 ml/min, while renal vascular resistance (RVR) increased by 69%. In contrast, acetylcholine-induced hypotension was associated with an increase in RBF, suggesting PEG-MNP-induced hypotension was not mediated by a vasodilator effect. In addition, PEG-MNPs did not alter endothelium-dependent vasodilation induced by acetylcholine. Calculated cross-sectional area (CSA) obtained from Poiseuille equation decreased by 26% with t_{τ} of 20 ± 2 min (n=6); similar CSA results were obtained based on ABF measurement. Intriguingly, tachyphylaxis of the hemodynamic responses to PEG-MNPs occurred with the 2nd dose. At the end of the experiments, renal histology analysis with Prussian blue staining revealed no significant iron retention in the kidney, suggesting that PEG-MNPs did not cause long-lasting obstruction in the renal vasculature. In conclusion, PEG-MNP-induced hypotensive effects were associated with decreased blood flow and increased vascular resistance, which may be due to PEG-MNP-induced agglomeration that causes transient occlusion in microvasculature.

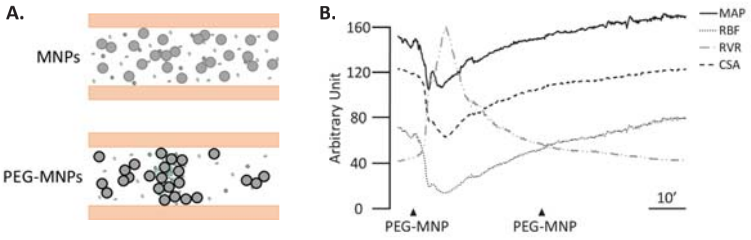

Figure. A) Schematic diagram of PEG-MNP agglomerate formation in microvessels. **B)** Representative hemodynamic and renal CSA responses to two consecutive doses of PEG-MNPs in one rat. Hypotension induced by PEG-MNPs is associated with a decrease in RBF and CSA.

Iron oxide polymer nanorattles as nanocarriers for targeted tumor therapy

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Designing an efficient system to deliver drugs to a tumor site and to minimize the systemic toxicity remains challenge. In the field of nanomedicine, extensive attentions from research groups all over the world have been given to investigating the possibility of using nanocarriers to tackle these challenges in targeted drug delivery. The low toxicity and magnetic properties of superparamagnetic iron oxide nanoparticles (SPION) have made them ideal candidates for applied research in imaging and drug delivery [1,2]. In this study, SPION contained polymeric nanorattles (Fig.1A) were synthesised using the same approach that was developed in our group to produce TiO_2 polymeric nanorattles [3]. These nanorattles can be loaded with a chemodrug such as doxorobucin and become more efficient in the targeted drug delivery as iron oxide nanoparticles play as magnetic vectors (Fig.1B). These nanorattles were tailored to be stable under physiological conditions and non-toxic to cells. The loading of doxorubicin into nanorattles was simple, effective and quantitative. Bench top and *in vitro* studies showed that the release of doxorubicin from the carriers was facilitated under the low pH conditions such as in the endosomes and lysosomes (Fig.1C,D). Furthermore, the drug release was triggered in response to an external alternating magnetic field due to the presence of SPIONs. Elevated local temperature in the presence of SPIONs when exposed to an alternating magnetic field can potentially contribute to the destruction of cancer cells for hyperthermia treatment. The simple and robust synthesis of the SPION-nanorattles and possibility of loading a variety of active ingredients to these nanorattles offer effective nanocarriers for targeted delivery in nanomedicine.

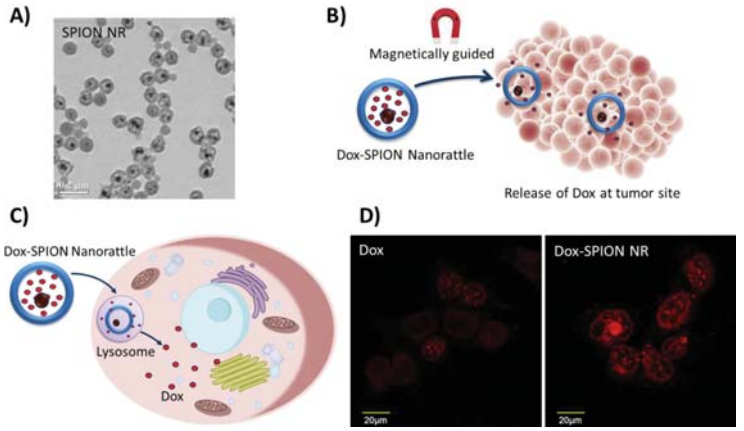

Figure 1. TEM image of monodispersed SPION-nanorattles (A); Scheme of targeted cancer therapy with doxorubicin loaded SPION-nanorattles (B); Scheme of doxorubicin released from SPION-nanorattles in cells (C); Confocal images of DLD-1 cells treated with doxorubicin alone (left) and doxorubicin loaded SPION-nanorattles (C)

References:
1. Krishnan, K.M. *Ieee T Magn* **2010**, *46*, 2523
2. Pham, B.T.T.; Colvin, E.K.; et al.. *Int J Mol Sci* **2018**, 19
3. Nguyen, D.; Such, C.; Hawkett, B. *J Polym Sci Pol Chem* **2012**, *50*, 346

Polyvinyl alcohol-based ferrogel system for magnetic field guided acid triggered delivery of omeprazole

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Smart hydrogels are macromolecular networks of crosslinked hydrophilic polymers able to absorb and release aqueous solutions in a reversible manner, in response to specific environmental stimuli finds promising applications as smart drug delivery carriers. It is proposed to develop polyvinyl alcohol-based ferrogel for sustained, magnetic field guided, site targeted and acid triggered delivery of omeprazole in the GI tract. Such a system is envisaged with properties like on-demand controlled drug release, improved bioavailability, drug stability from acidic environment and prevent burst release effects.

Polyvinyl alcohol, a hydrophilic, biocompatible and pH sensitive polymer was dissolved in water under magnetic stirring at 80°C and incorporated with magnetite as ferro fluid. Omeprazole was dissolved in methanol and added to the polymeric dispersion and exposed to freeze thaw cycles. Polyvinyl alcohol-based ferrogel was developed based on the formation of physical crosslinks. Further the pharmaceutical properties such as drug content, drug release kinetics; physicochemical parameters such as swelling, TGA, XRD, particle size and surface charge analysis and morphological parameters using SEM; magnetic properties such as magnetic susceptibility were studied. The experimental results from swelling studies revealed that the hydrogels displayed definite pH sensitivity under physiological conditions, as well as sharp changes in the mesh size of their network and looks as a promising carrier for the sustained magnetic field guided acid triggered delivery of omeprazole in the GI tract.

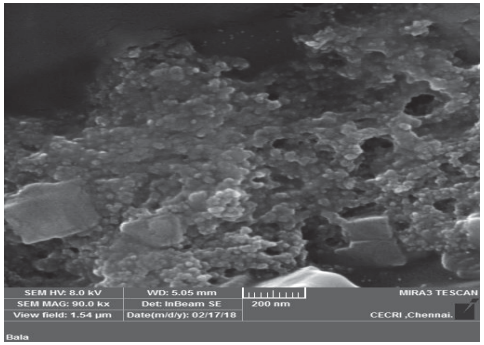

Fig 1. SEM images of Polyvinyl alcohol-based ferrogel

Fig 2. Polyvinyl alcohol-based ferrogel stored in an inverted vial

Development of Fe_3O_4-CuS nanocomposite modified with Poly-L-Lysine for drug delivery and imaging

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Iron oxide nanoparticles (IONPs) possess several important physical and chemical properties which make them valuable tools for biomedical applications such as cell labeling, imaging contrast agents, and hyperthermia. Semiconducting nanomaterials whose properties such as light emission, charge transport, thermal diffusion etc are a function of their size have gained importance in the last few years; especially CuS, due to its excellent optical and electrical properties. Thus, a nanocomposite of IONPs and CuS can be used for a combination of applications such as drug delivery, hyperthermia, phototherapy and imaging.

IONPs were synthesized by co-precipitation method under anaerobic conditions using $FeCl_2$ and $FeCl_3$ as precursors. Synthesized bare IONPs were then refluxed along with precursors for CuS nanoparticles to form Fe_3O_4-CuS nanocomposites. Synthesized nanocomposites were coated with poly-l-Lysine (PLL) in a shaker for surface functionalization. UV-Visible spectrum of nanocomposite showed a peak at 280nm thus showing the presence of CuS in the composite. FTIR spectrum for bare IONPs showed a strong peak at 581cm^{-1} which relates to Fe-O bond vibration, whereas the nanocomposite showed peak at 618 cm^{-1} which shows the presence of the Cu-O bond. Thus, this confirmed the formation of the Fe_3O_4-CuS nanocomposites. The PLL coated nanocomposite showed peaks at 1646 and 1541 cm^{-1} which represents the amide I and II groups, respectively thus confirming the coating of PLL on the nanocomposite. Model cancer drug, 5-Fluorouracil were encapsulated within the polymeric layer by incubation under shaking conditions at room temperature. The loading efficiency were optimized by varying drug:nanocomposite ratio, incubation time, temperature and pH. Release study were performed *in-vitro* using reservoir-sink method. The photoluminescence of the nanocomposites were also studied for bioimaging applications.

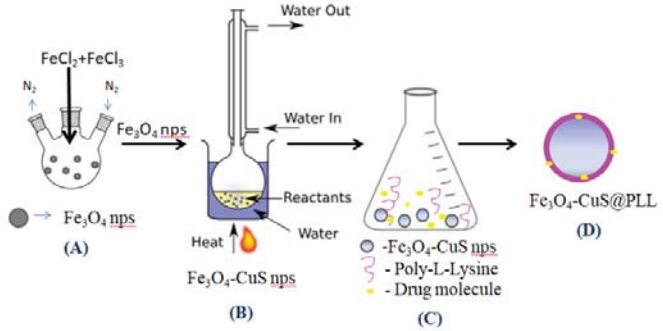

Fig: **(A)**: Synthesis of IONPs (Fe_3O_4 nps) by co-precipitation method under anaerobic conditions. **(B)**: Synthesized Fe_3O_4 nps were then refluxed with precursors for copper sulfide nanoparticles (CuS nps) to form the Fe_3O_4-CuS nanocomposite. **(C)**: Nanocomposites were then coated with poly-L-Lysine (PLL) and drug molecule was encapsulated into them. **(D)** Formed Fe_3O_4-CuS nanocomposite coated with PLL and containing drug molecule.

Magnetically responsive liposomes for delivery and remote-controlled release of high-molecular compounds.

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Liposomes, combined with magnetic nanoparticles, give an approach to use vesicles for medical applications, such as controlled drug delivery and release, diagnostic imaging. The most significant and less investigated issue is the remote-controlled drug release. Controlled release for magnetoliposomes achieves by using magnetic hyperthermia-triggering approach on the thermosensitive components on the membrane, where high-frequency alternating magnetic field (in the range of hundreds of kilohertz or higher) is often applied. However, such design may be not suitable for the cases where temperature changes are detrimental, such as in the brain tissues and highly perfused organs (kidney, liver, lung) (Jordan, Scholz, Wust, Fahling, & Felix, 1999). In this case, a low-frequency alternating magnetic field (LF-AMF) would be highly desirable in which no hyperthermia is produced. The mechanism of MNPs behavior in liposomes under LF-AMF exposure isn't investigated fully, but theoretically nanoparticles rotate and mechanically destroy the lipid membrane. Moreover, the effect depends on magnetic core size – the bigger diameter, the higher effect. Hence, use of MNPs for magnetoliposomes is limited by particles size. The aim of this work was to create magnetoliposomes, conjugated with core-shell MNPs@Au on its surface, and study the effect of LF-AFM (30-400 Hz) exposures on it. The effect was observed by TEM, ATR-FTIR and release kinetics.

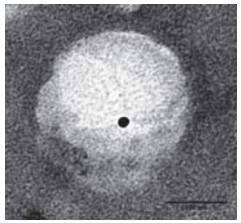

Figure 1. TEM picture, showing the structure of magnetic liposome.

Liposomes were produced via thin film method. MNPs@Au (mean diameter 35 nm) were stabilized with cystamin and then covalently bonded to the liposomes surface, using carbodiimide method. According to the TEM analysis, there are 1-3 MNPs@Au per a liposome. The ATR-FTIR analysis showed a high-frequency shift of CH2 asymmetric and CH2 symmertic bands under LF-AMF, that means an increase in the alkyl chains mobility in the liposome membrane as a result of the phase transition from the gel to the disordered liquid crystalline. The inhibitor Aprotinin (6 kDa) release study showed the increase of the rate of protein release under LF-AMF exposure in 2 times during 15 min. The dependence of the released inhibitor amount on AMF parameters was studied as well and the maximum effective frequency and intensity were found. It should be noted, that all observed effects were higher in the case of electrostatically adsorbed MNPs@Au. Moreover, we did not detect any effects of the release of a compounds with high molecular weight in the case of liposomes with MNPs of 5-6 nm. It proved the influence of magnetic core size on mechanical effects of MNPs under MF exposures. TEM and DLS analysises of the systems before and after MF exposures did not show membrane destruction or changes in liposome size. We assume that MNP just slightly 'stir' the membrane, increasing its permeability due to production of local small pore, without destruction of liposome. To conclude, we demonstrated new horizons for the development of systems for triggered high-molecular compound release.

Investigation was supported by RSF-14-13-00731 and RFBR 17-54-33027 OHKO_a grants.

Experimental quantification of volume loss rate and flow dynamics due to a magnetically localized fluid region in a laboratory model blood vessel flow

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Magnetic nanoparticle (MNP) based therapies often require long-term localization of high concentration MNP fluid within the blood; one example of this is for enhanced extra-vasation into tumor cells. The many difficulties of clinical studies of such therapies demands a realistic *in vitro* substitute. We have designed an experimental framework for pulsatile flow in a rigid tube of circular cross section. Within this framework, we have developed a mechanism to introduce and capture concentrated volumes of MNPs, a photographic system to quantify instantaneous volume, and a PIV-based analysis of the flow. In the present study, the pulsatile fluid is water, although optically transparent blood models would work equally well; also, we introduce a water-based, and thus miscible, ferrofluid as the theraputic volume. Our goal is to quantify the rate of loss of ferrofluid volume as a function of the flow characteristics (Reynolds and Womersley numbers) and the magnetic field characteristics.

The volume loss rate is quantified using a novel photographic method. First, we construct an accurate empirical "volume function" by building a library of images, top view and side view, of precisely known volumes. During pulsatile flow experiments, we found that area derived from a single top view image can be used to deduce the instantaneous volume at any time. For image analysis, we maintain consistent lighting (intensity, angle) so colors will map to ferrofluid density and subsequently, volume. To map colors in the images to ferrofluid density, we make use of KMEANS clustering in MATLAB, finally binarizing the images to achieve a projected area, measured in pixels (see figure).

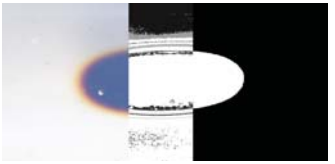

Figure 1: 3 stages of image analysis

Particle Image Velocimetry (PIV) is an accurate technique to obtain quantitative flow details, typically as a two-dimensional cut of velocity vectors. Our flow framework has been designed to allow PIV in either a vertical or horizontal plane, just as we can (at the same time) take optical images, for volume quantification, from a top or front view. This is performed by briefly stopping the flow to capture an optical image (Fig. 2 shows a front view PIV field). With these methods, the time-averaged rate of volume loss for an initial 10 μL Ferrofluid aggregate is presented under Reynolds and Womersley numbers ranging from 200-2000 and 15-45, respectively.

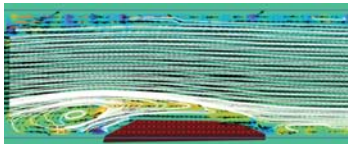

Figure 2: PIV velocity and shear rate

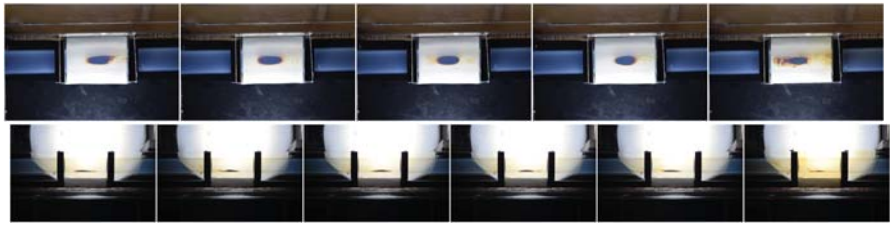

Figure 3: Top view (top) and front view (bottom) of consecutive stages of wash-away of miscible ferrofluid in pulsatile flow experiments. A permanent magnet below the ferrofluid provides a gradient field holding the fluid in place.

Cetuximab-conjugated thermosensitive magnetic liposome for targeted delivery of Irinotecan in glioma treatment

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Objective: This study aims to develop a thermosensitive dual targeted liposome for antitumor drug (Irinotecan, CPT-11) delivery in glioma treatment. Thermosensitive liposomes co-encapsulating iron oxide and CPT-11 will be modified with Cetuximab (CET) to recognize the epidermal growth factor receptor (EGFR) that is highly expressed on tumor cell surface. Dual targeted delivery of CPT-11 followed by thermo-responsive drug release at the tumor site is expected to increase the anti-tumor efficacy of the nanodrug. The therapeutic efficacy will be demonstrated in an orthotopic xenograft brain tumor mouse model.

Methods: Thermosensitive magnetic liposome (TML)/CPT-11/CET was first prepared through co-encapsulating superparamagnetic iron oxide nanoparticles with CPT-11 in the liposome (magnetic targeting) and then conjugating CET on liposome surface (ligand targeting). The chemical and physical properties of TML/CPT-11/CET were characterized, including DLS, DSC, TEM, XRD, FTIR, zeta potential, TGA, SQUID and ICP. Cellular uptake and cytotoxicity experiments were carried out in vitro to test the targeting ability and cytotoxicity toward tumor cells. Apoptosis-related proteins and apoptotic status were also examined by western blot and flow cytometry. Finally, the in vivo evaluation of TML/CPT-11/CET antitumor activity was assessed by non-invasive in vivo imaging system (IVIS) and nuclear magnetic resonance imaging (MRI) with U87 (human glioblastoma cell)-implanted BALB/c nude mice model.

Results: Drug loading and releasing experiments showed 72.51% (w/w) CPT-11 could be encapsulated in TML/CPT-11/CET. The drug release of CPT-11 from TML/CPT-11/CET was temperature sensitive, with around 6-fold increase in drug release at 43°C compared to 37°C after 30 min. By applying an alternating magnetic field (AMF) for heat induction, total drug release was achieved within 15 min compared with 20% release in 30 min without an AMF. In cellular uptake and cytotoxicity studies, the presence of CET on liposome surface enhanced cellular uptake of fluorescence-labeled TML by U87 cells. The results that intracellular uptake was drastically reduced by blocking the EGFR on cell surface before incubating with TML/CPT-11/CET further proved CET-mediated endocytosis. From in vitro cytotoxicity tests, enhanced cytotoxicity was shown for CET-conjugated TML/CPT-11 at 37°C, which further increased at 43°C due to the thermosensitive nature of drug release. The expression of Caspase-3 and pERK proteins, combined with PI/AnnexinV staining, indicating CPT-11 induced apoptosis. In the orthotropic xenograft mouse brain tumor model, IVIS results revealed a reduction in tumor volume after 15 days of treatment with intravenous injection of TML/CPT-11/CET, or combined with magnetic guidance in the presence or absence of an AMF. A marked shrinkage of tumor size could be also confirmed from MRI.

Conclusion: These results suggest that the dual targeting liposome developed in this study is an efficient drug carrier for brain tumor treatment. Furthermore, this smart drug delivery system is expected to provide a unique platform for controlled drug delivery in treatment of other diseases.

Development of a magnetic field applicator for magnetic nanoparticle targeting to the eye

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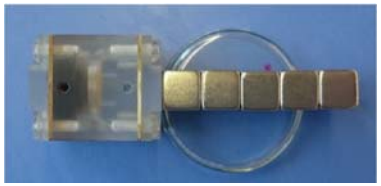
A lot of diseases of the eye (e.g. macular degeneration) are treated by intravitreal injection of a pharmaceutical agent by a syringe, where the vitreous serves as a drug depot. Since this injection is connected with important risks, i.e. endophthalmitis, and discomfort for a patient, the aim of our study is to replace the syringe injection by a “magnetic injection” where the drugs are coupled to magnetic nanoparticles and will be targeted into the eye magnetically.
In the first part of our study it has to be investigated, whether magnetic nanoparticles can be transported through different tissue types of the eye magnetically. A setup had to be designed and built up to perform these experiments. Main part of this setup is the magnetic field applicator which generates the magnetic field gradient to move the magnetic nanoparticles. For the first approach, different arrays consisting of permanent magnets were designed and the resulting magnetic field gradient at the front of the eye was calculated by means of numerical simulations. The second part of the setup is a device which holds the biological tissue and enables the experimental investigation of the nanoparticles transport through this tissue. Furthermore, the magnetic nanoparticles itself have to be optimized for the targeting application. For this purpose, magnetic iron oxide cores were prepared in single- and multicore structures and coated by a variety of organic materials. The targeting efficiency and the influence of the core type as well the agglomeration of particles on the targeting behavior was investigated in a simplified setup, consisting of a container filled with a ferrofluid and a permanent magnet bar.



Two chamber system with mounted bar magnet for investigation of particles transport through different tissue of the eye.

Numerical calculations revealed, that a magnetic field gradient of 5 T/m towards the vitreous can be obtained when permanent magnets are used for the setup of the magnetic field applicator. With this field gradient, the first tests for the targeting efficiency of the prepared particles were performed. It was found, that this gradient is not strong enough for targeting of well dispersed single-core particles but good targeting results were obtained for larger cores. Therefore we prefer to use multicore particles for further investigations. In this contribution we present the setup to perform the study as well as the first results. Ongoing work is focused on the application of superconducting magnets for application of stronger magnetic field gradients as well as the development of a novel setup for magnetic nanoparticles targeting by using pushing magnets.

Acknowledgements
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Temperature controlled Camptothecin release from magnetic PLGA microspheres

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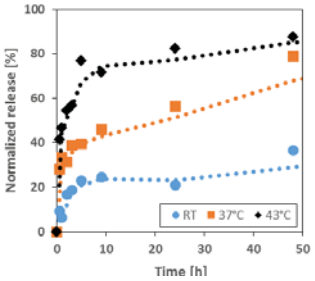
Magnetic microspheres (MMS) are essential for magnetic drug targeting and consist typically of a polymeric matrix material, which encloses magnetic particles and a loaded drug. MMS can be magnetically guided to a target area within the body where the pharmaceutical agent is released. Typically, the release takes place by degradation of the polymer matrix. For a faster release, the microsphere has to be disintegrated actively, e.g., by an increase of the temperature of the MMS. For this, the MMS can be exposed to an alternating magnetic field resulting in magnetization reversal losses within the magnetic particles leading to a temperature increase of the MMS. In order to guarantee optimal clinical magnetic targeting and defined drug release, MMS with uniform and consistent particle sizes and high specific heating power are necessary.



Camptothecin release from PLGA MMS as function of time and temperature.

Therefore, the aim of our investigation was the preparation of biodegradable MMS showing a homogeneous size distribution and well dispersed magnetic particles with a high magnetic heating performance. These MMS have to be loaded with a pharmaceutical agent and the release of the drug has to be investigated. In previous investigations, we developed magnetic multicore nanoparticles, which show a high magnetic heating efficiency. In the here presented study, these particles were coated by different materials to enable a homogeneous dispersion of the magnetic cores within the poly(lactic-co-glycolic) acid (PLGA) matrix of the microsphere. The drug camptothecin was also embedded into the PLGA matrix. MMS sets were prepared from this hydrophobic dispersion (of three PLGA with different lactid-co-glycolid ratios) by formation of micro-droplets within a hydrophilic phase using a mechanical homogenizer, followed by drying of the droplets. MMS were characterized by means of light scattering, differential centrifugal sedimentation spectroscopy, scanning electron microscopy, magnetometry, magnetic calorimetry, and UV/Vis spectrophotometry for determination of the drug release as a function of time and temperature (water bath: 20 to 43 °C). The MMS diameter can be tuned in the range of 1 to 2 µm and the MMS show a content of magnetic material of up to 30% by mass and a drug loading of about 0.5% by mass. The MMS have a specific heating power of 112 W/g_{MMS}, which enables a sufficient magnetic heating for enforced disintegration of the PLGA at a MMS in tissue concentration of 2 % by mass. Depending on the applied temperatures and the used PLGA type, the loaded drug can be released within hours to days. In ongoing studies the drug loading has to be increased and the temperature controlled release has to be realized by magnetic heating.

Acknowledgements
This work was supported by German Academic Exchange Service (DAAD) in the frame of an Alumni Project of Research Mobility (PPP) and in the frame of DFG Priority Program (SPP) 1681 (FKZ: MU2382/4-2).

Detection of *Legionella pneumophila* in Porous Monolithic Filter Systems by a Miniaturized Nuclear Magnetic Resonance System

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Worldwide legionellosis outbreaks and tightened laws for monitoring of Legionella concentrations in evaporating cooling system as well as drinking- and service water show a great need for rapid detection and quantification systems. Nowadays, the gold standard is culture-based that takes several days. Detection by nuclear magnetic resonance (NMR) after bacteria were labelled with magnetic nanoparticles or antibody based methods on microarrays can be much faster and with preconcentration sensitive enough to detect and quantify Legionella in legal limits [1].

An efficient method for Legionella concentration is based on the use of polymeric monoliths. The monolithic adsorption filtration (MAF) can be used with different filter sizes and allows filtrations at high flow rates, low back pressure and high retention capacity [2]. However, efficient elution of bacteria for later analysis can display a bottleneck.

To overcome this drawback and to avoid the necessary elution a NMR based system for direct detection of bacteria on the porous filter is presented. A MAF-module with a volume of 10 µL is integrated into a miniaturized NMR system with permanent magnet. After filtration, adsorbed bacteria are labeled with magnetic iron oxide nanoparticles conjugated with monoclonal antibody. The quantification is based on the change in transverse relaxation time (T_2) of water proton spins in the porous filter system. An automated system for nanoparticle injection and regeneration of the filter is under consideration to potentially apply the system for rapid detection of Legionella.

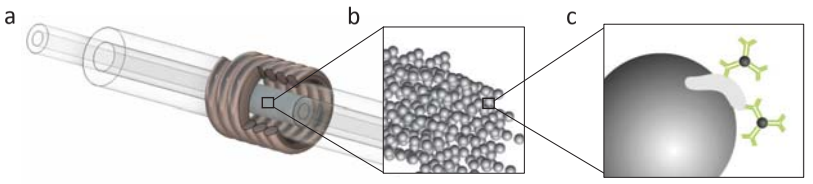

Figure 1: Sliced view of NMR coil (a) filled with porous monolithic filter material (b). Adsorbed legionella are labelled with antibody conjugated magnetic nanoparticle for detection on the filter (c).

[1] A. Wunderlich, C. Torggler, D. Elsässer, C. Lück, R. Niessner, M. Seidel, Anal. Bioanal. Chem. 408 (2016) 2203-2213.
[2] C. Peskoller, R. Niessner, M. Seidel, J. Chrom. A 1216 (2009) 3794-3801.

The Magnetic Response of Magnetoactive Elastomers via the FORCs Method

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Magnetoactive elastomers are systems consisting of magnetisable particles embedded in a non-magnetic elastomeric matrix. External magnetic fields strongly influence the rheological properties of these materials, similarly to what is observed in ferrofluids. The difference from magnetic fluids is that the response of magnetoactive elastomers also depends notably on the restoring forces and torques exerted by the polymer matrix. Such systems are attracting an increasing attention due to their potential for numerous applications, such as the design of magnetically controlled adaptive damping devices, stiffness tunable mounts, vibrational absorbers, force sensors and artificial muscles, soft actuators and micromanipulators.

We study the properties of magnetoactive elastomers by means of computer simulations with a minimal bead-spring model. We model magnetically hard particles as soft-core spheres with a fixed permanent magnetic moment located in their centres. The elastic matrix is modelled implicitly using harmonic springs with different rigidities K, anchored to fixed points in space. The rigidity constants have a normal distribution within a given range. With this simple approach we can model two types of elastic constraints: only on the translations (model M1) or on the translations and rotations of the particles (model M2). These minimal elastomer models exhibit dynamical magnetic hysteresis that fit qualitatively the experimental behaviour.

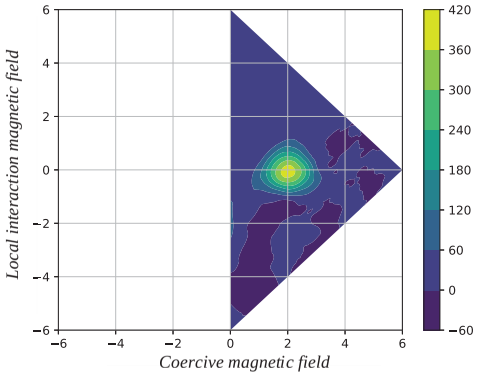

Figure 1. The FORCs diagram.

In order to study in more detail the effect of the matrix on the internal magnetic interactions, we use the first-order reversal curves (FORC) method [1]. Figure 1 presents an example of FORC diagram obtained for model M1, in which a broad peak corresponding to a magnetisation jump can be seen. In our results for model M1 we observe that the position of the peak shifts to the region of lower coercitivity and becomes broader along the coercive field axis with growing rigidity of the constraints. Therefore, the rigidity of the matrix decreases the coercitivity of the magnetic particles. Importantly, in systems with low rigidity the aggregation of magnetic particles is more likely. For model M2 we observe a shift of the peak position towards larger coercivity with increasing magnetic moments. We also observe that the peak spread along coercive field axis becomes smaller.

Support by the projects DFG Ref.No.: OD 18/24-1, RFBR 16-52-12008, FWF START-Projekt Y 627-N27, and the Ministry of Education and Science of the RF (project 3.1438.2017/4.6) is acknowledged.

[1] C.R. Pike, A.P. Roberts, and K.L. Verosub J. Appl. Phys., 85 (1999) 6660-6667.

Temperature dependence of harmonic spectra studied by Magnetic Particle Spectroscopy

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Magnetic Particle Spectroscopy (MPS) is an integral characterization method for investigating the nonlinear properties of magnetic nanoparticles (MNP) using comparatively high magnetic field strengths, which is of utmost significance for biomedical applications such as Magnetic Particle Imaging (MPI) and magnetic hyperthermia. Since the dynamic characteristics of MNP are influenced by both the Néel and the Brownian relaxation mechanism, harmonic spectra in MPS measurements are directly linked to ambient influences like temperature or viscosity of the surrounding medium. Experimental data of multiparametric measurements helps one to evaluate and validate mathematical models of dynamic particle magnetization. Typically, the dependence of harmonics ratios (e.g. A5/A3) is addressed [1] but the complex behavior of higher harmonics is neglected, although higher harmonics contain sensitive information and are of particular importance for functional MPI.

This contribution deals with the investigation of temperature-dependent harmonic spectra of different commercially available single-core and multi-core particle systems as is exemplarily shown for Ocean NanoTech SHP-30 and Micromod synomag-D in Fig. 1. A custom-built MPS setup [2] with selectable excitation frequencies in the range of 100 Hz $< f_0 <$ 25 kHz and excitation field amplitudes up to 30 mT provides a wide sample temperature control range of -20 °C $< T <$ 120 °C and enables freezing the sample. Therefore, a direct comparison of mobile and immobile states of the same sample is feasible. Experimental data were acquired at f_0 = 1 kHz and B = 25 mT for a Brownian (Fig. 1 (a)) and a Néel dominated (Fig. 1 (b)) particle system. As can be seen in Fig. 1 (a), the higher harmonics of SHP-30 drop by trend for decreasing temperatures as a result of slowing Brownian relaxation times. At -17 °C a non-continuous jump is observed, which is due to the phase transition of subcooled water to ice. Harmonic spectra of synomag-D for the same measurement parameters are depicted in Fig. 1(b). The frozen state is reached at -15 °C. Importantly, the temperature dependence of both particle systems is qualitatively inverse. The measured harmonic spectra and their temperature dependence are compared with common theory (e.g. Fokker-Planck equation, Effective Field method).

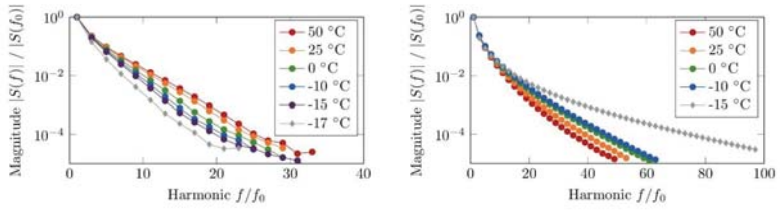

Figure 1: Harmonic spectra of Ocean NanoTech SHP-30 (a) and Micromod synomag-D (b), acquired at 1 kHz excitation frequency and 25 mT field amplitude for different temperatures.

Acknowledgment
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References
[1] J. B. Weaver and E. Kuehlert. Med. Phys. 39(5), 2012.
[2] S. Draack et al. Int. J. Magn. Part. Imag. 3, No. 1, 2017.

Magnetic Parameters Evaluation of Magnetic Nanoparticles for Use in Biomedical Applications

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Magnetic nanoparticles (MNPs) have been widely studied for use in biomedical applications such as hyperthermia and magnetic particle imaging (MPI). The key parameters that determine MNPs performance in these applications are their magnetic moment m and relaxation time τ. The m value determines the signal strength and nonlinearity. The relaxation time τ determines the response to high frequency field. For m, it is given by the relation $m = \frac{\pi}{6} d^3 M_s$, where M_s is saturation magnetization, and d is particle diameter. The Néel relaxation time τ_N is given by the relation $\tau_N = \tau_0 \frac{\sqrt{\pi}}{2} \frac{1}{\sqrt{\sigma}} exp(\sigma)$ and $\sigma = \frac{KV}{k_B T}$, where K is anisotropy energy density, V is particle volume, and τ_0 is the characteristic time.

Therefore, we evaluate the parameters of M_s, d, K and τ_0, which determine m and τ_N values. First, we measure M-H curve of suspended and immobilized MNP samples (Resovist, Fujifilm Pharm.), as shown in Fig.1. From the M-H curve of suspended sample, the value M_s=300 kA/m is determined by the value of M at $\mu_0 H$ = 1 T. In addition, the distribution of d in the MNP sample is estimated by analyzing the M-H curve using singular value decomposition (SVD) method [1]. Distribution of d had two peaks at d_{lp} = 7 nm and d_{hp} = 26 nm. Next, the M-H curve of the immobilized sample is compared with a previously developed empirical expression [2], where distribution of d is taken into account and the value $\mu_0 H_k = 2K/M_s$ is taken as an adjustable parameter. As shown in Fig. 1, $\mu_0 H_k$ = 53.3 mT gave the best fit between experimental result and empirical expression. We then can obtain K = 8 kJ/m^3 by using M_s = 300 kA/m. Finally, we measure the AC susceptibility (ACS) of suspended and immobilized MNPs samples as shown in Fig. 2. The real part of ACS of immobilized sample is compared with a previously developed analysis [3], where distribution of d is taken into account and τ_0 is set as an adjustable parameter. As shown in Fig. 2, the value $\tau_0 = 1*10^{-12}$ gave the best fit between experimental result and ACS analysis.

Table 1 shows summary of magnetic parameters evaluated for Resovist MNPs. The Resovist MNPs were then magnetically fractionated into two MNPs, i.e., MS1 and MS3. MS1 contained large MNPs, while MS3 contained small MNPs: Distribution of d had a peak at d_{hp} = 26 and d_{lp} = 7 nm for MS1 and MS3, respectively. As shown in the table, we obtained different values between MS1 and MS3. It was shown that the Resovist MNPs contains 40% of MS1 and 60% of MS3. It was also shown that the parameters of Resovist MNPs were given by the volume-weighted average of those of MS1 and MS3.

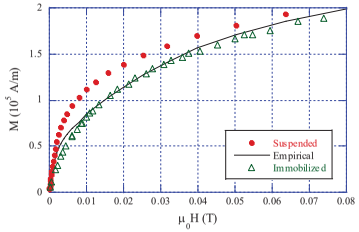

Fig. 1. M-H curve of suspended and immobilized Resovist samples and analytical result of empirical equation.

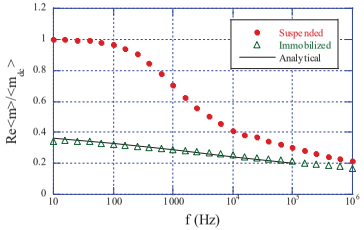

Fig. 2. AC susceptibility of suspended and immobilized Resovist samples and analytical result.

Table. 1. Summary of magnetic parameters for the different MNP samples.

MNP sample	Magnetic Parameters
Resovist	M_s = 300 kA/m $\mu_0 H_k$ = 53.3 mT K = 8 kJ/m^3 $\tau_0 = 1*10^{-12}$
MS1	M_s = 370 kA/m $\mu_0 H_k$ = 64.8 mT K = 12 kJ/m^3 $\tau_0 = 1*10^{-12}$
MS3	M_s = 250 kA/m $\mu_0 H_k$ = 40 mT K = 5 kJ/m^3 $\tau_0 = 1*10^{-12}$

References

[1] T. Sasayama, T. Yoshida, M. M. Saari, and K. Enpuku, "Comparison of volume distribution of magnetic nanoparticles obtained from M-H curve with a mixture of log-normal distributions", J. Appl. Phys., 117, 17D155, 2015.
[2] A. L. Elrefai, T. Sasayama, T. Yoshida, and K. Enpuku, "Empirical expression for DC magnetization curve of immobilized magnetic nanoparticles for use in biomedical applications", AIP Advances, Vol. 8, No. 5, 2018.
[3] K. Enpuku, T. Sasayama, and T. Yoshida, "Estimation of magnetic moment and anisotropy energy of magnetic markers for biosensing application", J. Appl. Phys., 119, 184902, 2016.

Transformations of nanoparticles composition, size and aggregation followed by AC susceptibility

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Alternating current (AC) magnetic susceptibility is a powerful characterization technique that allows following dynamic transformations of magnetic nanoparticles (MNPs). Due to the strong influence of parameters such as the nanoparticles size, composition or aggregation on the temperature dependence of the out-of-phase susceptibility, this technique allows studying small variations of these parameters in different scenarios of nanoparticles transformations such as synthesis procedures, aggregation effects or degradation processes.

In addition, the out-of-phase susceptibility profile can be used as a fingerprint to track magnetic nanoparticles transformations in biological matrices. This technique is especially relevant due to its low detection limits, being able to identify, quantify and follow the transformations of magnetic nanoparticles in biological samples, generally at very low concentrations, and with almost no need for sample processing. Importantly, these measurements can distinguish between nanoparticles and endogenous iron.

Several series of samples characterized by AC magnetic susceptibility in which a single parameter (composition, size or aggregation) has been modified will be presented including:

- The synthesis of iron oxides with different size[1].
- The growth of ferrihydrite nanoparticles[2].
- The synthesis of nanoflowers with the same particle size but growing core sizes[3].
- The effect of the aggregation in agar, cell and tissue samples[4].
- The effect of Mn doping in magnetite nanoparticles.
- The degradation of nanoparticles in tissue samples and the formation of ferritin[5].

Results from the characterization of all these series have allowed us to gain understanding on how these relevant parameters affect the magnetic properties of the nanoparticles (Fig. 1).

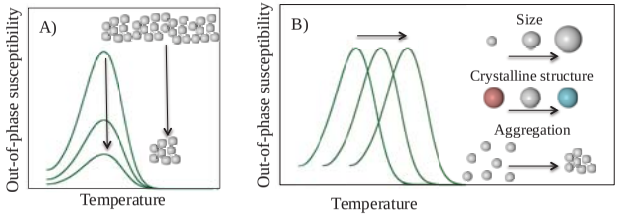

Fig. 1. A) Effect of the MNPs concentration of the out-of-phase susceptibility maximum height. B) Effect of the MNPs size, composition and aggregation on the out-of-phase susceptibility maximum location in temperature.

[1]J. Pellico, J. Ruiz-Cabello, I. Fernández-Barahona, et al., *Langmuir*, 2017.
[2]L. Gutiérrez, V. Barrón, M. Andrés-Vergés, et al., *Journal of Geophysical Research: Solid Earth*, 2016, **121**, 4118-4129.
[3]H. Gavilán, E. H. Sánchez, M. E. F. Brollo, et al., *ACS Omega*, 2017, **2**, 7172-7184.
[4]J. M. Rojas, H. Gavilán, V. del Dedo, et al., *Acta Biomater.*, 2017, **58**, 181-195.
[5]A. Ruiz, L. Gutierrez, P. R. Caceres-Velez, et al., *Nanoscale*, 2015, **7**, 16321-16329.

Mechanical performance of magnetic nanoparticle assemblies under low frequency rotating field

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Magnetic nanoparticles (MNPs) are currently the subject of intense research because of their fundamental properties and potential applications especially in medicine. Submitted to a high frequency magnetic field, MNPs release heat—the so-called magnetic hyperthermia which can be used to destroy cancer cells [1]. One major drawback of this approach is the use of high-frequency magnetic fields, which is technically difficult and uncomfortable for the patient. Very recently, results of cell death using MNPs and low-frequency magnetic fields has been obtained by a few groups [2], which lifts one major disadvantage of magnetic hyperthermia. When placed in a homogeneous magnetic field, MNPs experience a torque. Calculating the torque undergone by MNPs submitted to a magnetic field is useful to interpret quantitatively such experiments but should also permit prediction of the parameters maximizing the torque, which is especially useful when the final objective is to destroy cancer cells.

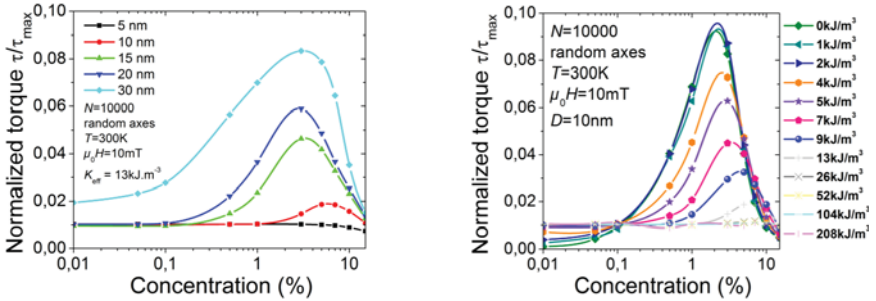

Figure 1: Influence of the concentration on the torque undergone by an assembly of N = 10 000 interacting superparamagnetic particles with randomly oriented axes; T = 300 K. μ0Hmax = 10mT, (Left) τ/τmax as a function of concentration for diameters varying between 5 and 30 nm. (Right) τ/τmax as a function of concentration for various values of K_{eff}.

On this presentation, we focus on torque generated by magnetic nanoparticles submitted to a rotating magnetic field. The results have been obtained using kinetic Monte Carlo simulations, in which thermal activation is taken into account, so the torque undergone by ferromagnetic and superparamagnetic nanoparticles could both be simulated [3]. As first, simple system with one MNP will be presented. Then, different parameters are added and analyzed (diameter, temperature, effective magnetic anisotropy (K_{eff}), amplitude of external magnetic field, number of MNPs). Finally, dipolar interactions are taken into account to be relevant with biological applications. Indeed, particles are generally found under the form of aggregates because they accumulate at the cell membrane, are internalized inside lysosomes, or are synthesized under the form of beads containing several particles. After a discussion about the microscopic phenomenon responsible for the torque amplitude in assemblies of MNPs, one can expect those results could help the synthetization of MNPs with enhanced torque properties.

[1] E. A. Périgo, G. Hemery, O. Sandre, D. Ortega, E. Garaio, F. Plazaola, and F. J. Teran, Appl. Phys. Rev. 2, 041302 (2015).
[2] E. Zhang, M. F. Kircher, M. Koch, L. Eliasson, S. N. Goldberg,and E. Renström, ACS Nano 8, 3192 (2014).
[3] J. Carrey, N. Hallali, Phys. Rev. B 94, 184420 (2016)

Nanorheological studies of xanthan/water solutions using magnetic nanoparticles

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The complete chewing and swallowing process of food is an intricate combination of voluntary and involuntary actions and it involves complex flow geometry as well as a mixture of shear and extensional flow during which aroma and taste are perceived. To study textural changes (determined by viscoelastic properties of the food) directly in the mouth generally means large experimental equipment that interferes with the oral processing mechanisms, and especially with the perception of the food. Instead, to use magnetic nanoparticles (MNPs) enables remote sensing of local viscoelastic properties by measuring and analyzing the dynamic magnetic response from the MNPs. This means that rheological properties, texture and aggregation could be followed remotely. Further, by modifying the particles and e.g. embedding MNPs in proteins, information on aggregation and food texture may be gained. Previous nanorheological studies using magnetic nanoparticles in various concentrations of PEG, gelatine and polymer melts have used AC susceptibility (ACS) measurements [1,2].

To study the viscoelastic properties, the MNPs used need to exhibit Brownian relaxation (e.g. particle magnetic moment locked in a specific direction of the MNP). Initially, different concentrations of xanthan in water were studied. One of the MNP systems used consists of multi-core particles with a particle size of about 80 nm (BNF, micromod Partikeltechnologie) with either starch or dextran matrix. The ACS response is measured, and the data is modelled using dynamic magnetic models and different viscoelastic models. In figure 1, we show the ACS response (in and out-of-phase ACS components) at different concentrations of xanthan and water, mixed with a constant concentration of MNPs. To clarify, the figure only shows two concentrations, MNPs in water and MNPs in 1 wt % of xanthan. ACS measurements are performed using the DynoMag system. As can be seen, the Brownian relaxation peak is shifting down in frequency and the ACS response is broadening and decreases due to changes in the viscoelastic properties around the MNPs in the xanthan solution. The in-phase and out-of-phase components of the shear moduli are determined at each excitation frequency and compared with traditional macroscopic rheological measurements with an oscillating shear.

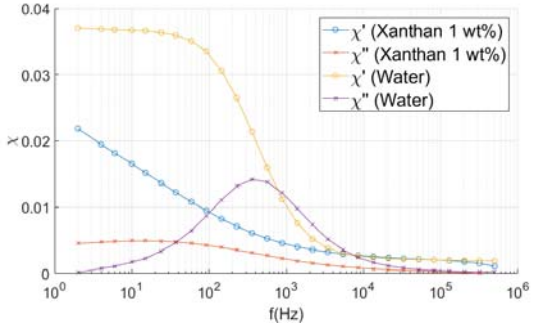

Figure 1 In-phase (yellow and blue) and out-of-phase components (purple and red) of the ACS vs frequency for different concentration of xanthan in water (0 and 1 wt %). The MNP concentration is constant (5 mg/ml). Measurement are carried out at room temperature.

[1] Hilke Remmer et al, Journal of Magnetism and Magnetic Materials, 427(2017)331-335.
[2] Sergio Sierra-Bermúdez et al, Journal of Magnetism and Magnetic Materials, 378(2015)64–72.

GoldMag®-Magnetic Lateral Flow Immunoassay for NT-proBNP Detection

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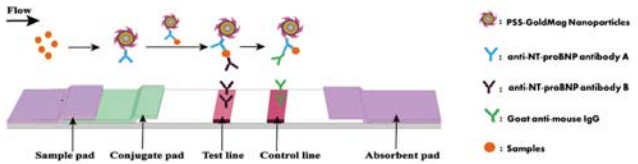
Lateral flow immunoassay (LFIA), typical point-of-care testing, has been used for several decades for clinical diagnosis due to its inherent advantages as being simple, less time consuming. Conventional LFIA tests are detected visually, providing qualitative result, or quantitative result by measuring reflectance, contrast, color change, or fluorescence. However, for optical signal detection, only signal from top 10 μm layer can be collected, making the conventional LFIA less sensitive. Magnetic particle-labeled detection system is able to improve LFIA sensitivity by measuring magnetization of whole volume of magnetic particle labels in the detection zone through Magnetic Assay Reader (MAR).

In previous study, a novel Fe_3O_4/Au composite nanoparticle (GoldMag® nanoparticles) has been prepared, and its physicochemical properties and applications in antibody/antigen immobilization have been reported. For further application in LFIA, sodium polystyrene sulfonate (PSS) was introduced to the surface of GoldMag® nanoparticles to improve its dispersity and stability.

Based on the PSS-GoldMag® nanoparticle, the quantitative magnetic LFIA system was established for detection of NT-proBNP, a critical biomarker of heart failure. Monoclonal anti-NT-proBNP antibody was conjugated to GoldMag® nanoparticles and the paired antibody was immobilized on test line of nitrocellulose membrane. The concentration of NT-proBNP was provided by measuring magnetic intensity Through MAR. A wide detection range (from 0 pg/mL to 8,000 pg/mL of NT-proBNP in sera sample) and high sensitivity (detection limit of 43 pg/mL of NT-proBNP in sera) were found with this LFIA system by testing 206 clinical samples. GoldMag® nanoparticle based LFIA is capable of serving as a promising point-of-care testing in the fields of medical diagnostics, food and environmental monitoring in near future.



Schematic representation of PSS-GoldMag® nanoparticles based LFIA for NT-proBNP detection

Novel platform for the multidimensional analysis of magnetic nanoparticles

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The unique magnetic properties of magnetic nanoparticles (MNP) make them ideally suited to a broad spectrum of biomedical applications in the field of therapy and diagnostics. These applications place particular demands on the magnetic properties of the nanoparticles. For these reasons, research, production, and quality assurance of MNP require powerful analytical techniques measuring all structural and magnetic properties relevant for the specific application. The coupling of chromatographic separation techniques with complementary detectors in a contained system allows for multidimensional, accurate and reproducible characterization of MNP. Although various methods have already been combined for this approach, so far, no detector for real-time magnetic analysis (online-detection) has been demonstrated.

The method for magnetic online-detection adopted here is based on the rapid and sensitive nonlinear magnetic susceptibility method, also known as magnetic particle spectroscopy (MPS). In a first step, the physical modelling of the magnetic signalling and separation is used for investigating detector characteristics, and to deduce design recommendations for a detector flow cell. Finally, this flow cell is designed and manufactured by 3D-printing and enables the magnetic online-detection by MPS. We then combined the magnetic online-detector with asymmetric low field-flow-fractionation (AF4) and detectors for size determination (multi-angle light scattering, dynamic light scattering). The operation of the developed system is almost entirely automated, including data acquisition and processing and yields time synchronized detector results.

The developed platform provides valuable, and previously unobtainable, insights in the magnetic behavior of MNP samples which will be demonstrated on commercial MRI contrast agents (e.g. Resovist®, Endorem®, and Feraheme®). The application of this novel platform may shed important insight in the physics of MNP and can be used as a standard analytical method for quality control of MNP in biomedical applications such as magnetic particle imaging.

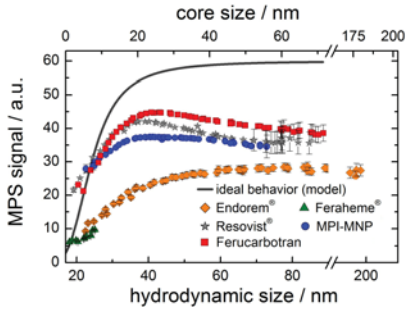

Fig.: Core and hydrodynamic size dependent MPS signal of different MNP systems as obtained by multidetector AF4.

Magnetic textile solid phase extraction of basic dyes

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Solid phase extraction (SPE) represents currently the most often used analytical procedure for the preconcentration, extraction and clean-up of both organic and inorganic compounds from variety of biological, clinical, food, environmental and other samples [1]. In order to simplify the SPE process, magnetic solid phase extraction (MSPE) was developed in 1999 and subsequently efficiently used for analytes extraction from difficult-to-handle samples [2].

Magnetic textile solid phase extraction, based on the use of magnetically modified non-woven textile impregnated with agarose-carrageenan solution, was successfully employed for the preconcentration of basic dyes from water solutions. Dye binding is mediated by the ionic interaction between the negatively charged surface carrageenan groups and the positively charged groups of dye. After the dye preconcentration photos of textile squares with the adsorbed dye were taken using a digital camera or a mobile phone. The image analysis of the photos was performed using appropriate free software. Using the HSB (hue-saturation-brightness) color space [3], the values of saturation were measured which are proportional to the dye concentration in the analyzed samples. Using this inexpensive, elution free assay it is possible to analyze dyes concentration in various solutions. This novel method has a potential to be a useful alternative to existing semiquantitative determination procedures, especially for dyes analysis.

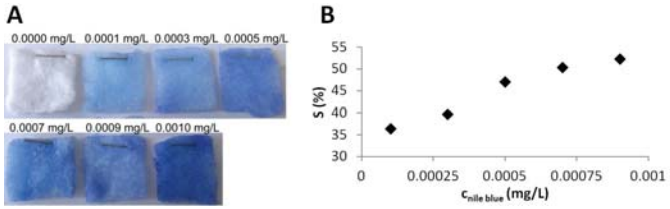

Fig. 1. A – Magnetically responsive agarose-carrageenan modified textile squares after nile blue extraction from water solution (100 mL; concentrations of original solutions are shown). B – Dependence of values of saturation (S) on concentration of analyzed nile blue solutions.

Literature:

[1] A. Andrade-Eiroa, M. Canle, V. Leroy-Cancellieri, V. Cerdà, Solid-phase extraction of organic compounds: a critical review (Part I), TrAC Trends in Analytical Chemistry, 80 (2016) 641-654.
[2] M. Šafaříková, I. Šafařík, Magnetic solid-phase extraction, Journal of Magnetism and Magnetic Materials, 194 (1999) 108-112.
[3] H.-D. Cheng, X.H. Jiang, Y. Sun, J. Wang, Color image segmentation: advances and prospects, Pattern Recognition, 34 (2001) 2259-2281.

Laboratory magnetorelaxometry device for characterization of magnetic nanoparticles

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Magnetic nanoparticles (MNP) are used in a variety of biomedical applications such as drug delivery, hyperthermia and imaging. The efficiency of these applications depends on structural and above all magnetic MNP characteristics (e.g. magnetic moment distribution, colloidal stability, hydrodynamic size distribution, binding on biological targets). These parameters need to be carefully determined before the use of the MNP for which a number of highly sophisticated measurement techniques for structural and magnetic characterization of MNP exist.

One of them is Magnetorelaxometry (MRX) which measures the relaxating magnetic response of the MNP sample after switching-off a polarizing magnetic field. MRX has been proven to be capable for magnetic characterization of MNP and their specific quantification in biological systems with outstanding detection limits down to a few nanogram [1]. To be able to detect feeble MRX signals as small as 10^{-15} T to 10^{-12} T typically occurring for MNP concentrations in biomedical applications, the operation of superconducting quantum interference devices (SQUIDs) is required. Additionally, the use of large magnetically shielded rooms for MRX measurements is indispensable.

Here, we adapted an existing six-channel SQUID system (Figure 1) with integrated superconductive shielding to be operational for MRX measurements of small MNP samples in a conventional laboratory environment without any additional shielding. The system originally was developed for conscious detection of magnetocardiograms in mice [2] and consists of a liquid helium Dewar vessel with a horizontal cylindrical warm bore (diameter of 27 mm and length of 700 mm). At the center there are six SQUID sensors circumferentially (detecting fields perpendicular to the bore axis) arranged at a cold warm distance of 16 mm. Close to the warm bore and the SQUID sensors are housed by a superconducting niobium cylinder for shielding.

For MRX measurements we developed a magnetizing support which is inserted into the warm bore of the device. It consists of a magnetization coil to provide magnetic fields up to 4 mT (parallel to bore axis) which can precisely be aligned vertically to the SQUID sensors, so that the detection of magnetizing fields by the sensors is strongly suppressed. A second insert is used to accurately and reproducibly place MNP samples (volumes up to 150 µL) within the coil close to the SQUID sensors.

We thoroughly characterized the performance of our MRX device in laboratory environment with measurements of fluidMAG-D (chemicell GmbH, Germany) at different concentrations. Measuring the same samples with our commonly used one-channel MRX system in the Berlin magnetically shielded room showed that the shielding of the six-channel SQUID system is more robust against magnetically and electrically interferences. This results in an improved signal to noise ratio of the relaxation curves and finally, a higher sensitivity for detection of MNP in our new system.

Our transportable MRX device allows the comfortable and sensitive characterization of MNP in laboratory environment without need of an additional magnetically shielded room.

***Figure 1**: One-channel SQUID system (left) commonly used for MRX measurements in the Berlin magnetically shielded room (middle). The new six-channel SQUID device with integrated shielding (right) for measurements in laboratory environment.*

[1] F. Wiekhorst *et al.*, *Pharm. Res.*, vol. 29, no. 5, pp.1189-1202, 2012.
[2] R. Ackermann *et al.*, *IEEE Trans. Magn.*, vol. 17, no. 2, pp.827-830, 2007.

Dynamics of magnetic nanoparticles in Newtonian and viscoelastic media

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The dynamics of magnetic nanoparticles (MNP) play an important role for many applications, such as magnetic hyperthermia and homogeneous MNP-based bioassays. In addition, the measurement of the dynamic magnetic properties of the MNP provides information on their embedding in and interaction with the matrix, proposed that the MNP are thermally blocked.

To study the MNP dynamics in various matrices, we apply ac susceptometry (ACS) as well as measurements in a rotating magnetic field (RMF). As matrix systems we exemplarily investigate Newtonian fluids like water and glycerol as well as more complex viscoelastic media such as gelatin. The utilized ACS setup, which was originally designed for RMF measurements, involves two Helmholtz coil pairs, allowing one e.g. to use the second coil for the application of an additional dc field perpendicular to the ac field.

The imaginary parts of ACS spectra for a suspension of FeraSpin XL multi-core particles (nanoPET pharma GmbH) in different water-glycerol mixtures are shown in Fig. 1(a). Despite the rather complex changes of the ACS spectrum with increasing viscosity, the generalized Debye model allows one to extract the dynamic viscosity in very good agreement with the theoretical expectation.

ACS measurements of the gelation process of aqueous gelatin solutions of $CoFe_2O_4$ single-core particles – after rapidly cooling the sample down from 313 K to 296 K – indicate a very complex behavior depending on the gelatin concentration (Fig. 1(b)). Viscoelastic parameters such as viscosity and shear modulus can be extracted applying the Voigt-Kelvin model. However, no qualitative differences in the variation of the characteristic frequency (maximum of the imaginary part of ACS spectrum) were observed when applying an ac, oscillating or rotating magnetic field.

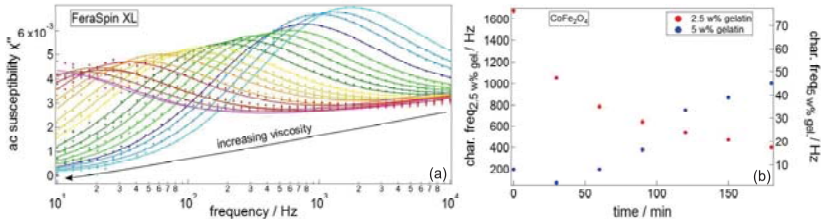

Figure 1: (a) Imaginary parts of ACS spectra for samples with FeraSpin XL particles in different water-glycerol mixtures. Brown peaks shift to lower frequencies with increasing viscosity.
(b) Characteristic frequencies of ACS spectra for samples with $CoFe_2O_4$ particles dispersed in aqueous gelatin solutions (2.5 w% and 5 w%) over time.

Acknowledgment:
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Using Multimodal Phosphor Particles to Monitor Radiopharmaceutical Release and Accumulation *in vivo*

[1]Gretchen B. Schober, [1]Hongyu Chen, [2]Thomas Moore, [1]Unaiza Uzair, [3]Dan C. Colvin, [3]John C. Gore, [2]Frank Alexis, and [1]Jeffrey N. Anker

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Rare earth doped gadolinium oxysulfide nanophosphors (e.g. $Gd_2O_2S:Eu^{3+}$) are promising candidates for multimodal optical, X-Ray, and MRI imaging. These particles are photostable and emit light with narrow spectral peaks when excited by blue light, X-Rays, or alpha/beta radiation. If a drug is radiolabeled (e.g. ^3H), radioactive decay will generate luminescence when in close proximity to a nanophosphor. Drug release can then be monitored *in vivo* by measuring the decrease in luminescence intensity as drug is released, provided that nanoparticle concentration and tissue extinction effects can be estimated. This is accomplished by using a secondary phosphor reference ($Gd_2O_2S:Tb^{3+}$) that has similar yet distinct emission peaks from the primary phosphor between 610-630 nm ($Gd_2O_2S:Eu^{3+}$). The $Gd_2O_2S:Tb^{3+}$ is encapsulated with a known concentration of radiolabel, and a ratiometric comparison of luminescence intensity is completed for calibration. Localized radiopharmaceutical accumulation can also be measured if nano- or microparticles are immobilized on a biomedical implant surface. To calibrate, a ratiometric comparison is done between a region encapsulated with known radiolabel concentration and a variable region exposed to local radiopharmaceutical concentration. Herein we present preliminary results for several features, including nanoparticle drug delivery to mouse brain tumor xenografts, X-Ray imaging, MRI, photoluminescence, and radioluminescence.

Multi-spectral Magnetic Particle Spectroscopy for the investigation of particle mixtures and particle mobility

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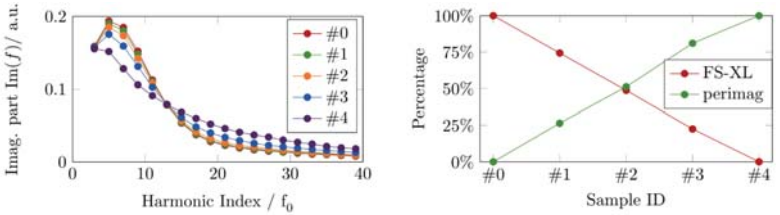
Magnetic Particle Spectroscopy (MPS) records the non-linear response of magnetic nanoparticles (MNP) to large ac magnetic fields. It obtains a harmonic spectrum for the characterization of MNP samples, especially in the context of Magnetic Particle Imaging (MPI). MPS is sensitive to smallest variations in magnetization response, thus different particle types or batches are easily discriminated. Also, since the dynamic magnetization of the particles is driven by both the Néel and the Brownian relaxation mechanisms, the harmonic spectra in MPS scale with ambient factors, such as temperature, viscosity or binding state of the particles. Previous studies mostly observed the dependence of low-frequency harmonic ratios (e.g. $5f_0/3f_0$) in order to obtain information on temperature or viscosity [1]. Here, we propose a multi-spectral analysis technique, using the full available spectrum, to decompose the collective response of different particles or particles in different states (i.e. temperature, viscosity/binding state, etc.) from an integral measurement on a MNP sample. Potential applications of the new method include the in-vitro analysis of particles in a cell culture, where cell-ingested particles can be distinguished from those still in the culture medium (e.g. cell update study), or the discrimination of different particle types or particle parameters. The proposed method borrows from multi-color MPI [2], where we can spatially resolve different spectral responses to constitute a functional imaging modality. Multi-spectral MPS helps to methodically investigate dependencies of the spectral response on the various particle parameters in a much simpler setting compared to MPI, while still being translatable, and it allows us to study the quantitativeness of proposed method about various factors. In this contribution, we apply the multi-spectral method to analyze binary and ternary mixtures of different particles (e.g. FeraSpin™ XL, perimag®, etc.) and viscosity series of FeraSpin XL regarding parameter estimation, separation quality and quantitativeness. A comparison of multi-spectral MPS to multi-color MPI, both from in-house custom-built measurement and imaging devices, will be provided as well.



(a) (b)

Figure 1: Binary mixtures of FeraSpin™ XL and perimag® with ratios (FS-XL:perimag) of #0 100:0, #1 75:25, #2 50:50, #3 25:75, #4 0:100: (a) imaginary part of the harmonic spectrum and (b) estimated mixing ratios of the mixtures obtained by multi-spectral MPS analysis.

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[1] J. B. Weaver and E. Kuehlert. Med. Phys. 39(5), 2012.
[2] J. Rahmer, A. Halkola, et al.: Phys. Med. Biol. 60(5), 2015.

Automated Synthesis of Magnetic Nanoparticles and Process Monitoring by Nuclear Magnetic Relaxation

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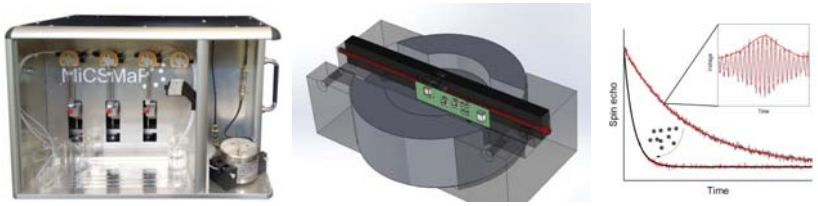
Microfluidic reactors show great potential for decentralized and on-demand synthesis of high quality nanomaterials. However, dealing with solid materials in microfluidic systems still involves a variety of challenges. The integration of analytical devices for on-line particle characterization in microfluidic systems facilitates fast reaction optimization, process monitoring and quality control.

This project combines the continuous synthesis of iron oxide magnetic nanoparticles by co-precipitation and subsequent surface functionalization with online characterization of nuclear magnetic resonance (NMR) relaxation properties in an automated manner. Microreactor, relaxometer and all pumps and valves are combined in one portable system, shown in Figure (a). The microreactor is easily constructed from tape and polymer foil and utilizes 3D flow focusing to avoid clogging. The NMR relaxometer, based on a 0.5 T permanent magnet allows for determination of transverse (T_2) and longitudinal (T_1) relaxation times by CPMG and inversion recovery sequences, respectively. The NMR probe head (Figure (b)) was designed such that integration into the flow synthesis of the nanoparticles was achieved. Furthermore, relaxivites, key characteristics of magnetic nanoparticles to assess their applicability as MRI contrast agents or as probes in immunoassays can be obtained by automated concentration variations.

Several particle characteristics like the primary particle size, saturation magnetization, aggregation state and coatings influence the relaxivity because they change the effective magnetic moment and hence the field inhomogeneities caused by the particles. The resultant change in transverse relaxation is shown in Figure (c). The integrated relaxometer was proven to be an efficient tool for process control during the nanoparticle synthesis, optimization of cluster sizes for maximum transverse relaxivity and characterization of final products. Clusters sizes were optimized for maximum transverse relaxivities. The impact of several synthesis parameters like pH and iron precursor was investigated.

For further optimization and more detailed quality control the integration of dynamic light scattering and zeta potential analysis is currently developed.



(a) *(b)* *(c)*

Figure: (a) Portable system including a microreactor, pumps, valves and an integrated NMR relaxometer (b) 3D-model of the NMR probe head suitable for on-line characterization of relaxation properties. Probe coil is wound around a tube and matched to system impedance. (c) Influence of field inhomogeneities caused by magnetic moments on T_2 relaxation and a single spin echo (zoom).

Inorganic-Polymeric Microdisks Loaded with Ferromagnetic Nanoparticles for Long-Term Cell Control

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Keywords. photolithography, microdisk, self-assembly, ferromagnetic-nanoparticle, magnetic-hyperthermia, endocytosis-resistance, fluorescence-thermometry.

The development of magnetothermal neuromodulation and magnetic hyperthermia therapies requires magnetic nanoparticles (MNPs) with high heating power that are able to form stable and lasting attachment with cells. Membrane-bound ferromagnetic nanoparticles (NPs) can be powerful magneto-thermal and magneto-mechanical cell actuators. However, their application in-vivo is challenged by colloidal stability and by cellular endo- or phagocytosis. Combining nanotechnology, photolithography, chemistry and bioengineering, we have produced versatile inorganic-polymeric microdisks loaded with ferromagnetic NPs, fluorophores and biomolecules. The diameter of the disks (>2µm), their thickness (>40nm), the inorganic phase and the NP density on the disk surface are fully customizable. These magnetic hybrid microdisks attach stably to the cell membrane and are endocytosis resistant. As the microdisks bind to the cells, the ferromagnetic NPs' arrangement remains unperturbed, ensuring precise control over the physical distribution of the NPs and their heating capacity under an alternating magnetic field. Local heat generated within the microdisks attached to the cell membrane has been measured by fluorescence thermometry. It has been found that the heating rate of the cell-attached microdisks increases linearly, up to 2 ºC/s, with the NP density on disk surface for non-interacting MNPs. Hence, the produced flat devices function as tunable, endocytosis resistant, stable sub-cellular implants for efficient magnetothermal actuation.

Surfactant modulated interactions of magnetic nanoparticles with biomolecules: An insight through electrochemistry and surface-enhanced Raman spectroscopy

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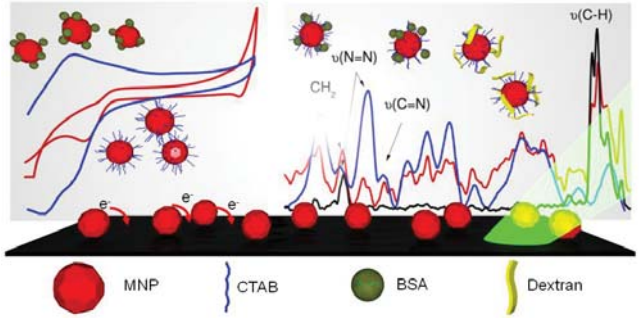
Magnetic iron oxide (Fe_3O_4) nanoparticles (MNPs) are used extensively in biomedical applications due to their unique nanoscale properties specifically superparamagnetism. However, there are certain issues of MNPs like stability, size and dispersibility which limits their use in biomedical arena. To address these issues, surfactants are widely used during the synthesis of nanoparticles since they act as a capping agents preventing nanoparticle aggregation and thereby enhancing stabilty and dispersiblity. Nevertheless, not much information is available on the interaction mechanism of the surfactants and the biomolecules with MNPs.

In this study, cyclic voltammetry (CV) has been used to depict the association of MNPs with two different biomolecules namely, bovine serum albumin (BSA) and dextran. The role of a cationic surfactant, hexadecyltrimethylammonium bromide (CTAB) in stabilizing the MNPs and augmenting the association with BSA and dextran is also investigated. In case of MNPs/CTAB, the presence of a surfactant interface shows a characteristic electrochemical behavior of magnetite nanoparticles towards the biomolecules. The diffusion coefficients for MNPs and MNPs/CTAB in presence of BSA, calculated using the Randle-Sevick equation, were reduced in presence of a surfactant. The CTAB surfactant interface thus acted as a diffusion barrier for the electroactive species to reach the electrode surface.

Raman spectra of the system added further insights to the structural changes due to molecular association. The Raman spectroscopy analysis in the low wavenumber region shows that MNPs/CTAB−BSA had an upscale shift, while MNPs/CTAB−dextran had a downscale shift with respect to MNPs/CTAB signifying the difference in the pattern of association in both systems. Surface-enhanced Raman spectroscopy (SERS) provided an insight into the mode of interaction by enhancing the otherwise weak signals. SERS analysis showed that the head group of the CTAB surfactant comprising of the $-N^+(CH_3)_3$ was involved in a hydrogen bonding interaction with the dextran molecule in the MNPs/CTAB−dextran system. In case of the MNPs/CTAB−BSA arrangement, 100-fold enhanced SERS spectra signified the BSA binding with CTAB at the hydrophobic tail. Hence the plasmon coupling effect facilitates lesser enhancement in MNPs/CTAB-dextran sample compared to MNPs/CTAB−BSA, as dextran molecules occupy superficial position while covering the MNPs/CTAB whereas BSA molecules diffuse through the polar head of CTAB to associate with the underlying hydrophobic tail and thereby establishing a proximity with the inner core of iron oxide as well.



Graphical representation of the interaction of magnetic nanoparticles with the biomolecules

FeCrNbB Ferromagnetic Particles with Shape Anisotropy for Cancer Cell Destruction by Magneto-Mechanical Actuation

Horia Chiriac[1*], Ecaterina Radu[1,2], Mihai Țibu[1], George Stoian[1], Gabriel Ababei[1], Luminița Lăbușcă[1], Dumitru-Daniel Herea[1], Nicoleta Lupu[1]

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Magnetic particles (MPs) were found useful in different cancer treatment applications such as magnetic hyperthermia, magnetic controlled delivery and release of antitumoral drugs at the targeted site of a tumour. Lately, cancer cell destruction techniques involving the movement of magnetic particles in alternative magnetic fields (MFs) came to the fore [1,2].

In this work, we aim to test the efficiency of newly developed ferromagnetic FeCrNbB MPs for magneto-mechanical destruction of cancer cells, MPs that are produced by a controllable process and in a large amount without involving costly technologies. We introduce a new type of glassy magnetic particles (MPs) prepared by wet milling of Fe-Cr-Nb-B precursor glassy ribbons for cancer treatment by magneto-mechanical actuation in low magnetic fields (1÷20 Oe). The rectangular shape of Fe-Cr-Nb-B MPs induces important magnetic shape anisotropies which, in association with a large saturation magnetization, generate an improved magneto-mechanical torque in a rotating magnetic field, producing important damages on the cellular viability of MG-63 human osteosarcoma (HOS) cells. The specific parameters such as MPs concentration, frequency and intensity of the applied magnetic field, or the time of exposure have a strong influence on the cancer cells viability, as one can see in Fig. 1. The high magnetic susceptibility of the MPs offers them destructive effect even in low magnetic fields such as 10 Oe, and this characteristic allows the use of coils systems which provide large experimental spaces. The novel MPs are used for the magneto-mechanical actuation alone or in association with hyperthermia, but also can be transported to the tumor sites by means of stem cells carriers. Understanding the importance of using ferromagnetic MPs with shape anisotropy to generate significant magneto-mechanical torque in low external variable magnetic fields offers a simple and efficient alternative to cure the cancer disease and the results will be discussed in detail.

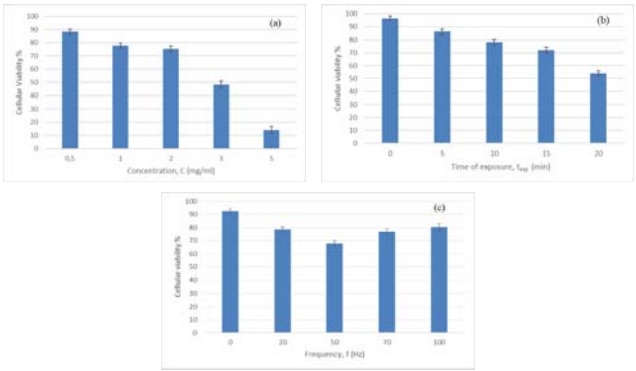

Fig. 1. The variation of the cellular viability of osteosarcoma cells with: (a) concentration of MPs in cell culture media, (b) time of exposure to variable magnetic field and (c) frequency of applied magnetic field.

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[1] D.H. Kim *et al*., Nat. Mater. **9** (2010) 165.
[2] D. Cheng, X. Li, G. Zhang, H. Shi, Nanoscale Res. Lett. **9** (2014) 195.

DNA ENGINEERED MAGNETICALLY TUNED COBALT FERRITE FOR HYPERTHERMIA APPLICATION

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Magnetic particles are getting immense attention for their versatile biological as well as device applications. We are especially interested in biological applications. The cobalt ferrite nanoparticles have suitable magnetic property which may provide a new direction in cancer treatment as these particles heating can be controlled by the magnetic fields from outside the body to kill the cancer cells. For such application these cobalt ferrite nanoparticles was properly engineered with DNA. Here different batches of cobalt ferrite nanoparticles were synthesized on DNA scaffold by wet chemical co-precipitation method varying the DNA concentrations. The cobalt ferrite nanoparticles attached with DNA was analyzed by infrared spectroscopy (IR), scanning electron microscope (SEM), Transmission electron microscope(TEM), squid, X-ray diffraction (XRD), Dynamic light scattering(DLS) etc. From XRD data it was confirmed that the above mentioned nanoparticles is cobalt ferrite in pure phase and from IR, SEM and TEM analyses, it was observed that cobalt ferrite nanoparticles were properly attached with DNA. Magnetic properties with change of DNA concentrations were investigated from SQUID magnetometer, which showed change in magnetic properties with change of DNA concentrations functionalized to cobalt ferrite nanoparticles. From DLS data the hydrodynamic size of the particles was investigated. Cytotoxicity and biocompatibility of these particles were also analyzed in Triple Negative Breast Cancer (TNBC) cell where different amount of doses of different batches were added to the cells. Here Cell viability is measured as the percent live cells compared with untreated control. From these studies it was seen that the CoFe2O4 NPs themselves showed cytotoxic effect in case of cancer cell but remained biocompatible in case of peripheral blood mononuclear cell (PBMC). Heating ability of different batches of particles was performed under AC magnetic field and was observed that the heating property was changed with change of DNA concentration. Hence DNA plays a very important role in tuning magnetic properties of particles which may give us opportunity to find out the optimum particles for hyperthermia therapy.

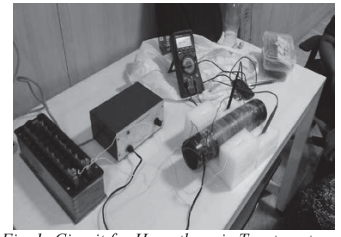

Fig. 1: Circuit for Hyperthermia Treatment

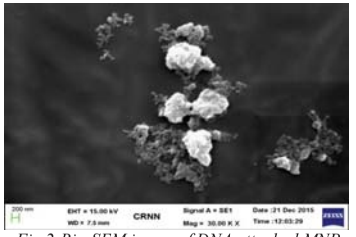

Fig.2:Bio-SEM image of DNA attached MNP

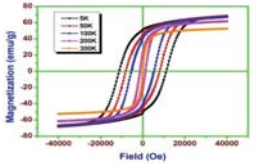

Fig 3: SQUID data for DNA attached magnetic nano particle

Self-assembly of magnetic particles and rheology of their dispersions studied by numerical simulations

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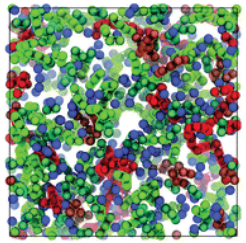
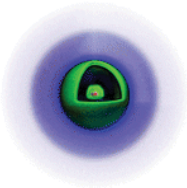
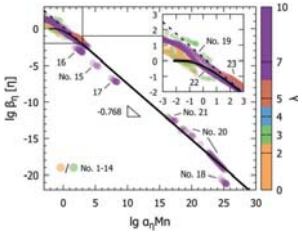
Magnetic particles are prospective candidates for various biomedical applications: as vehicles for drug targeting, hyperthermia etc. For these purposes they are injected into the body and interact with different body fluids in the confined space of vasculature and interstitium often experiencing the simultaneous action of magnetic field and shear flow. In these conditions the presence of even minor amount of magnetic particles can have a profound influence on the flow behavior [1-4]. However, the precise role of the microstructure in the development of the rheological response has not been reliably quantified.







Here we will report the new results of numerical simulations of the structure and rheology of magnetic colloids in a magnetic field. Using hybrid molecular dynamics and multi-particle collision dynamics simulations with explicit coarse-grained hydrodynamics we observe the self-assembly of magnetic particles in various aligned structures and their destruction by the shear flow. We will present quantitative comparison of our simulated rheometric studies with micromechanical models and experimental data published in the past by different groups, showing evidence of the universality of the structural behavior governed by the competition between the bonding (dipolar) and erosive (thermal and hydrodynamic) stresses. The simulations display viscosity changes across several orders of magnitude in fair quantitative agreement with various literature sources, substantiating the universality of the approach, which seems to apply generally across vastly different length scales and a broad range of physical systems.

Acknowledgments

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References

[1] J. Nowak, D. Wolf, S. Odenbach, JMMM 354 (2014) 98
[2] M.T. Lopez-Lopez et al., Langmuir 28 (2012) 6232
[3] D. I. Santiago-Quinonez, C. Rinaldi, Soft Matter 8 (2012) 5327
[4] D. Zablotsky et al., Soft Matter 13 (2017), 6474

Synthesis of Magnetic Iron Oxide and Modified Iron Oxide Nanoparticles with Starch and Study of Their Effectiveness in the Separation of Phosphate Ion from Real Sample

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Due to the importance of magnetic nanoparticles, the synthesis of these nanoparticles with the morphology and particle size of the nanoscale has long been considered by researchers. In this study, iron oxide nanoparticles and iron oxide modified iron oxide nanoparticles were synthesized in a coherent manner. X-ray diffraction, Fourier transform spectroscopy, scanning electron microscopy and transmitted electron microscopy were used to study the properties and specifications of the nanostructures. Magnetically coated magnetic stannous nanoparticles have special properties such as high surface area, low penetration resistance, superparamagnetic properties, and ease of separation by an external magnetic field without the need for filters and centrifuges. The synthesized nanoparticle has been used as a new and effective absorbent for phosphate removal in standard phosphates and in real samples. Zirconium chloride can also be used as the core for coating nanoparticles, as well as by the use of magnetic nanoparticles, to improve the efficiency of phosphate removal. Absorption rate was investigated under different conditions such as pH, adsorbent contact time, adsorbent amount and initial concentration of phosphate. After examining the parameters affecting the absorption process, it was observed 0.1 g of adsorbent in a 90 minute period with a pH of 3 and a concentration of 50 PPm of phosphate could eliminate 98% of the phosphate from the actual sample. Finally, the kinetic and isothermic models affecting absorption were investigated. The results showed that the effective model on the phosphate absorption process is the Freundlich isotherm model, and the experimental data have the most correspondence with the second-order kinetic model.

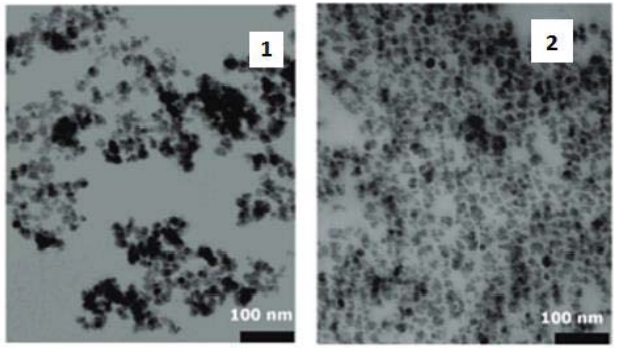

Fig 1. TEM image of 1- Fe_3O_4 and 2- Fe_3O_4@starch

Mastering active targeting, alleviating unspecific cellular uptake and tailoring *in vivo* fate of USPIOs through a dendritic coating

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and Delphine Felder-Flesch*

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Cancer is growing worldwide and its incidence continues to increase as well as mortality associated with a + 51% increase of deaths related to cancer in the world between 2002 and 2030. The move towards personalized/precision medicine[1] and new targeted therapies[2] requires at first the development of highly efficient diagnostic tools able to diagnose and monitor the evolution of the pathology, evaluate the structural modifications but also physiological changes, even cellular. The stakes are to speed up the diagnosis, to increase its sensitivity, reliability and specificity for a better management of the disease (patient's care) thus an impact on the patient survival probability.

Designing nanoparticles for targeted cancer diagnosis and therapy is therefore of the utmost importance. The wish list of such systems is long: they should selectively home on in the cells and organs of the body that are involved in the disease process, specifically targeting their potent healing effects on these cells and organs, while sparing cells not involved in the disease process. They should be completely non-toxic, biodegradable or capable of natural excretion, not be recognized or eliminated by the body's own immune system before they have reached their target, and not induce any allergic reactions. Ideally, they are generic, i.e. they can be "programmed" to combat a wide variety of cancers by docking onto any target structures one chooses and being capable of carrying any medicines. To improve tumour targeting efficacy and to obtain better *in vivo* imaging properties, our studies explored the multivalency effect of a dendritic surface functionalization of SPIONs. Grafting of dendritic molecules on the surface of 10 nm spherical SPIONs using a phosphonate group as coupling agent has led to a new generation of contrast agents for MRI. The appeal of such strategy is due to the unique properties of the dendritic structures which can be chemically tuned to reach ideal biodistribution or highly and efficient targeting efficacies. Our dendronized SPIONs (DUSPIOs) display good colloidal stability in isoosmolar media. Their MRI contrast enhancement properties were found higher than those of commercial products (polymer-decorated) and no evident adverse effect was observed in rat after injection, even at high concentrations and a long time after injection. Their biodistribution was also studied by optical imaging thanks to Alexa labelling and in this case, a fast hepatobiliary, together with a low urinary eliminations were observed. Luckily, no reticuloendothelial system (RES) uptake could be highlighted.

Besides the challenge to synthesize NPs optimized for imaging, the efficiency and resolution of these imaging modes require the internalization of a sufficient amount of NPs and their homogeneous 3D internalization in tumors. We have recently shown that, after i.v. injection in melanoma mice model, DUSPIOs coupled with a melanin targeting ligand were specifically uptaken by melanoma tumor cells with very favorable biodistribution and biokinetic properties (no unspecific macrophage uptake and fast elimination in few hours of the untargeted DUSPIOs fraction).

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Study of Brownian motion of Magnetic Nanoparticles in Viscous Media by Mössbauer spectroscopy

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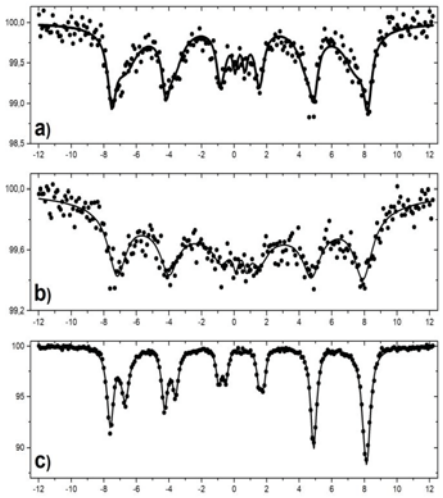
In previous works, we developed an experimental method based on Mössbauer spectroscopy that allowed us to study the biodegradation of iron oxide nanoparticles in animals and to reveal the evolution of the magnetic properties of the nanoparticles during this process [1]. In these works the magnetite nanoparticles were injected into the vessels of experimental animals. At certain time after the injection, the animals were sacrificed, their organs extracted and lyophilized. Then the dried samples of organs were used for Mössbauer studies.

Study of biodegradation of magnetic nanoparticles in the live cells [2] and development of the Mössbauer brachytherapy method [3] require an accurate study of the nanoparticle dynamics inside the liquid cell cytoplasm. In this case, the nanoparticles may undergo Brownian motion, which additionally complicates the Mössbauer spectra.

In this work we demonstrated the ability of Mössbauer spectroscopy to study the Brownian motion of magnetic nanoparticles in viscous media, simulating cell cytoplasm. We used samples of a ferrofluid based on the magnetite nanoparticles with average size 25 and 130 nm. The nanoparticles were enriched by ^{57}Fe isotope to compensate the decrease of the probability of the Mössbauer effect in the liquid media. In order to maximize the viscosity coefficient we dissolved the both samples in glycerol to receive more than 90 % glycerol solution. Another experimental series were carried out on the dried powders of the samples, in which Brownian motion is absent. Mössbauer spectra were measured in the temperature range of 78−300 K. The analysis of the spectra allowed us to reveal the Brownian movement of the particles and to evaluate intrinsic dynamical parameters of the model viscous medium. The developed technique opens the possibility for studying the local thermodynamic properties of the cytoplasm of living cells with the help of probing magnetic nanoparticles.



The figure shows ^{57}Fe Mössbauer spectra of a) ferrofluid in glycerol at 240 K; b) ferrofluid in glycerol at 280 K; c) dried ferrofluid at 300 K.

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References

[1] Gabbasov, R., et al. JMMM, 427 (2017): 41-47.
[2] Yurenya, A., et al. JMMM, 427 (2017): 111-113.
[3] Gabbasov, R., et al. *Hyp. Int.*, 237.1 (2016): 34.

ORDERING OF MAGNETIC NANOPARTICLES ON SURFACES: NEUTRON AND X-RAY REFLECTOMETRY DATA

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Ordered arrays of magnetic nanoparticles (MNP) are of current interest due to various potential applications in biomedicine [1], catalysis [2], optics and data storage [3]. Additionally, the effects of self-assembly of nanosized objects are interesting from the fundamental point of view. In this regard, the study of ferrofluids (FF), suspensions of colloidal magnetic nanoparticles coated with various stabilizing agents (surfactants, polymers) is an important step in understanding of the discussed processes. At the same time, the behavior of magnetic nanoparticles in bulk and at interfaces can be very different due to specific adsorption properties, which should be considered in a variety of applications. An open question is the possible differences in the FF stability in bulk and at interfaces.
This study was aimed at obtaining structural information about MNP ordering at interfaces with ferrofluids. The FF stability in bulk and on surfaces was compared. Small-angle neutron scattering experiments were used to obtain structure of FF in bulk, and X-ray and neutron reflectometry experiments were done in the frame of the interface study [4, 5]. It was observed that the structural organization of nanoparticles at interface depends on the MNP concentration in ferrofluids, as well as on the structural organization of MNP in bulk. Also, the impact of the stabilization type of FF together with the kind of magnetic components were investigated by neutron reflectometry. The influence of gravity and external magnetic field on the adsorption properties of magnetic particles and their behavior at interface was considered including free surfaces (air/FF). Mono- and multi-layered structures of ferrofluids on the silicon surface after evaporation of the liquid carriers were studied (Fig.1). The possibility of anchoring MNP from FF on substrates by external magnetic fields is discussed.

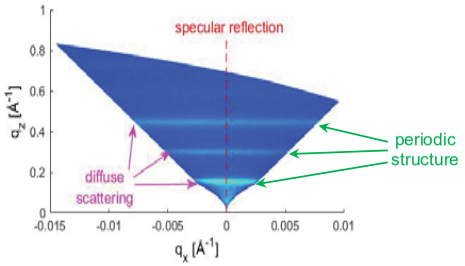

Fig.1. 2D scattering map for ferrofluids after evaporation of the solvent.

References

1. Ito A., Shinkai M., Honda H., Kobayashi T., J. Biosci. Bioeng. **100**(1), 1 (2005)
2. Roy S., Pericàs M.A., Org Biomol. Chem., **7,** 2669 (2009).
3. Nie Z., Petukhova A., Kumacheva E., Nat Nanotechnol., **5,** 15 (2010)
4. Avdeev M.V., Petrenko V.I., Gapon I.V. et al., Appl. Surf. Sci. **352**, 49 (2015).
5. Kubovcikova M., Gapon I.V., Zavisova V. et al., J. Magn. Magn. Mater. **427**, 67 (2017).

Magnetic microbead sample handling integrated with optomagnetic nanobead detection

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Microfluidics provides different possibilities for high throughput nucleic acid tests to be performed in miniaturized "lab-on-a-chip" devices.[1] Magnetic microbeads (MMBs) coated with streptavidin are a standard substrate to handle biotinylated DNA targets, which allow for on-chip transportation of DNA anchored to the MMBs between microfluidic chambers by use of an external magnet. In this work, a DNA target was captured on a MMB, amplified by rolling circle amplification (RCA) using phi29 polymerase to form a long single stranded concatemer (the rolling circle product, RCP), which was subsequently detected via depletion of capture oligo-functionalized magnetic nanoparticles (MNPs) in a detection chamber as they bind to the RCP.[2] Here, we present the use of 1 µm MyOne magnetic beads (ThermoFisher) for magnetic transportation of DNA in a polymethylmetacrylate (PMMA) chip and investigate protocols with different polymerase concentrations during the RCA as well as the effect of adding EDTA to the detection chamber. We label the conditions P1, P2, P4, and P2 EDTA corresponding to the relative polymerase concentrations and as to whether EDTA is added.

The chip consists of three connected chambers of different sizes that contain the liquids for the reactions described below (Fig. 1a). The chip was fabricated using a CO_2 laser cutting and engraving process. In chamber I, circularized templates linked to the MMBs are magnetically separated from non-circularized probes by the use of an external magnet. In chamber II, isothermal RCA takes place for 45 min to produce large RCPs from the circularized probes. Then, the MMB-anchored RCPs are transported to chamber III that contains MNPs and the depletion of free MNPs was measured real-time for 1 hour using optomagnetic measurements. Fig. 1b shows the relative signal from free MNPs for conditions P1, P2, P4 and P2 EDTA as function of DNA target concentration (including no target controls, NTCs). The results showed capture of more MNPs for higher polymerase concentration indicating that more or longer RCPs were formed. As a compromise between signal and cost (the phi29 polymerase is expensive) we selected condition P2 for further studies. We further investigated impact of EDTA, which binds and dissolves magnesium salts formed during the RCA. Previous studies have shown the sponge-like RCPs to open and become more accessible for binding in the presence of 50 mM EDTA.[3] Our experimental results in Fig. 1b show that addition of EDTA results in a higher depletion of single MNPs, i.e., that the RCPs bind more MNPs, which is consistent with this. We therefore found that the P2 EDTA condition was optimum for our work.

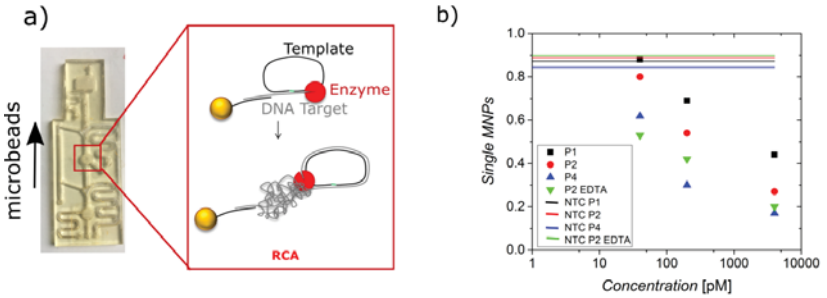

Figure 1 (a) Picture of PMMA chip. The arrow shows the direction of transport of the microbeads from chamber I to III. A schematic of the RCA process taking place in chamber II is shown. (b) Depletion of free MNPs obtained for the indicated conditions and DNA concentrations (including no template controls.

1. Lafleur JP, Jönsson A, Senkbeil S, Kutter JP. Recent advances in lab-on-a-chip for biosensing applications. Biosens Bioelectron. 2016;76:213–33.
2. Fock J, Jonasson C, Johansson C, Hansen MF. Characterization of fine particles using optomagnetic measurements. Phys Chem Chem Phys 2017;19(13):8802–14.
3. Roh YH, Lee JB, Shopsowitz KE, Dreaden EC, Morton SW, Poon Z, et al. Layer-by-layer assembled antisense DNA microsponge particles for efficient delivery of cancer therapeutics. ACS Nano. 2014;8(10):9767–80.

AFM and SANS studied on the interaction of magnetic nanoparticles with lysozyme amyloid fibrils

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Amyloids are associated with more than 20 human diseases, where the abnormal accumulation of amyloid fibrils in organs may lead to amyloidosis, and therefore, play a role in neurodegenerative disorders like Alzheimer´s or Parkinson´s disease [1]. Initially, amyloid fibrils were investigated because of their involvement in such degenerative diseases. However, fibrils have undergone a wide interest for potential application such as new types of nanomaterials in bio-nano-technology and materials science. The recent literature indicates a huge interest in developing technologies based on nanoparticles to detect, prevent or even treat protein-level diseases [2,3]. Such biohybrid materials not only can lead to the invention of nanoelectronic devices, but also allow promising materials and application in other fields such as biosensors or biomedical applications. Nevertheless, it is still a matter of controversy to assess the impact of nanoparticles on amyloids. Experimental studies suggest that nanoparticles interfere with the protein amyloid aggregation in different ways and the final impact depends on diverse chemical and physical properties of nanoparticles such as size, charge, structure and surface composition. In the presentation will be illustrated experimental data regarding the interaction of an assorted shape of magnetic nanoparticles with lysozyme amyloid fibrils using small-angle neutron scattering and atomic force microscopy. When the fibril solution is doped with spherically shaped magnetic nanoparticles, the particles had a tendency to get adsorbed on the surface of the amyloid fibrils. In the case of spindle-like magnetic nanoparticles as dopants no adsorption has been observed, which reveals subtle difference in fibrillary interaction with spherical particle that are smaller than the pitch size of the fibrils, can be explained via geometrical and dimensional considerations. Finally, these finding opened a new field of research that is related to the utilization of the magnetic properties of MNP in the potential application of such biohybrids in the future.

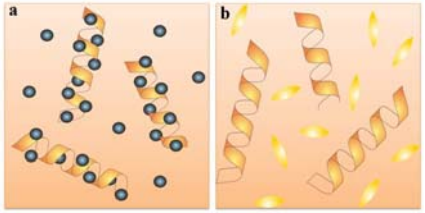

Figure. Cartoon of the possible schemes of MNPs-fibrils organisation. The resultant fibrillary complexes of doping LAF with (a) spherical MNPs and (b) spindle-like MNPs were illustrated.

Reference

[1] Sunde, M.; Blake, C. C. Q Rev Biophys 1998, 31, 1–39. doi: 10.1017/S0033583598003400
[2] Kogan, M. J.; Bastus, N. G.; Amigo, R.; Grillo-Bosch, D.; Araya, E.; Turiel, A.; Labarta, A.; Giralt, E.; Puntes, V. F. Nano Lett 2006, 6, 110–115. doi: 10.1021/nl0516862
[3] X, J.; Naistat, D.; Li, C.; Orbulescu, J.; Leblanc, R. M. Colloid Surf B: Biointerfaces 2006, 50, 104–111. doi: 10.1016/j.colsurfb.2006.02.010

Magneto-functional DNA Nanostructures using Ferritin-based Magnetic Nanoparticles

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Over the last years, a lot of progress was made in engineering dynamic DNA nanostructures. The major challenges are to precisely arrange the single machine parts and to control their motion. Recently, Kopperger *et al.* developed a platform consisting of a moving arm, which can freely rotate by 360°, using the DNA-Origami technique.[1,2] Here, the rotation is controlled via an external electric field. But it is also conceivable to actuate such macromolecular DNA machines using external magnetic fields. To this end, magnetic nanoparticles need to be attached to these DNA structures.

In order to reliably attach such magnetic nanoparticles, we utilized a semisynthetic magnetic nanoparticle based on the protein ferritin, which is highly compatible with our DNA structures. Ferritin is a protein of 24 subunits and usually acts as an iron storage in living organisms.[3] Because of this property, it enables the synthesis of a magnetic crystals into its octahedral cage. The resulting crystals are highly monodisperse and exhibit a diameter of around 8 nm. Experiments have shown, that the particles can be attracted by a magnetic tip.[3] This principle is adapted here to a DNA-based machine. The coupling to the DNA-structure is performed in two steps. First, ferritin based magnetic nanoparticles are coupled to a Dibenzocyclooctene-linker using NHS-chemistry. They are then connected to a single stranded azide-DNA using a copper-free click reaction. In order to demonstrate successful binding of the ferritin nanoparticle to DNA, we attached them to a convenient rectangular DNA origami structure.

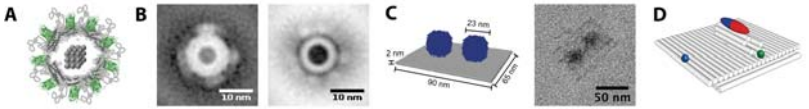

Figure: A) Scheme of ferritin magnetic nanoparticle, consisting of the protein cage with magnetite core and coupled mEGFP and PEG. **B)** Class averages of TEM-images taken from ferritin nanoparticles, without (left and with (right) magnetic core. **C)** Left: Schematic illustration of two ferritin nanoparticles bound to a rectangular DNA structure. Right: TEM image of ferritin nanoparticles on DNA origami. **D)** Illustration of the self-assembled DNA nanostructure with nanoscale robotic arm, as published by Kopperger *et al.*[2]

References:

1. Rothemund, P. W. K. (2006). Folding DNA to create nanoscale shapes and patterns. *Nature*, **440**(7082), 297–302.
2. Kopperger, E., List, J., Madhira, S., Rothfischer, F., Lamb, D. C., & Simmel, F. C. (2018). A self-assembled nanoscale robotic arm controlled by electric fields. *Science*, **359**(6373), 296–301.
3. Liße, D.*, Monzel, C.*, Vicario, C., Manzi, J., Maurin, I., Coppey, M., et al. (2017). Engineered Ferritin for Magnetogenetic Manipulation of Proteins and Organelles Inside Living Cells. *Advanced Materials*, **29**(42), 1700189. * equal contribution

The magneto-mechanical effect of barium-hexaferrite nanoplatelets on cancer cells in low-frequency magnetic field

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Studies of advanced cancer treatment often exploit magnetic nanoparticles as carriers for drug delivery or for hyperthermia treatment. In contrast, our studies were focused on a proof of a novel concept of cancer treatment, which is based on a transformation of low-frequency magnetic-field energy (1 Hz to 100 Hz) to mechanical energy. Such effect could be achieved through actuation of anisotropic magnetic nanoparticles, e.g., barium-hexaferrite nanoplatelets (BFNPs) internalized into cancer cells with the applied field. Barium hexaferrite is a hard magnetic hexagonal ferrite that grows in the form of platelets. The BFNPs display a high, uniaxial magnetocrystalline anisotropy with an easy axis perpendicular to the nanoplatelet; a very rare property, which enables effective alignment of the platelet with an applied magnetic field. Exposure to a low-frequency alternating magnetic field causes the rotation of the nanoplatelets, which subsequently results in a mechanical torque that can be transferred to the surroundings (Figure 1). The BFNPs internalized into a cancer cell could, therefore, transfer the mechanical force to the cell organelles, causing damage to the cell.

The BFNPs, approximately 50 nm wide and 3 nm thick, were hydrothermally synthesized [1] and subsequently coated with a thin silica layer, using a modified Stöber process [2]. The silica-coated BFNPs were then grafted with dextran that was pre-reacted with (3-Glycidyloxy-propyl)trimethoxy-silane. Covalently bound dextran coating ensured the BFNPs colloidal stability by steric repulsive forces in physiological media. Highly invasive, breast adenocarcinoma (MDA-MB-231) and cervical adenocarcinoma (HeLa) cancer cells were used for nanoplatelets cytotoxicity screening. Preliminary studies on biocompatibility and cytotoxicity of BFNPs were evaluated in in vitro studies using Presto blue assay. Cells were treated incubated with different concentrations of BFNPs and treated with an alternating magnetic field (2 Hz or 10 Hz) for short period of time. Without the exposure to the field, the cell viability did not decrease significantly, proving the BFNPs non-toxic. After the treatment with the field, the cell viability decreased significantly compared to the control without BFNPs (Figure 2 and 3).

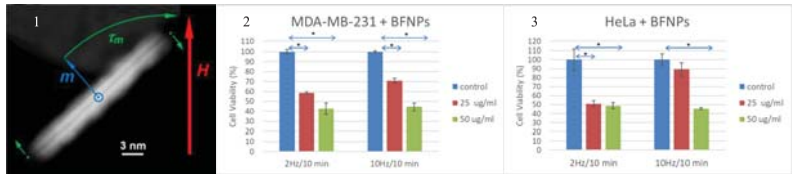

Figure 1: Atomic-resolution HAADF-STEM image of BFNP oriented edge-on with a schematic representation of the nanoplatelet's magnetization (m) and its rotation in a magnetic field (H) producing a torque τm; Figure 2 and 2: Viability of MDA-MB-231 and HeLa cells assayed by Presto blue assay (*shows that the pair is statistically different: $p < 0,05$)

References

[1] D. Lisjak and M. Drofenik, Crystal Growth & Design vol. 12, no. 11 (2012) 5174–5179.
[2] S. Kralj, D. Makovec, S. Campelj, and M. Drofenik, Journal of Magnetism and Magnetic Materials, vol. 322, no. 13 (2010) 1847–1853

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Experimental setups for the low frequency rotating magnetic field approach in cancer treatment

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Significant research has been conducted on magnetic hyperthermia, based on the combined use of high-frequency magnetic field and magnetic nanoparticles (MNPs) to induce cell death. One major drawback of this approach is the use of high-frequency magnetic fields, which is technically difficult and uncomfortable for the patient. Very recently, results of cell death using MNPs and low-frequency magnetic fields has been obtained by a few groups, which lifts one major disadvantage of magnetic hyperthermia.

To achieve low frequency rotating magnetic field experiments, we developed two types of setup (see figure). The first one consists on a Halbach array magnet fixed to an electrical motor allowing its rotation from 1 Hz to 20 Hz. The magnetic field amplitude is modified by changing the magnets and a maximum amplitude of 380 mT was reached. This device is adapted to either in vitro or in vivo experiments. To perform in vitro experiment a thermo-regulated room was designed to maintain living cells to 37°C during magnetic field application. The second setup is an electromagnet which fit under a confocal microscope and permits to perform real-time observations of cells submitted to a rotating magnetic field. Magnetic field generation is based on phase shifted electrical current operating with frequency range from 1 Hz to 100 Hz and generating magnetic field amplitude range from 0 to 100 mT. To focus magnetic field on cell epithelium the ferromagnetic core is immersed in cells medium.

Examples of experiments and data obtained using the two setups will be shown.

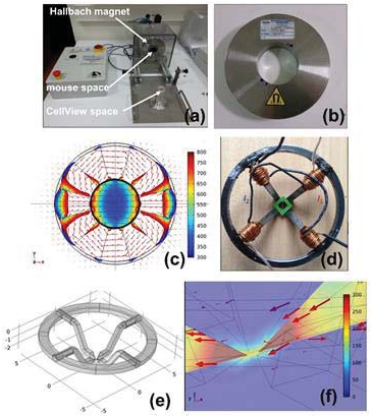

Figure: (a) Overview of the current setup used for in vitro experiments and suitable for in vivo experiments. (b) Example of a Halbach magnet used on the setup (c) Comsol® simulation of the magnetic field generated by one of the Halbach magnets. This map has been validated by magnetic field measurements. (d) Prototype for the generation of a rotating magnetic field under a confocal microscope. Its diameter is 8.5 cm. (e) Comsol® modelisation of the setup (f) Mapping of the magnetic field generated by the prototype in the zone of interest. The gap between the tips is 1 mm wide.

Magnetic Force-Based Tissue Engineering of Skeletal Muscle

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Tissue engineered skeletal muscle constructs have been designated as an attractive tool in the field of regenerative medicine, bioactuator, and drug discovery for patients with injured, diseased and age-related muscle. For drug testing, conventional two-dimensional (2D) cell culture systems are based on formation of myotubes on tissue culture dishes. However, the systems have limitations to mimic in vivo skeletal muscle functions due to lacking the architecture of muscles. One of the most important features of skeletal muscle is contractile force generation ability, and tissue-engineered skeletal muscle constructs should mimic the architecture of muscles and reproduce contractile force generation. In the present study, we demonstrate an *in vitro* system for drug testing using tissue-engineered skeletal muscle constructs.

As shown in Figure 1, we developed a fabrication method of functional skeletal muscle tissue constructs by using a magnetic force-based tissue engineering (Mag-TE) technique, in which myoblasts were labeled with magnetite nanoparticles, and assembled by magnetic field to form a cell-dense and aligned skeletal muscle-like structure.

The skeletal muscle constructs fabricated by the Mag-TE technique generated contractile forces in response to electrical stimulation. In response to small-molecular drugs as a model drug, myotube differentiation of myoblasts were promoted in 2D cell culture. However, the levels of contractile force generation of tissue-engineered skeletal muscle constructs treated with small-molecular drugs were not consistent with those of myotube differentiation in 2D cell culture. On the other hand, there was a high correlation between sarcomere formation as well as contractile activity in 2D cell culture and contractile force generation of tissue-engineered skeletal muscle constructs. These observations indicate that the contractility data is indispensable for in vitro drug screening. Additionally, as a drug testing model for neuromuscular disorders, we successfully fabricated neuron-muscle tissue constructs by co-culturing neural cells and myoblasts. These results indicate that skeletal muscle tissues constructed by the Mag-TE technique are useful for drug screening.

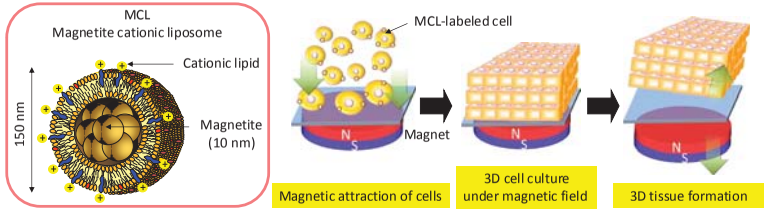

Fig. 1 Scheme of Mag-TE. Functional magnetite nanoparticles, MCLs, were added to target cells to label them magnetically. MCL-labeled cells were accumulated by a magnet and cultured in a 3D manner under magnetic field to form 3D tissues. This technique is useful for fabricating cell-dense tissues like skeletal muscles.

Simulation of the Magnetophoresis of Magnetic Nanoparticles under Cylindrical Permanent Magnet Using Coupled Particle-Fluid Model

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Abstract

A coupled particle-fluid analysis is proposed to investigate the magnetophoresis of magnetic nanoparticles (MNP) under cylindrical permanent magnet. More specifically, the closed-form field approach, which is obtained by using equivalent current source (ECS) method, is adopted to analyze the Kelvin force, playing a pivotal role in controlling the behavior of MNP. As combined with the closed-form force analysis, the non-linear convection-diffusion differential equation is used to investigate the influence of the Brownian motion on the behavior of MNP, in which the carrier fluid is assumed to be static. Besides, by integrating the Navier-Stokes equation into the existing magnetophoresis model, we reveal that the interaction between the particles and the carrier fluid not only accelerate the sedimentation of MNP, but also force more particles to deposit into the center. Moreover, this phenomenon will be more evident with the increasing of the initial particle concentration. However, this phenomenon is opposite to that of convection-diffusion equation, in which majority of the particles are captured at the margin of the magnet, presenting a ring structure. Based on these results, we derive that the effects such as the fluid-induced enhanced particle capture efficiency and the flow vortex can be predicted by using the coupled particle-fluid method, which provides a more efficient and accurate model in investigating the magnetophoresis of MNP.

Keywords: magnetic nanoparticles, magnetophoresis, coupled particle-fluid method;

Directing the transport of active particles using magnetic nanoparticles in an applied field

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Microscopic active particles, including self-propelled cells and microorganisms, artificial swimming colloids and nanomotors, have gained a lot of attention due to their relevance in such important fields as biology, biomedicine, nanoscience and nanotechnology [1]. One important aspect in systems of microscopic active particles is to reach an effective external control of their motion. Whereas artificial active particles can be designed to allow such an external control, this feature is difficult to realise for self-propelled cells and microorganisms. Here, we explore theoretically the possibility to control the main direction of motion in a dispersion of generic microscopic active particles by adding to the carrier fluid a viscoelastic bath of ferromagnetic nanoparticles that are known to self-assemble in chains under the influence of external magnetic fields [2], creating an anisotropic environment with a preferred axis defined by the field direction. By means of extensive computer simulations (see the snapshot in Figure (a)), we study the influence of the field-assembled structures of nanoparticles on the motion of the active particles, characterising the conditions that provide a higher control of the system. We show that an active particle tends to move along the channels built by chains of ferroparticles and that, depending on the size ration between active and magnetic particles, one can tune the transport efficiency (the ratio of diffusion coefficients parallel and perpendicular to the magnetic field direction) by changing the ferroparticle concentration, ρ_d (Figure (b)). The explanation of the phenomena can be found in the structure of the system: we performed the triangulation of the ferroparticle positions in the direction perpendicular to the field and extracted the radii of the inscribed circles as a measure of the characteristic size of the channels. It turned out that the radii dramatically change with ρ_d and the maximum of the efficiency is reached when the radius mean value is comparable to that of the active particle (Figure (c)). If these channels are too broad or too narrow, the directionality of the transport decreases.

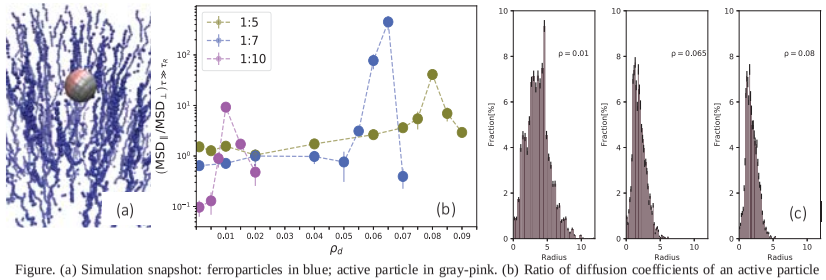

Figure. (a) Simulation snapshot: ferroparticles in blue; active particle in gray-pink. (b) Ratio of diffusion coefficients of an active particle parallel and perpendicular to the magnetic field. (c) Distributions of inscribed circle radii.

[1] C. Bechinger et al., Rev. Mod. Phys. 88, 045006 (2016)
[2] Y. Lalatonne et al., Nat. Mater. 3, 121 (2004)

Effects of interparticle magnetic correlations on the ferrofluid dynamic response

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We present experimentally measured magnetic response of specially obtained four ferrofluid samples S1– S4, different in magnetic material concentration (grows from S1 to S4), but identical in granulometry and rich with large, strongly magnetically interacting particles. Both static (χ_s) and dynamic ($\chi_d = \chi' - i\chi''$) initial magnetic susceptibilities were measured in a broad range of temperatures (T). In the static regime, on cooling, S1-S4 exhibit unusually low values of χ_s. It is the first time, to the best of our knowledge, that a clear maximum of the χ_s on cooling was experimentally obtained (Figura (a)). Non-monotonic behaviour of χ_s with decreasing T indicates the presence of structural transformations leading, first, to an enhancement of the response due to the magnetic correlations, and then to the decay of this response caused by the closure of magnetic flux within the clusters [1]. The fraction of particles in such clusters was estimated to reach 50 percent for S4 at the lowest T. In order to verify the stability of these clusters, a weak probing AC field was applied in the broad range of frequencies. Debye-like spectra were only obtained for the highest T. On cooling, the maximum of the χ'' of the samples rapidly shifts to the region of ultra-low frequencies (Figure (b)), unveiling the presence of highly correlated magnetically passive clusters, whose relaxations are very slow. Moreover, the signs of vast expansion of such magnetically inert clusters were discovered for the most concentrated sample, making the percolation in the systems of dipolar hard spheres [2], a possible explanation of the dramatic slowing of the magnetic response.

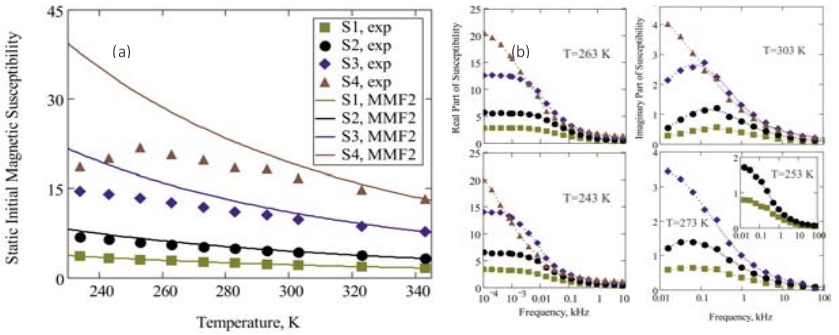

Figure. (a) Static magnetic susceptibility, symbols – experiment, curves – predictions of mean-field approach [3]. (b) Real part of the zero-field magnetic susceptibility, lines are guides for the eye. Legend is the same.

[1] S. Kantorovich et al., Phys. Rev. Lett. 110 (2013) 148306.
[2] L. Rovigatti, J. Russo, F. Sciortino, Phys. Rev. Lett. 107 (2011) 237801.
[3] A. O. Ivanov, O. B. Kuznetsova, Phys. Rev. E 64 (2001) 041405.

Magnetic particle labelling of breast cancer cells for immunohistochemistry

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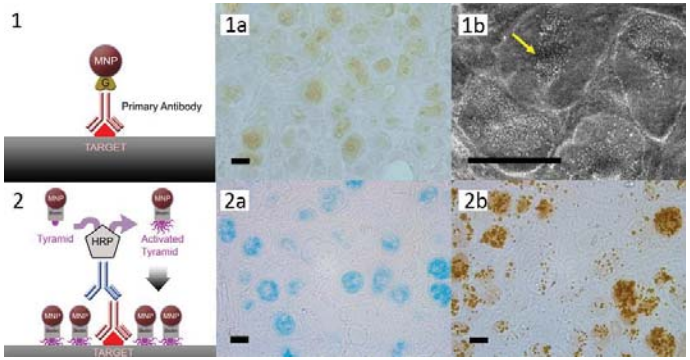
Using magnetic nanoparticles (MNPs) as antigen-specific markers for breast cancer is a promising opportunity for immunohistochemistry (IHC). The unique magnetic characteristics of MNPs offer new ways to image and identify malignant cancer cells in tissue samples. Through our work, we hope to bring the advantages of MNP-based methods (high sensitivity, large dynamic range, robustness, quantification, and multiplexing) to anatomical pathology.

A standard procedure in breast cancer IHC is to preserve biopsy samples through formalin fixation and paraffin embedding (FFPE), and to later analyze tissue for the prevalence of high-priority antigens in cancer, such as estrogen receptor alpha (ER), or human epidermal growth factor receptor 2 (Her2).

In this work, we introduce iron oxide particles as direct and indirect magnetic markers for FFPE tissue slides. In this pilot study, we used ER+ MCF-7 human xenograft tissue grown in mice that was dewaxed and rehydrated using standard xylene and ethanol baths. Direct labelling of ER was performed with Protein G-coated single-core nanoparticles (Miltenyi Biotec), which were further functionalized with an ER-specific antibody (Cell Marque) (graphic 1). The labelling success was first confirmed with 3,3'-diaminobenzidine (DAB) staining (picture 1a), and subsequently analyzed using a scanning electron microscope (picture 1b, scale bar = 10 µm). In the direct labelling approach, the total amount of bound particles is limited by the number of antigen receptors in the tissue because each receptor can only participate in one binding event.

We also explored an indirect approach in which ER is first labelled with a primary antibody, which is subsequently labelled with a secondary antibody that is conjugated to horseradish peroxidase (HRP). HRP can initiate binding events of MNPs functionalized with tyramide. Activated tyramide binds to neighboring proteins in proximity to the site where HRP is bound. Hence, multiple binding events can occur per ER antigen (graphic 2). We performed indirect tyramide signal amplification with two types of particles: single-core MNPs with a diameter of approximately 50 nm (Miltenyi Biotec), and multi-core particles with a diameter of approximately 1.5 µm (M-PVA, Chemagen Technologie). Binding of the single-core particles was confirmed using Prussian blue staining (picture 2a). The labelling success of the microparticles was confirmed using an optical microscope (picture 2b).



Statistical analyses on optimisation of high saturation magnetisation of iron nanoparticles synthesized from iron oxide by hydrogen reduction

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In this study, Taguchi method, response surface methodology and regression analyses have been applied to assess the effects of synthesis parameters on saturation magnetisation, M_s of iron nanoparticles produced by hydrogen reduction of iron oxide nanoparticles.

For Taguchi method, several experiments have been carried out based on an orthogonal array L9 with three parameters (temperature, reaction time and H_2 flow rate) at three levels (low, medium and high). Based on the signal to noise (S/N) ratio considering the condition larger is the better approach and the mean response, the highest M_s condition has been obtained at $A_3B_2C_3$ i.e. temperature is 800 ^{0}C m/min, reaction time is 60 min and H_2 flow rate is 1000 ml/min. Analysis of Variance (ANOVA) is applied to find out the F-ratio and percentage contribution of each parameter by using experimental trials and S/N ratios. It was found that the temperature was the most significant parameter on the M_s of the nanoparticles.

And, the response surface method was employed to determine the significance and interactions of the independent parameters. Also, mathematical models of multiple linear and quadratic regressions M_s were employed to derive the predictive equations of the M_s achieved via experimental design.

The results of the response surface method and the regression analyses verify the effects of the each parameter on M_s values obtained with Taguchi method, main response characteristics, and significance of each parameters on M_s values with ANOVA. A confirmation run has been carried out with 95% confidence level to verify the optimized result. Furthermore, the quality losses for M_s obtained at the highest combinations were 70.7%.

The predicted values from the developed mathematical models and experimental values are found to be very close to each other justifying the significance of the models. And, the presented quadratic interactive regression model provided the best statistical performance with high R^2 and R^2(adj) values of 100 and 100%, respectively between the experimental and predicted values of M_s. Also, more intensive predicted values were obtained by the quadratic regression models as compared to the multiple linear regression models. Taguchi prediction method was also very successful in the optimization of synthesis parameters for the highest M_s of nanoparticles within the prescribed limit.

Biodegradable and Highly Elastic Superparamagnetic Iron Oxide Nanoparticle Composite Hydrogels

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In this studies, we developed a new biocompatible magnetic polysaccharide-based hydrogel system. λ-carrageenan (Car) and cationic derivative of chitosan (CCh) are the main backbones of the hydrogel networks, denoted as Car-CCh hydrogel. In situ-gelling magnetic composite materials have been produced by using λ-carrageenan to cross-link superparamagnetic nanoparticles (SPION) surface-functionalized with N-[(2-hydroxy-3-trimethylammonium) propyl] chitosan chloride (CCh) . The ξ-potentials of SPION-CCh(+) was found be highly positive (>35 mV), similar to the ξ-potential of the cationic chitosan (+53 mV). Owing to the modified synthesis co-precipitation of superparamagnetic iron oxide nanoparticles in presence cationic derivative of chitosan and dynamic cross-links by carrageenan, the hydrogel possesses the magnetic ability. The applied nanoparticles were received in the polycation solution thanks to which uniform distribution of the nanoparticles in the hydrogel structure was obtained. The obtained composites show high water contents (80–83 wt.%) and also displaying significantly higher elasticities (G′ >50 kPa) than other hydrogels reported. M–H curve at room temperature showed the prepared sample was superparamagnetic in nature, which is also confirmed by the doublets of Mössbauer spectroscopy. The interaction between magnetic field and magnetic hydrogel was studied (AMF). Once the magnetic state was changed, mechanical and structural properties of hydrogel changed, too. The magnetic Car-CCh-SPION hydrogels hold great promises for biomedical application.

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Ferro-phasing and Potential Uses

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Highly magnetic biocompatible colloidal nanoparticles that can have sizes ranging from 35 – 160 nm such as those developed by Liberti et al.[1] have many properties in common with classical ferrofluids[2] [FF] and have previously been reported to act as phases in the presence of magnetic gradients[2]. These FF have been used in various applications. [3,4,5,6,7,8]

Phase behavior was first demonstrated as follows: 100 µL of an 8 µg/mL solution of 135nm particles tinted blue with food coloring and densified with 0.5% w/v sucrose was placed into a microtiter well, which was overlaid with buffer to form a stable two-layer system. When that microtiter well was placed into a quadrupole magnet[9]of appropriate bore, a blue-colored annular cylinder immediately formed on the periphery of the well surrounding a clear central cylinder of buffer. Removing the well immediately caused the layers to revert to their original positions. For experiments where cells were included in the bottom layer, cells therein completely moved with that layer. For both the dye and included cells, it was shown that there was no significant interaction with these FF. From these experiments, where FF act like a phase under the influence of magnetic gradients such that molecules or cells within them transport with them, the term "Ferro-phasing" was coined. Cycles of Ferro-phasing could be repeated with minimal mixing of the phases.

For this extension, BSA-coated FF having mean diameters of 58 and 135 nm were used. Original observations were reproduced in troughs, 1.0 x 4.0 cm and 1.0 cm high, for layering solutions. Experiments involved placing 2 mL FF aliquots (10 – 25 µg/mL),tinted blue by the addition of food coloring, over 2mL aliquots of buffer containing different concentrations of sucrose (0.5 – 4% w/v). Troughs were then placed on top of a strong magnetic gradient created by a bucking magnet arrangement to create a downward-pulling force. When the lower buffer layer contained 0.5% w/v sucrose, the top ferrofluid layer (for both 58 and 135 nm preparations) immediately moved to the bottom of the trough such that the blue dye remained in that phase; layers reverted to original positions when removed from the gradient. Increasing the sucrose concentration to 1 – 2% w/v resulted in partial migration of the phase, and the fraction of the phase that migrated could be increased by increasing the ferrofluid concentration or decreased by decreasing the ferrofluid concentration or reducing the magnetic force. At sucrose concentrations of 3– 4% w/v, Ferro-phasing was completely inhibited. Furthermore, for the 58 nm preparation, the ferrofluid remained in the top layer indefinitely when left on top of the magnetic device due to their Brownian motion and smaller magnetic moment. These results demonstrate some fundamental properties of Ferro-phasing and that it can be eliminated by altering solution density, ferrofluid concentration, and/or magnetic force.

To determine if a stable Ferro-phase could be established in a large vessel and be controlled externally by a magnetic gradient, aliquots (1 – 3 mL) of blue-tinted FF (15 – 25 µg/mL) were pumped through micro-bore tubing submerged in 500 mL graduated cylinders filled with buffer. The tubing tip was positioned ~ 2 cm from the vessel wall, against which an N52-grade rare-earth magnet (70x13x13 mm) was placed. As the FF exited the tubing, it remained in an intact stream and moved towards the magnet, forming a blue-tinted bolus against the vessel wall adjacent to the magnet that could be maintained overnight at RT. By moving the magnet up or down the side of the cylinder (ca. 5 cm/s), the bolus – and the dye contained therein – moved with the magnet. When RBC suspensions at 10^7/mL were included in buffered FF in experiments identical to the above, RBC remained within the bolus and could be moved or held in position without lysis for up to 72 h. Alternatively, when pumped into a vessel containing deionized water, RBC lysis was evident after about 6 h. After about 30 h, a bright red color (presumably hemoglobin) was observed diffusing out of the phase.

These results indicate that FF form structures under the influence of magnetic fields that can entrap within them cells and macromolecules, and that those entities can be moved or held in position by an external magnet. Since RBC lysis occurs when phases are established in DI, small ions must diffuse in/out of phases. Molecules like blue food coloring (MW 792) appear to be retained within the phase. The ability to retain or move non-magnetic entities within Ferro-phases may have use in drug delivery or localization and applications that require the ability to translate or move reactants/elements.

References: [1] P.A.Liberti, Rao,C, and Chiarappa, J. US Patent Number 5,698,271, [2] R.E.Rosensweig,"Ferrohydrodymamics", Dover Publications, 1997 [3] P.A. Liberti and Wang, Y. US Patent 5622831 [4] L. Terstappen, Rao,C, Uhr, J, Racilla,E and Liberti, P. US Patent Number 6,645,731 [5] E. Racilla, Euhus D., Weiss A.J., Rao C., McDonnell J., Terstappen L.W.M.M., Uhr J Proc Natl AcadSci USA 95, 4589 (1998) [6] C. Rao and Liberti P., US Patent No 5660990 [7] C. deHaenTopics in Magnetic Resonance Imaging 12(4): 221–230 [8] P. Liberti,BioreceptorFerrofluids: Novel Charisteristics and their utility in medical applications Fine Particle Science and Technology, E. Pellizzetti, Ed. 777 - 798 (1996) Springer [9] P. Liberti, Wang, Y, Tang,W, Feeley,B and Gohel, D, USPatent number 5466,574 (1995)

Study the stability of protein-magnetic nanoparticle composite

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Amyloid fibrils are considered to be the initial cause of a number of devastating neurodegenerative diseases such as the Alzheimer, Parkinson, and Huntington diseases. Fibrils doped with nanoparticles can receive benefical physical properties. At present, there is increasing interest for investigation of biocompatible composites of containing magnetic nanoparticles due to the vast variety of biomedical applications such as the targeted delivery of drugs, hyperthermia, magnetic resonance imaging and chelation therapy.

We present colloidal nanocomposite formed by combining a magnetite nanoparticles Fe_3O_4 and lysozyme amyloid fibrils from hen egg white. Two types of solutions were prepared: with and without the salt addition with the aim to study effect of a salt presence, nanoparticle´s and protein´s concentrations on stability of fibril-nanoparticle nanocomposite. To obtain the original information about the structural morphology of amyloid fibrils and their interactions with magnetic nanoparticles, atomic force microscopy AFM and small angle x-ray scattering SAXS techniques were used.

Results show that conformational properties of fibrils depend on protein concentration and that for stability is very important accurate aspect ratio between protein/nanoparticle concentration. Our results show importance of the salt role in stability and conformation both in pure fibril solution and nanoparticles-modified LAF solution too. Fibrils without the salt addition are longer, but on the other side this solutions are less stabile. The morphology of fibrils changes with changing the protein concentration. After doping of fibrils with nanoparticles, nanoparticles adsorb on fibril surfaces. Length of fibrils does not change by doping them with nanoparticles. Importantly, the resulting hybrid was shown to have excellent physical stability. It has been demonstrated the stability of some of the samples even in one year period.

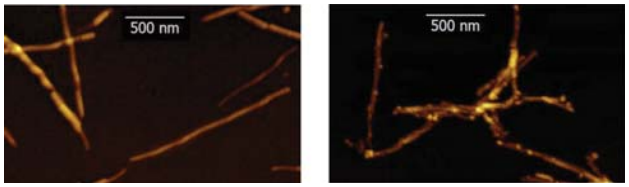

Fig. 1. AFM images of a) lysozyme amyloid fibrils and b) lysozyme amyloid fibrils decorated with magnetite nanoparticles.

Acknowledgments

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Enzymatic reaction management via Brownian relaxation of magnetic nanoparticles under low-frequency magnetic field

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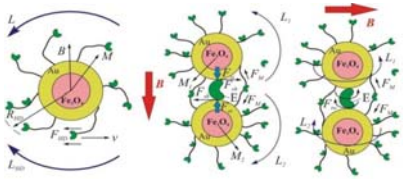
Application of magnetic nanoparticles (**MNPs**) for therapy, drug delivery, cells separation and as a contrast agent for MRI has undergone explosive development recently. Nanomechanical approach for biochemical reaction management is a promising one for biomedical application and biocatalysis with the use of MNPs [1, 2]. In this approach, Brownian relaxation of MNPs meaning their mechanical rotation under low-frequency non-heating magnetic field applied for changing of immobilized on MNP's surface enzyme structure-activity relationship occurs (Fig. 1). Here, we present the effect of super low-frequency magnetic field (50-77 Hz) exposed in increase and decrease of the catalytic activity of immobilized onto MNP's surface different enzymes.



(a) **(b)**

Figure 1.Different forces and deformations that undergo enzyme macromolecule (E) immobilized on a single-domain core@shellFe_3O_4@Au MNP with magnetic moment (M) and hydrodynamic radius (R_{HD}) as a result of Brownian relaxation under expose of low-frequency magnetic field (B). **(a)** In case of enzyme immobilized on individual MNP, it susceptible hydrodynamic forces (F_{HD}). **(b)**In case of enzyme cross-linked between two or more MNPs, it undergoes stretching (F_M), twisting (F_{tw}) and shifting (F_{sh}) forces.

The work was supported by RSF-14-13-00731 and RFBR 17-54-33027 grants.

[1] N.L. Klyachko, M.Sokolsky-Papkov, N. Pothayee, et al. Angew. Chem. Int. Ed. 2012. 51. 12016 – 12019.
[2] A.Majouga, M.Sokolsky-Papkov, A.Kuznetsov, et al.Colloids and Surfaces B: Biointerfaces 2015. 125. 104–109.

Microgels in computer simulations

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Colloidal particles, consisting of cross-linked polymers called microgels, are able to swell or shrink as a response to their external environment [1]. The fact that the swelling can be controlled makes microgels promising materials for many applications.

However, the system behavior can be drastically changed by embedding magnetic particles into the system as it is demonstrated in the resent works on the novel magnetic dipolar materials like magnetic gels, brushes and filaments [2,3,4].

In this work, we study the behavior of both nonmagnetic and magnetic microgels. The latter are obtained by embedding magnetic dipolar particles in cross-linked polymers comprising microgels. We elucidate how dipolar interactions alter structural and elastic behavior of microgels. To this aim, we employ Molecular Dynamics simulations where all microgels initially have a spherical shape and observe how the strength of electrostatic interactions and dipole-dipole interactions influence these systems changing the size of microgels and their polymer conformations. We show that embedding magnetic particles in microgels gives us an additional tool to manipulate their swelling along with changing the solvent of the system.

[1] A. Fernandez-Nieves, H. M. Wyss, J. Mattsson, and D. A. Weitz. Microgel Suspensions: Fundamentals and Applications. 2011.
[2] R. Weeber, S. Kantorovich, and C. Holm, J. Chem. Phys. **143 (15)** (2016).
[3] P.A. Sanchez, E.S. Pyanzina, E.V. Novak, J.J. Cerda, T. Sintes and S.S. Kantorovich, Faraday Discuss. **186**, 241 (2016).
[4] P.A. Sanchez, E.S. Pyanzina, E.V. Novak, J.J. Cerda, T. Sintes and S.S. Kantorovich, Macromolecules **48 (20)**, 7658 (2015)

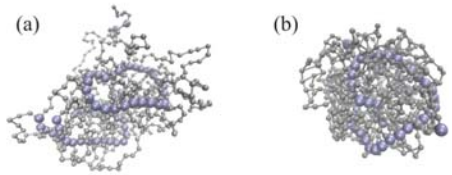

Fig.: Self-assembly of magnetic particles in magnetic microgels at strength of dipolar interactions λ=8. Magnetic particles are depicted in red. Fraction of crosslinked monomers is (a) 7% and (b) 67%. Fraction of magnetic particles is 10%.

Study of Magnetic Particle Interaction with Eukaryotic Cells Using Imaging Flow Cytometry, Scanning Electron Microscopy and MPQ-Cytometry

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Magnetic nanoparticles are attractive tools for biomedicine. Their unique ability to be controlled with external magnetic fields makes them useful in such applications as targeted drug delivery, bioimaging, hyperthermia, etc. Nanoparticles functionalized with various recognition bioreceptors are especially promising as nanosensors and therapeutic payload carrying vehicles. Understanding the intricacies of nanoparticle-cell interactions is essential for efficient drug delivery. In this work, we synthesized a variety nanoparticles functionalized with antibodies, lectins, and other bioreceptors and investigated their interactions with eukaryotic cells by several methods, namely, imaging flow cytometry [1], scanning electron microscopy and MPQ-cytometry based on magnetic particle quantification (MPQ) method [2,3].

Using imaging flow cytometry, which required additional fluorescent labelling of the nanoparticles, we evaluated with high statistical significance various parameters of interaction of the nanoparticles with the target cells in presence of large number of non-target cells. We investigated nanoparticle internalization, membrane targeting, clustering, etc. Furthermore, for high resolution structural examination of the nanoparticle-cell complexes, we used scanning electron microscopy (Fig. 1). Depending on the surface properties of the nanoparticle conjugates we observed either high or low particle binding, clustering or more uniform distribution of the nanoparticles on the cell surface.

Then, the total binding of the magnetic nanoparticles to cells surface were quantified with MPQ-cytometry. This method allows precise direct quantification of the magnetic nanoparticle bound to the cells without additional labels. The nanoparticles are quantified via subjecting the sample to magnetic field generated at two frequencies f_1 and f_2 and recording the non-linear magnetic response of the particles at combinatorial frequencies $f = mf_1 \pm nf_2$, where n and m are integers.

The obtained data will be useful for development of the advanced magnetic nanoagents with high specificity to cellular targets.

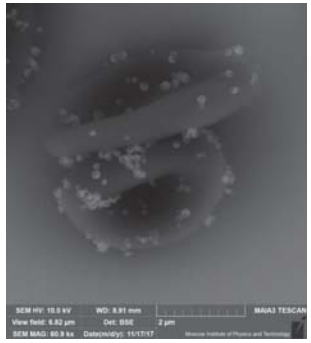

Fig. 1. Scanning electron microscopy image of cell targeting with 200 nm magnetic particles.

[1]. S.E. Headland, et al., Scientific Reports, 4, 5237, 2014.
[2]. V.O. Shipunova, et al., Nanoscale, 8, 12764-12772, 2016.
[3]. P.I. Nikitin, P.M. Vetoshko, Patents RU2166751, RU2177611 (2000), EP1262766 (2001).

The Influence of External Fields on the Self-Assembly of Magnetic Janus Particles

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Currently designed soft functional materials open up perspectives for various technical applications, from actuators to sensors and printed electronics. In this contribution we investigate silica particles with a magnetic thin film coating on one hemisphere, so-called Janus particles (Figure (a)). They are characterised by an extended magnetisation distribution, with the local magnetization pointing perpendicular to the particle surface. We study the influence of low-frequency external magnetic fields on the self-assembly of these colloidal particles. The formation of branched clusters of staggered chains, compact clusters, linear chains, and dispersed single particles can be selected in experiments. The experimental findings are complemented by molecular dynamics simulations, performed with a system of model colloidal particles that represent the extended magnetisation distribution as an off-centred, radially symmetric arrangement of five point dipoles (Figure (b)). The results demonstrate that the diversity of controllable structures formed under external fields can be increased by means of two ingredients: the magnetic particle anisotropy and the spatial extension of the magnetization distribution on the surface of the particles. The precise control of structure formation and reconfiguration under external fields of only a few μT exhibited by this system opens up the possibility to use it in responsive materials for highly sensitive magnetic and optical applications.

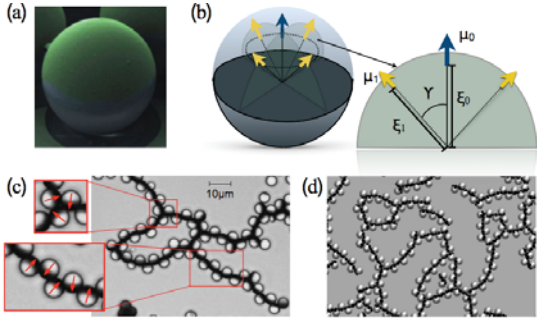

Figure. (a) Experimental view of a particle with the magnetic cap in green. (b) Model of a sphere with five shifted dipoles (5sd), a central one, μ_0 and two side dipoles, μ_1, with enclosed angle γ and radial shift $\xi_{0,1}$. (c) Microscopy image of self-assembled Janus particles. Open structures built from staggered chains and branching points (insets). (d) Simulation snapshot with 5sd particles, parameters used here are $\mu_0 = \mu_1 = 0.9$; $\gamma = 6\pi/25$ and $\xi_0=\xi_1=0.94$.

Structure of magnetoferritin solutions and its impact on amyloid aggregates

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An attractive biocompatible system for drug binding is the iron storage macromolecule – ferritin – due to its nano-sized spherical-like protein shell (internal and outer radii 8 and 12 nm respectively) providing an internal cavity where drug molecules can be encapsulated. The surface of this shell (apoferritin) contains numerous binding sites for coupling with various agents. The artificial superparamagnetic analogue of ferritin, magnetoferritin (MFer), offers promise as a potential magneto-pharmaceutical nanomaterial. In contrast to low-magnetic ferrihydrate core in ferritin, in MFer magnetic nanoparticles are placed into the shell cavity by controlled *in vitro* Fe^{3+} loading. This type of macromolecules is of current interest for targeted drug delivery using external magnetic fields, magnetic hyperthermia therapy and other applications.

We report about the detailed study of the structural stability of magnetoferritin in solutions at various pH levels and various loading factors (number of magnetic atoms per molecule) (see photo with visible sedimentation at pH<5.85). The analysis is based on the data of small-angle X-ray and neutron scattering, dynamic light scattering, zeta potential measurements and other methods. Thus, at pH 3–6 a reduction of electrostatic repulsion in the suspended colloids resulted in aggregation and sedimentation of MFer. At neutral and slightly alkaline conditions (pH 7–9) the MFer structure is stable only for comparatively low iron loadings, while at pH 10 – 12 the destabilization of the protein structure and dissociation of subunits occur. An increase in the loading factor in MFer leads to a decrease in the stability versus pH [1].

An interesting phenomenon found is the interaction of MFer with model amyloid fibrils (here, lysozyme) [2], which are actively studied regarding a potential impact on neurodegenerative diseases (e.g. Alzheimer's disease). It has been shown that MFer molecules affect the size, structure and amount of amyloid fibrils. This influence is strongly determined by the presence of iron in solutions and is sensitive to its amount in MFer.

[1] L. Balejcíková, et al. *Coll. Surf. B* 156 (2017) 375–381.
[2] P.Kopcansky, et al. *J. Magn. Magn. Mater.* 377 (2015) 267–271

Magnetically modified electrospun nanotextile and its bioapplication

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The preparation of magnetically modified electrospun nanofibrous textile based on polyamide, polycaprolactone and polyurethane is reported. The magnetic nanotextile materials were prepared by several modification procedures employing various types of magnetic fluids; both immersion of the nanotextile into diluted ferrofluid solutions or a spraying procedure were used. Magnetic modification led to the deposition of magnetic iron oxide nanoparticles on the surface of textile nanofibres. Magnetic nanotextile exhibited response to external magnetic field (see Fig. 1) and was stable for a long time. Magnetic modification has not substantially changed the structure of the modified nanotextile.

Magnetically modified nanotextile was especially applied for the immobilization of technically important enzymes (trypsin and lipase); the immobilized enzymes exhibited long-term stability and could be used repeatedly. In addition, magnetically modified nanotextile exhibited peroxidase-like activity.

Magnetically modified nanotextile represents a promising composite material applicable in various biochemical, biomedical and biotechnology applications.

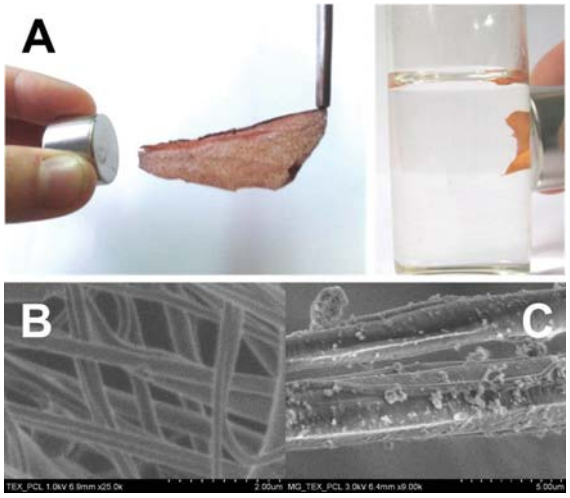

Fig. 1. Appearance of magnetically modified polycaprolactone nanotextile and its response to external magnetic field (A); SEM images of native (B) and magnetically modified (C) polycaprolactone nanofibers.

Self-assembly In Magnetic Filament Dispersions: Influence of Internal Parameters

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Magnetic filaments or supracolloidal magnetic polymers (SMPs) are semiflexible polymer-like chains of magnetic nanoparticles permanently crosslinked with polymers which have been recently shown to be promising building blocks for the creation of sophisticated magnetoresponsive materials. Using Langevin dynamics simulations, we focus on low-concentration solutions of filaments, analysing in detail their self-assemblyunder the influence of various internal parameters: dipole-dipole interaction, particle sizes, additional attraction potentials, conformations and lengths of chains. Simple open chains, closed rings and branched structures with “X” and “Y” junctions are used. Extensive cluster analysis was performed using graph theory.Clustersize distribution, amount of additional connections, their distribution and defects were calculated. We define defect particles with more than two bonded neighborsand defect cluster, or simply defect, a set of neighboring defect particles. We introduce two parameters to categorize the defect clusters: «s» (the number of defect particles in the defect, namely the size of the defect) and «w» (the number of ways out from the defect). We also compare the structures formed by filaments dispertions to those observed in conventional magnetic fluids containing non-crosslinked nanoparticles. For example, clustersize distribution has totally different behavior for ferrofluid and filament’s dispersion. We have shown that permanent links in the system of magnetic particles and their conformation can dramatically change microstructure of dispersion. The next step was to consider the behavior of such a system under the influence of additional attraction potential to investigate the difference in the existent scenario of self-assembly. Also, we were interested in the impact of polydispersity in the self-assembly of filament dispersion. Following the seminal theoretical work on the effects of polydispersity on the properties of ferrofluids [A. O. Ivanov and S. S. Kantorovich, Phys. Rev. E, 2004], we consider a bidisperse model as a first approximation to a polydisperse system. As a initial step for dispersion, we study four types of individual magnetic polymer chains: consisting of only large particles (0); with all large, but one small particle located at one chain end (1); with two small particles at the chain ends (2); with three small particles, two of them at the chain ends and one in the middle (3). Using replica-exchange molecular dynamics simulations, we study the radius of gyration and magnetic moment of a single linear SMP in a wide range of temperatures. We observe that the presence of even a little fraction of small particles in the chains significantly affects their structural behaviour. With the addition of small particles at the ends of the chains (configurations (1) and (2)), the radius of gyration is larger than the corresponding to the monodisperse system (0). On the contrary, the presence of a small particle in the central part of the chain tends to decrease the radius of gyration with respect to the same reference system.All results will form the basis for developing theoretical models and provide recommendations for the design of novel magnetoresponsive systems.

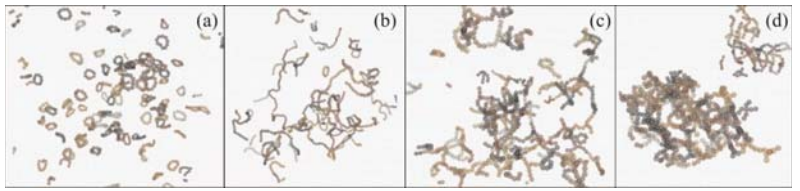

Figure.Snapshots for 4 types of SMP. (a) – rings, (b) – chains, (c) – Ys, (d) – Xs.

Magnetite-coated polystyrene nanospheres as a potential platform for magnetic guidance applications

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It is known that external stimuli might be utilized to drive responsive micro- and nanostructures towards a specific area. Magnetic, electric, and thermal fields are indeed examples of the use of physical stimuli to direct locomotion. It can be envisioned that the direction of micro- or nanomotors, which navigate through a continuous medium, for biomedical applications can be controlled by externally applying one of these fields, as long as they are kept below the physiological limits, for example in the case of a magnetic field.[1] Moreover, many magnetic nanomaterials, such as transition metal ferrites, have been demonstrated to be not harmful when interacting with cells or tissues, and they are finally eliminated from the biological systems.

Accordingly, the design of magnetically-controlled nanoarchitectures becomes an important approach for directed cargo-release therapies. In the last few years, many reports have described the assembly and diffusion properties of magnetic particle-based architectures for different applications.[2,3] Despite the increasing number of publications in this area, there exists however a lack of the reported values concerning magnetophoretic mobility coefficients, a key parameter when studying these magnetoresponsive systems.

Herein, we describe a synthetic approach to provide magnetically guided nanometer-sized architectures, towards their potential use in magnetic guidance, and evaluate these structures in terms of mobility.

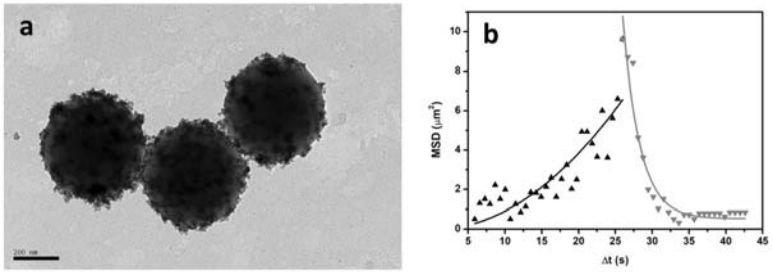

Figure 1. TEM image (a), and mean-squared displacement plot (b) of the magnetite-coated polystyrene nanoparticles. The graphical tendency reveals a guided diffusion motion of the nanoassemblies (black symbols), and a subsequent decay due to particle sedimentation (grey symbols).

1. Pankhurst, Q. A., Connolly, J. Jones, S.K., Dobson, J. *J. Phys. D: Appl. Phys.* **2003**, *36*, R167.
2. Zhou, C., Boland, E.D., Todd, P.W., Hanley, T.R. *Cytometry Part A* **2016**, *89A*, 585.
3. Schattling, P.S., Ramos-Docampo, M.A., Salgueiriño, V., Städler, B. *ACS Nano* **2017**, *11*, 3973.

Magnetic, electronic and optical properties of Zn doped Fe_3O_4 nano-hollow spheres

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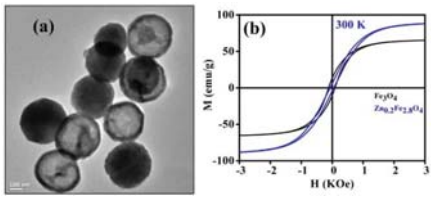
Abstract

Potential use of Fe_3O_4 (magnetite) nanosturctures in biomedical and other applications can be enhanced by increasing their magnetic saturation and making hollow structures which are light, more dispersible and compressible and can be used to encapsulate drugs, DNA and cosmetics [1].

In this work we have synthesized a series of Zinc doped Fe_3O_4 ($Zn_xFe_{3-x}O_4$, x=0.0, 0.1, 0.2, 0.3, 0.4, 1.0) nano-holllow spheres (NHSs) via solvothermal technique [2]. The structural and morphological characterizations of as-synthesized $Zn_xFe_{3-x}O_4$ NHSs were performed through X-ray diffraction, transmission electron microscope, Fourier transform infrared and energy dispersive X-ray spectroscopy. Detailed temperature dependent magnetic studies of $Zn_xFe_{3-x}O_4$ NHSs using a vibrating sample magnetometer point out their increase in saturation magnetization (M_S) with Zn doping, attaining a maximum at x=0.2 (M_S=92.52 emu/g at room temperature), and then decreasing with further increase in Zn content due to spin canting. Nonmagnetic Zn^{2+} ($3d^{10}$, S=0) ions replace antiferromagnetically coupled Fe_A^{3+}on the A site without affecting the magnetic exchange on the B site. As Zn^{2+} ion replaces Fe_A^{3+}, it transforms the Fe_B^{2+} ion on B site to Fe_B^{3+} to maintain the charge neutrality. Hence there is an increase in the saturation magnetization at low substitution level.

Dielectric properties as a function of temperature have been investigated over a frequency range of 10KHz to 100MHz by analyzing dielectric spectroscopy, dielectric loss and electrical conductivity. Dielectric permittivity and electrical conductivity of the samples are found to decrease with increasing Zn content. With the increase in Zn substitution the electrical conductivity is reduced both by a reduction in the density of itinerant charge carriers (Fe_B^{2+}) as mentioned above and their hopping amplitude due to spin canting. Optical properties of these NHSs were studied through UV-Vis spectroscopy revealing decrease in band gap with increasing Zn concentration.

Figure below shows (a) TEM image of prepared NHSs and (b) M-H loop of Fe_3O_4 and 20% Zn-doped Fe_3O_4 at room temperature showing enhancement of saturation magnetization with doping.



Reference:

[1] YanQi Zhang, LinLin Li1, Fangqiong Tang, and Jun Ren, Journal of Nanoscience and Nanotechnology Vol.6, 3210–3214, 2006

[2] Debasish Sarkar, Madhuri Mandal, and Kalyan Mandal, JOURNAL OF APPLIED PHYSICS 112, 064318 (2012)

Detailed toxicity evaluation of bio-functionalized magnetic tracers with reduced signal loss using magnetic particle imaging (MPI)

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The utilization of superparamagnetic iron oxide nanoparticles [SPIONs] in magnetic particle imaging [MPI] is progressively increasing, leading to the rapid development of biocompatible and surface modified SPIONs. However, there is still a need of information pertaining to its cellular and acute toxicity profile to be used as tracer material for MPI. This work reports the synthesis of β-cyclodextrin coated SPIONS and their characterization using spectroscopic (FT-IR), thermal (TGA) and surface analysis (TEM, SEM, BET and Zeta potential). βCD-SPIONs obtained were rod-shaped of ~45nm. Time dependent cellular uptake of βCD-SPIONs was evaluated using Prussian blue staining. The cytotoxicity analysis of βCD-SPIONs was done in mouse fibroblast cell line (NIH 3T3) using MTT and LDH assays, which did not show any cytotoxicity. Further, acute toxicity was carried out in female Wistar rats according to OECD guidelines 420. Rats were exposed to the highest dose (2000mg/kg) of βCD-SPIONs along with control and observed for 14 days. After two weeks of administration, tissues and blood were collected and subjected to histopathological and biochemical analysis (SGOT, SGPT and ALP). It has been seen that βCD-SPIONs did not have any significant toxic effect at the cellular level. Finally, βCD-SPIONs were employed for MPI along with the marketed Resovist. In cellular imaging, βCD-SPIONs showed higher spatial resolution as well as higher sensitivity than Resovist. Furthermore, it has been found that the choice of bio-functionalization strongly influences sensitivity, localization properties and spatial resolution in cellular imaging using MPI.

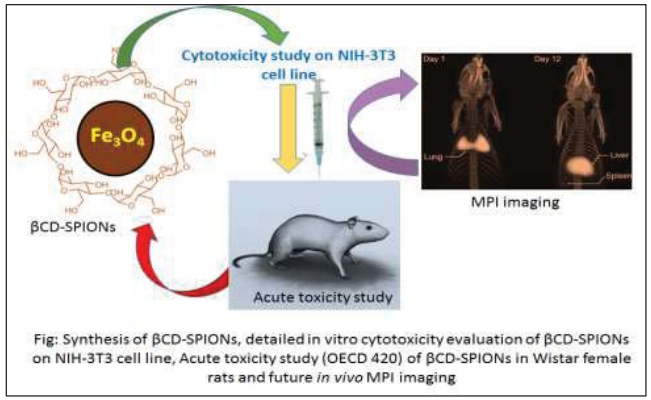

Fig: Synthesis of βCD-SPIONs, detailed in vitro cytotoxicity evaluation of βCD-SPIONs on NIH-3T3 cell line, Acute toxicity study (OECD 420) of βCD-SPIONs in Wistar female rats and future *in vivo* MPI imaging

Investigation on the Self-assembly of Magnetic Core-Shell Nanoparticles Under Soft-Magnet Element by Using Discrete Element Method

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The self-assembly of magnetic core-shell nanoparticles in the presence of a magnetic template, which consists of an array of soft-magnetic elements embedded in non-magnetic substrate, is analyzed by using discrete element method. An external bias magnetic field is used to magnetize the soft-magnetic elements to saturate state. The high-gradient field produced by elements combined with biased uniform magnetic field provides a flexible way to control the behavior of particles. An equivalent source method is adopted to obtain the closed-form magnetic field analysis, which not only improves the calculation efficiency but also enables accurate prediction of the Kelvin force. In the presence of magnetic field, the behavior of the magnetic nanoparticles is dependent on the magnetic and hydrodynamic forces. Therefore, the assembled structures of magnetic nanoparticles are firstly investigated without considering the magnetic dipole interaction force, in which an unordered nanostructure is formed. As a contrast, the self-assembly of particles is also simulated by taking all forces into account. In this case, the magnetic nanoparticles assemble into an ordered 3D structure, which presents a hexagonal close packed structure. A comparison between the results of the mentioned two cases denotes that the magnetic dipole interaction force plays an important role in controlling the self-assembly of magnetic nanoparticles.

Experimental Analysis of the Magnetophoresis of Magnetic Nanoparticles under Permanent Magnet

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Magnetic nanoparticles (MNPs) are drived to the source of magnetic field along the magnetic field gradient in carrier fluid by external magnetic field, which is known as magnetophoresis. Magnetophoresis of MNPs is a complicated physical process, in which the carrier fluid can be influenced by the motion of the MNPs and in turn the behavior of MNPs also can be modified by the fluid. Thus, investigation of the interaction between the carrier fluid and the MNPs plays an important role in illustrating the intrinsic mechanism of magnetophoresis of MNPs. In this work, an experiment platform, including a cylindrical NdFeB permanent magnet, a UV- visspectrophotometer and cuvettes with different concentrations of MNPs, is performed for studying the magnetophoresis of MNPs. Firstly, the magnetic force is computed by using an equivalent moment method in which the MNPs are regarded as equivalent magnetic point dipoles. Then, the velocity of MNPs is obtained by calculated the Newton's law in which the Kelvin force and viscous force are included. Thirdly, the stream of fluid is indirectly observed at the vertical direction and the bottom of cuvette through adding methylene blue into magnetic nanoparticles suspension. Finally, by means of UV-vis spectrophotometer, the variation of concentration of MNPs is indirectly reflected by measuring light absorption. The results show that obvious vortexes can be witnessed at the vertical direction of the fluid. The tendency of the carrier fluid motion is all direction at the bottom of the cuvette. Besides, the MNPs, which are far away from the magnet, mainly follow the fluxion of their surrounding fluid. Hence, this experiment is useful for understanding and analyzing the magnetophoresis of MNPs.

Keywords: magnetophoresis, magnetic nanoparticles, Kelvin force, magnetic field

Development of a Superparamagnetic and Luminescence Ferrite@Quantum Dot System for Cellular Imaging

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Magnetic resonance imaging (MRI) is known as a non-invasive technique to detect tumors or trace stem cells *in vivo*. Low sensitivity of this technique as its limitation that leads to unclear diagnosis of healthy tissues from abnormal tissues is due to the long relaxation time of magnetic spins in water protons. Attempting to resolve this problem, more studies focus on magnetic systems as potential MRI contrast agents. Superparamagnetic nanoparticles can alter relaxation time, and thereby affect the MRI signals due to their uniform particle size and high superparamagnetic moment. Besides the superparamagnetic nanoparticles, functionalized-graphene quantum dots (GQDs) with glycol, dithioglycol and amine compared to GQDs can not only enhance the luminescence emission in near infrared (NIR) regin , but also increase the solubilityof system in biological medium.

In the present investigation, a superparamagnetic and luminescence system based on $MnFe_2O_4$@N-GQDs core-shell coatd with polymer was prepared via an *in situ* thermolysis procedure at 200 °C. Photoluminescen (PL) spectra of the $MnFe_2O_4$@N-GQDs/polymer presented in Figure (1a) are located in NIR regin and independed upon the excitation wavelength. As shown in Figure1 (b) vibrating sample magnetometer(VSM) spectra of the $MnFe_2O_4$@N-GQDs core/shells with and without polymer indicate soft and super-paramagnetic behavior for both samples. The M_s value of 9.12 emu/g for the $MnFe_2O_4$@N-GQDs/polymer is lower than the value of 46.27 emu/g for the $MnFe_2O_4$@N-GQD.

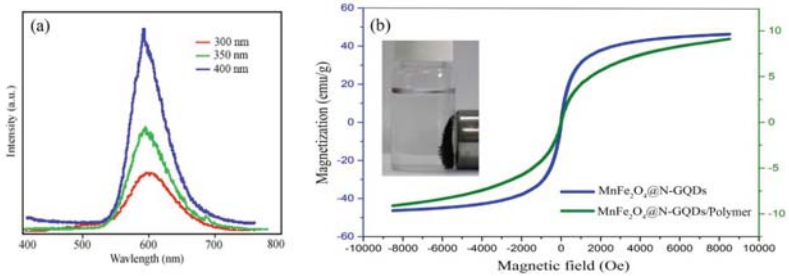

Figure 1. (a) PL spectra and (b) VSM spectra of $MnFe_2O_4$@N-GQDs/polymer at room temperature.

Development of Lumino-Magnetic Iron Oxide Nanoparticles for Multimodal Applications

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The optimization of magnetic and optical characteristics is an essential requirement to enhance the applicability of magnetic nanomaterials in various fields of biomedicine and imaging. The encapsulation of magnetically active iron oxide nanoparticles by optically active silver- revealing a core shell nanostructure- offers an excellent combination of both optical and magnetic properties in a single component. The structural, optical and magnetic properties of magnetite nanoparticles of size 6 nm as well as coated magnetite with thickness 2 nm has been investigated via different characterization techniques. The structural and magnetic analysis of uncoated and coated nanoparticles emphasize on occurrence of an intermediate disordered magnetic layer which arises on the upper surface of magnetic core formed due to diffusion from non-magnetic shell material. The presence of blocking temperature and imperceptible values of coercivity confirms the superparamagnetic nature of coated and uncoated particles. The non-magnetic coating on the magnetic material leads to the arousal of canted spins at the interface as well as exhibits an increase in magnetoelastic anisotropy. The optical study shows remarkable increase in luminescence intensity of magnetite nanoparticles along with a blue-shift after coating by optically active silver. The hyperthermic measurements revealed a temperature raise from 29°C to 43°C in approximately 10 minutes.

References

(1) R. Thomas, I. Park, Y.Y. Jeong, Magnetic Iron Oxide Nanoparticles for Multimodal Imaging and Therapy of Cancer, Int. J. Mol. Sci. 14 (8), (2013) 15910–15930.
(2) D.A. Balaev, S. V. Semenov, A.A. Dubrovskiy, S.S. Yakushkin, V.L. Kirillov, O.N. Martyanov, Superparamagnetic blocking of an ensemble of magnetite nanoparticles upon interparticle interactions, J. Magn. Magn. Mater. 440 (2017) 199–202.
(3) D.L. Dexter, A Theory of Sensitized Luminescence in Solids, J. Chem. Phys. 21 (1953) 836–850.
(4) P. Singh, C. Upadhyay, Role of silver nanoshells on structural and magnetic behavior of Fe_3O_4 nanoparticles, J. Magn. Magn. Mater 458 (2018) 39-47.

Synthetically-driven enhancement of the magnetophoretic mobility of iron oxide nanocrystals

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Owing to their unique magnetic properties and low cytotoxicity values, iron oxide nanoparticles have drawn a lot of attention in the field of biomedicine, where they postulate as excellent candidates for magnetic guidance, drug delivery and as contrast agents for imaging.[1] In this regard, their potential applicability relies on the effective magnetic anisotropy, which ultimately determines the magnetic behavior of the nanostructure. Therefore, it becomes necessary to exert control over the anisotropy when designing iron oxide nanocrystals for a given bio-application, fact that can be assessed by reconsidering the synthetic approach.[2]
Herein, we report a one-pot synthesis of core-shell FeO@Fe_3O_4 nanoparticles by thermal decomposition in oleic acid. Given the high temperature of the reaction and the particular synthetic conditions, the attained nanostructures display an octopod-like geometry and are endorsed with both a high saturation magnetization and a low coercivity, rendering them optimal for magnetic guidance due to their enhanced electrophoretic mobility values.[3]

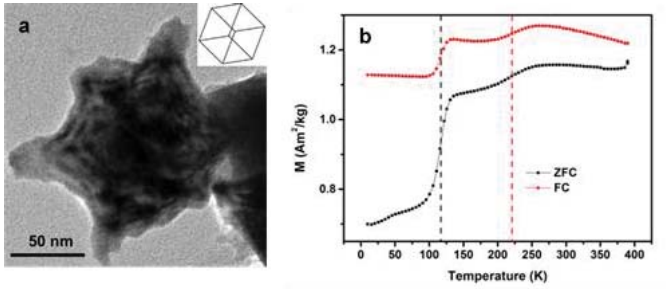

Figure 1. (**a**) HRTEM image of a single FeO@Fe_3O_4 nano-octopod and (**b**) temperature-dependent ZFC (black symbols) – FC (red symbols) (H = 5mT) magnetization curves of the as-synthesized iron oxide nanocrystals

1. Q. A. Pankhurst, J. Connolly, S. K. Jones, J. Dobson, *J. Phys. D: Appl. Phys.*, **2003**, *36*, 167-181
2. H. Khurshid, W. Li, S. Chandra, M-H. Phan, G. C. Hadjipanayis, P. Mukherjee, H. Srikanth, *Nanoscale*, **2013**, *5*, 7942-7952
3. M. Testa-Anta, S. Liébana-Viñas, B. Rivas-Murias, B. Rodríguez-González, M. Farle, V. Salgueiriño (in preparation, 2018)

Genotyping Platform Based on GoldMag Nanoparticles for Clinical Diagnostic Application

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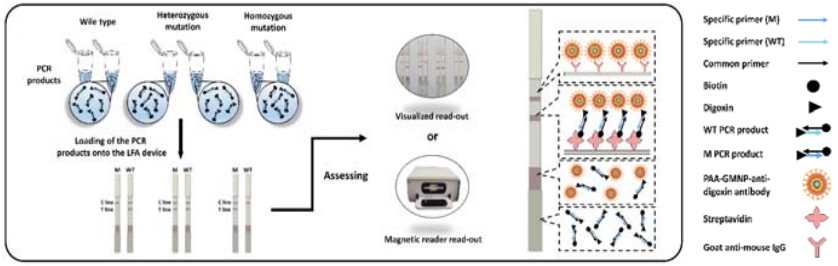
In clinical diagnosis, affordable on-site techniques for the detection of disease-associated biomarkers with high efficiency are urgently needed. However, current techniques often require expensive, sophisticated instruments that may not be available in laboratories with limited resources. Gold magnetic nanoparticles (GoldMag) are characterized by specific optical properties, a versatile surface chemistry and superparamagnetism. As a result, GoldMag-based lateral flow test systems distinguish themselves by their ease of use and their flexibility for various applications.

Here, we report an ARMS-LFA system that enables single-nucleotide polymorphism (SNP) genotyping via combining amplification-refractory mutation system (ARMS) with GoldMag-based lateral flow assay (LFA). Using GoldMag nanoparticles as carriers, a LFA device was constructed to detect SNP polymorphism in 5 minutes with PCR products. The detection sensitivity can reach 15 ng (genomic DNA). The final genotyping result can be provided by using a magnetic reader automatically and visual inspection of colors on the LFA strips quantificationally and qualitatively. We further directly amplify genomic DNA from whole blood without tedious DNA extraction and purification process. The genotyping result can be obtained within 90 min. Results of double-blind clinical trials indicated that ARMS-LFA system has a concordance rate up to 99% compared with DNA sequencing.

Currently, on our diagnostic platform, we have applied ARMS-LFA system for genotyping of multiple genetic disease-related SNPs, such as MTHFR C677T which associated with folate metabolism and risk of stroke and birth defects, CYP2C19 genotype on outcome of clopidogrel treatment, EGFR mutation which related to risk of many types of cancer, and multiple SNPs detection for phenylketonuria diagnostic. On the whole, ARMS-LFA is an easy, cost-effective, rapid method with high specificity, sensitivity and stability, which making it a valuable molecular diagnostic tool and useful in clinical practice.



Schematic diagram of ARMS-lateral flow assay (LFA) system.

Reference

1. A novel lateral flow assay based on GoldMag nanoparticles and its clinical applications for genotyping of MTHFR C677T polymorphisms. *Nanoscale*. 2016 Feb 14;8(6):3579-87.
2. Sensitive genotyping of mutations in the EGFR gene from NSCLC patients using PCR-GoldMag lateral flow device. *Sci Rep*. 2017 Aug 21;7(1):8346.
3. Multiple SNPs detection based on lateral flow assay for phenylketonuria diagnostic. *Anal Chem*. 2018 Mar 6;90(5):3430-3436.

Metamagnetic self-assembled FeRh nanoparticles

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Magnetic nanoparticles are intensely investigated in the realm of biomedicine, with prominent applications including targeted drug delivery, bioseparation, sensing, contrast enhancement in MRI, or hyperthermia [1]. The vast majority of magnetic nanoparticles used in practice are based on ferromagnetic compounds and mainly rely on their superparamagnetic behavior upon size reduction to avoid undesired effects such as agglomeration. These particles can then be remotely controlled via magnetic field gradients, or heated by means of high-frequency alternating magnetic fields for subsequent therapeutic applications.

Here we focus on the potential properties of another class of magnetic materials, namely those featuring metamagnetism. Specifically, the equiatomic FeRh-alloy presents a first-order phase transition *between antiferromagnetic and ferromagnetic states* just above room temperature (~360 K) [2]. The versatility to trigger and control the transition makes FeRh an interesting material platform where the magnetic order can be controlled not only by magnetic fields, but also via external agents such as temperature, electrical currents, hydrostatic pressure, or (pulsed) optical beams. Another remarkable property of FeRh consists in the extraordinary amount of latent heat released across the transition [3].

In this presentation, we explore the option to grow self-assembled FeRh nanoparticles based on the observation that metallic films deposited on oxide substrates often tend to grow in the shape of nanoislands as a way to minimize their surface energy. We have sputter deposited FeRh on MgO substrates using different temperature protocols, finding that deposition at elevated temperatures as well as during increasing temperature ramps causes FeRh films to segregate into sub-micron-sized islands. We have analyzed their size and morphology in connection to their metamagnetic behavior, identifying temperature protocols for which a large fraction of particles undergoes the phase transition slightly above room temperature. Moreover, we have studied the individual nanoparticle behavior across the thermally driven transition via magnetic force microscopy (Fig. 1). This allows us to examine interesting features of FeRh under spatial confinement, similar to the ones previously observed in nanostructures patterned from films [4, 5]. We further comment on the option to release these nanoparticles into solution, upon which we can envision numerous applications for theranostics, such as sensing and remote actuation via magnetic field independent approaches.

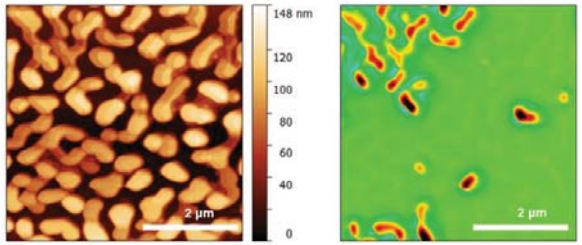

Fig. 1: Topography (left) and magnetic force microscopy (right) images of FeRh nanoparticles at room temperature over an area of 5 × 5 µm2.

[1] Q. A. Pankhurst *et al.*, *J. Phys. D: Appl. Phys.* **36**, R167 (2003)
[2] S. Maat, J.-U. Thiele, and E. E. Fullerton, *Phys. Rev. B* **72**, 214432 (2005)
[3] M. P. Annaorazov *et al.*, *J. Appl. Phys.* **79**, 1689 (1996)
[4] V. Uhlíř, J. A. Arregi, and E. E. Fullerton, *Nat. Commun.* **7**, 13113 (2016)
[5] J. A. Arregi *et al., J. Phys. D: Appl. Phys.* **51**, 105001 (2018)

NRL-coated magnetic Fe_3O_4 nanoparticle for MRI contrast enhancement

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Recent technology advances in nanotechnology, and imaging technology allow the application of nanomaterials for early cancer diagnosis and therapy. As early detection is prerequisite for being successful, magnetic resonance imaging (MRI) has become one of the most extensively used and powerful tools for noninvasive clinical diagnosis owing to its high degree of soft tissue contrast and spatial resolution. Magnetic nanoparticles generate either "positive" or "negative" contrast in MRI figure. Among the various MNPs under investigation, superparamagnetic iron oxide nanoparticles (SPIONs) have attracted considerable interest due to their excellent magnetic properties and biocompatibility. In this work, Iron oxide nanoparticles (Fe_3O_4) were synthesized by a simple co-precipitation method in mild temperature (90°C) using natural rubber latex (NRL) as capping agent for MRI contrast agent. Magnetic nanoparticles (MNPs) were prepared by mixing ferric and ferrous ions in highly basic solutions (NH_4OH) in different NRL volumes. The properties of the obtained MNPs were characterized using various techniques. TEM images showed that core size and size distribution of magnetic nanoparticles can be controlled by the NRL concentration. Two important functions that cis-isoprene molecules or proteins in NRL can have influence in the growth and passivation of the particles. They may act as a capping agent, thereby avoiding agglomeration of the nanoparticles, improving the particle size distribution and decreasing the particle size. The Zeta potential measurement of the NRL-coated and uncoated MNPs revealed higher colloidal stability for the NRL-coated MNPs. FTIR spectra suggested the presence of hydroxyl groups in the uncoated MNPs. Absence of hydroxyl groups in the NRL-coated samples suggests that the binding of MNPs to NRL molecules protects the magnetic cores from water, that is very important for biomedical applications. The presence of bands related to the cis-1,4-polyisoprene confirmed that the MNPs were successfully coated with NRL latex. Hall magnetometer measurements revealed that the iron oxide nanoparticles are superparamagnetic and that the NRL-coated magnetic nanoparticles have higher magnetization compared to the bare magnetic nanoparticles. We used these prepared samples as contrast agents in MRI. MRI images of bare and NRL-coated MNPs were done by a Philips Achieva 3.0T MRI scanner. MRI sequence for T_2 was performed using a 2D multi-spin echo, 8 echoes in steps of 14 ms per each 20 slices. For T_1 map, a single shot of one echo with a repetition time (TR) steps, 100 ms from 450 ms to 1050 ms and 200 ms from 1050 to 2050, total of 15 echoes was performed. The final image resulted in a FOV of 110 x 110 pixels, effective pixel size of 0.52 mm. Figure 1 shows a T_2 map of the first echo with different concentrations of MNPs (0.25, 0.5, 1, 2 and 3 mM) on gelatin. R_2 and R_1 rates were calculated by a MATLAB code**.** The relaxivity ratios (r_2/r_1) of MNPs were shown in Table 1. As it can be observed the ratio significantly decreases by increasing the NRL volumes in the synthesis from 100 µL to 800 µL. Therefore, it can be concluded that NRL-coated MNPs can enhance the image contrast in MRI.

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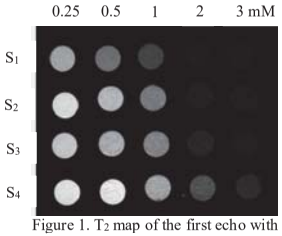

Figure 1. T_2 map of the first echo with different concentrations of MNPs

Table 1. Summary of Physical Properties of NRL-coated MNPs.

Samples	NRL (µL)	TEM size (nm)	Zeta potential (mV)	Ms (Am^2/Kg)	r_2/r_1 (s^{-1})
S_1	0	12±4	-4.4	80	2.6
S_2	100	13±2.8	+32.2	107	3.6
S_3	400	10.3±2.2	+33.4	93	2.3
S_4	800	7.9±1.5	+32	89	0.51

Development of continuous synthesis of iron oxide nanoparticles as magnetic carriers for biomedical and clinical applications

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The synthesis of effective single-core iron oxide nanoparticles intended for use in biomedical applications has attracted much interest over the last decades. Several synthetic routes to obtain particles of certain size, shape, crystallinity, dispersity and magnetic behavior were reported in the literature such as sol-gel synthesis, microemulsion, hydrothermal reactions, etc. Nevertheless, most of them are time-consuming, costly, partially rely on the use of organic solvents and offer only limited control over the particle characteristics. Therefore, the search for an efficient synthetic route that is simple, economical, environmentally friendly and rapid is one of the most challenging issues related to the synthesis of well-crystallized and size-controlled iron oxide nanoparticles. Herein, we report the development of an aqueous, micromixer based synthesis route, which enables, in a very short period of time, the production of large amounts of stable, reproducible and biocompatible single-core nanoparticles. Synthesized magnetic nanoparticles display a narrow size distribution and suitable surface chemistry for further functionalization. Cell tests also confirm their good biocompatibility as important prerequisite for the different biomedical and clinical applications

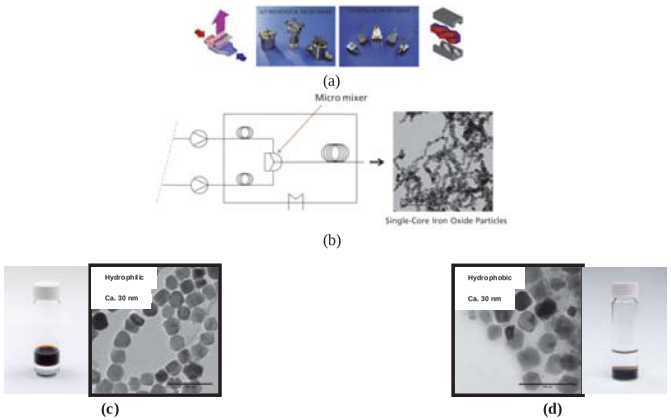

Figure (a) Different micro mixers and their mixing profiles: Left: Slit Interdigital Micro Mixer, Right: Caterpillar Micro Mixer (b) Schematic representation of continuous manufacturing of magnetic single-core iron oxide nanoparticles. (c) Photograph and TEM image of continuously synthesized nanoparticles in upper-water phase (hydrophilic) (d) in the lower-chloroform phase (hydrophobic)

Microstructural and magnetic properties of stable suspensions of $Co_xFe_{3-x}O_4$ magnetic nanoparticles for utilization in life sciences

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Like many magnetic oxides, the cobalt-ferrites caught the attention of scientists due to their controllable magnetic proprieties that make them convenient for tumor hyperthermia in experimental medicine. This work was focused on the influence of core chemical composition on the microstructural and magnetic properties of core/shell mixed iron and cobalt oxides nanoparticles. We presented a cost-effective one-step synthesis of $Co_xFe_{3-x}O_4$ (x = 0, 0.25, 0.5, 0.75, 1) magnetic cores, via chemical co-precipitation method that were further stabilized in water with organic acid shell, as designated to be used in various biomedical applications. As-prepared ferrites were characterized in detail by X-ray diffraction (XRD), transmission electron microscopy (TEM), vibrating sample magnetometry (VSM) and small angle neutron scattering (SANS) technique. For all five samples crystallinity and spinel structure were revealed by XRD. The influence of cobalt content was discussed in the case of magnetic properties based on VSM data. The nanometric size and quasi spherical shape corresponding to studied suspensions of particles was evidenced by TEM investigation that revealed also some rare larger particles or particle agglomeration. SANS data analysis was carried out to get complementary information on colloidal particle structure size, stabilizer layer thickness, the situation of larger particle or particle agglomeration in colloidal suspension. The experimental data approach was performed with adequate theoretical model that provided new data on particle interaction (interparticle potential, magnetic moment correlation, phase separation) and cluster formation (aggregation and chain formation). We concluded that the prepared cobalt ferrite sample array, with various cobalt content and consequently various magnetic properties could offer appropriate possibility of choosing suitable core composition for tumor hyperthermia in modern oncology.

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Structural studies of Co and Cu-doped ferrihydrite nanoparticles

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Ultrafine iron oxide, hydroxide, and oxyhydroxide powders are subjects of active interest of scientists for their potential applications in catalysis, waste recovery, water purifying, biomedicine, etc. [1]. Among the iron oxyhydroxides, special attention is given to metastable ferrihydrite, the properties of which are dependent on their composition, structure, and the preparation method [2]. Due to the unusual properties acquired during the transition from bulk to a nanodispersed state, the ferrihydrite particles can contend with nanoparticles of conventional ferromagnetic and ferrimagnetic materials used in different applications [3], including the targeted drug delivery, and the contrast matching in magnetic resonance imaging [4].

The present work reports the results of structual investigation of cobalt-and copper-doped ferrihydrite nanoparticles. Co- and Cu-doped synthetic ferrihydrite particles were prepared at room temperature by slowly adding NaOH alkali solution to iron nitrate Fe(NO3)3 and cobalt or copper salt solution at continuous intermixing to attain the neutral pH value [5].

The samples were investigated by means of small-angle neutron scattering (SANS), X-ray diffraction (XRD) and scanning electron microscopy (SEM) at the facilities in function at the Joint Institute of Nuclear Research Dubna. The results are analyzed and discussed in comparison with the magnetic properties reported earlier [5].

Acknowledgements. The work is supported by the Special Program of the Ministry of Education and Science of the Russian Federation for the Siberian Federal University and 2017-2018 RO-JINR projects (Theme 04-4-1121/ 2015-2020).

[1] A.-H. Lu, E. L. Salabas, and F. Schüth, Angew. Chem., Int. Ed. 46, 1222 (2007).
[2] S. V. Stolyar, O. A. Bayukov, Y. L. Gurevich, V. P. Ladygina, R. S. Iskhakov, and P. P. Pustoshilov, Inorg. Mater. 43 (6), 638 (2007).
[3]Yu. L. Raikher, V. I. Stepanov, S. V. Stolyar, V. P. Ladygina, D. A. Balaev, L. A. Ishchenko, M. Balasoiu, Phys. Solid State, 52(2), 277 (2010).
[4] E. V. Inzhevatkin, E. V. Morozov, E. D. Khilazheva, V. P. Ladygina, S. V. Stolyar, and O. V. Falaleev, Bull. Exp. Biol. Med. 158, 807 (2015).

Engineering of magnetic-based nanoplatforms for theranostic

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In the field of the synthesis and functionalization of inorganic nanoparticles (NPs) for biomedical applications, most researches aim at developing multifunctional theranostic NPs which can both identify disease states and deliver therapy and allow thus following the effect of therapy by imaging. One current challenge for iron oxide based NPs is the design of NPs able to combine in one nano-object both magnetic hyperthermia (MH) and MRI with the best efficiency in order to reduce the dose injected in the patient. IONPs are already commercially used as T_2 contrast agent for MRI. The use of MH as therapy for cancer is closer to be a reality thanks to the positive results achieved by the clinical trials carried out by *Magforce*^{TM}(Germany). Nonetheless, there is currently a need of improving NPs for MH.

On that basis, NPs with different shapes were prepared by controlled thermal decomposition of home-made iron stearate and the *in vitro* and *in vivo* MRI and MH experiments have allowed demonstrating their targeting potential, their biodistribution and their therapeutic properties. A start-up company is currently under construction. Starting from these promising NPs, multimodal imaging and other therapeutic functions have been added by:

- encapsulating them in carbon nanotubes or associating them with gold or carbon materials: they were so capable of absorbing and efficiently converting NIR light into heat to generate thermoablative temperatures and cell lysis. They can be used as contrast agents for X-Ray and/or MR image-guided therapy.
- coating them with some mesoporous silica displaying different pore sizes: such nano-objects are able to load large amount of drug which are released by an external stimuli such as pH, enzyme and of course temperature induced by magnetic hyperthermia. The obtained results open the possibility of using these systems as *theranostic* platforms thanks to the exhibited performance in hyperthermia, drug release and MRI.

Chem. Mater., 2014, 26 (18), 5252; *ACS Nano* 2014, 8, 11290; *Chemistry – An Asian Journal*, 2015, 10, 160; *ACS Nano*, 2015, 9, 10113; *Nanomedicine (Future Medicine) 2016,* 11, 1889; *Scientific reports*, 2017, 7, 40997; *Biochimica et Biophysica Acta,* 2017, 1861 (6), 1617; Adv. Funct. Mater, under press DOI: 10.1002/chem.201705845.

Metalloporphyrins-sensitized titania-silica-iron oxide nanocomposites with high photocatalytic and bactericidal activities under visible light irradiation

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Multifunctional photocatalyst system of meso-tetra(4-carboxyphenyl)porphyrin (TCPP) with different metal centers [Mn(III), Fe(III), Cu(II), Zn(II) and metal-free] adsorbed on titania-silica-iron oxide magnetic photocatalyst nanocomposites (TSI) that unite superparamagnetic behavior and high photoactive activity under visible light irradiation have been prepared. The superparamagnetic nanocomposites have been characterized and analyzed using UV-visible absorption spectroscopy, X-ray diffractometry (XRD), transmission electron microscopy (TEM), Fourier transform infrared spectroscopy (FT-IR), and X-ray photoelectron spectroscopy (XPS). The results showed that copper(II)tetra(4-carboxyphenyl)porphyrin - sensitized titania-silica-iron oxide nanocomposites (CuTCPP-TSI) can effectively degrade methylene blue (MB) and kill *Escherichia coli* (*E. coli*) under irradiation with an incandescent lamp. The magnetic photocatalysts could be used at least four times without regeneration. Therefore, the CuTCPP-TSI nanocomposites can be applied in catalytic photodegradation and used for magnetically-guided antibacterial application.

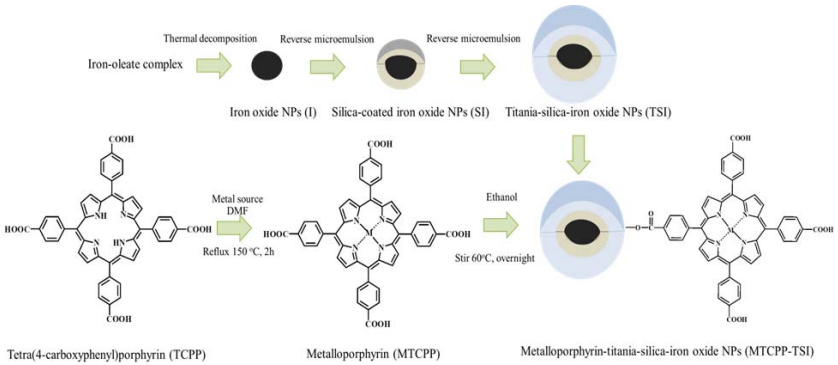

Figure1. Scheme for the synthesis of metalloporphyrin-sensitized titania-silica-iron oxide nanocomposites

Influence of sterilization and preservation procedures on the integrity of serum protein coated magnetic nanoparticles

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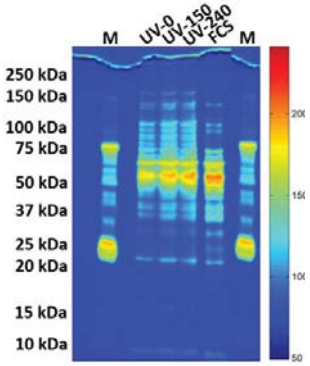
For a potential application of protein coated magnetic nanoparticles (MNP) in animals or humans, one has to guarantee that the particles are free from biological pathogens or any biological contamination. Additionally, beside this sterilization, it is important to bring the particles into a condition, which makes them storable for several weeks. For these purposes, several procedures like freezing, lyophilisation, autoclaving, and UV-irradiation were evaluated with regard to their effect on the composition of the protein coating.
To produce the protein coating, PEI-coated MNP were incubated at 37°C for 10 min in fetal calf serum (FCS), serving as natural protein source. Afterwards they have been magnetically washed with distilled water and possible aggregates have been dispersed by ultrasonication. Next, the protein-coated MNP were treated with one of the following procedures: freezing at -15°C, deep freezing at -80°C, lyophilisation (with and without PEG or TMAH as additives), autoclaving (121°C for 20 min), or UV-irradiation (λ ~ 200 - 280 nm for 150 to 240 min). Possible effects on the particles and their protein coating like degradation, agglomeration or cross-linking have been investigated by size measurement via dynamic light scattering (DLS). The zeta potential was used as an indicator for surface protein amount and composition. To determine the composition of the protein coating, a sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and a grey value intensity analysis by means of ImageJ was performed.



SDS-PAGE of original sample (UV-0) as well as samples exposed to UV-irradiation for 150 minutes (UV-150) or 240 minutes (UV-240).

After freezing and deep freezing and thawing the sample after two, four and six weeks, DLS shows a significant agglomeration for all storing times. The lyophilisation of protein coated MNP with PEG as additive and re-dispersion after one, three and six weeks leads to a similar particle size and particle size distribution compared to the original sample for all respective times. The TMAH-supplemented samples show instability and big agglomerates as well as a fraction of very small protein fragments. Autoclaving is not suitable at all, as it damages the integrity of the proteins of the protein corona. After UV-irradiation for 150 and 240 min a stable zeta potential of -30 mV as well as comparable results for SDS-PAGE show, that there are no major changes in composition and amount of the protein coating (see Figure). Thus, the exposure to UV radiation causes no relevant changes of protein content and composition.
In conclusion, only UV-irradiation and lyophilisation were suitable for sterilization and conservation of the protein coated MNP. Freezing and deep freezing lead to the degradation of large proteins and agglomeration. Thus, they are only suitable for short term storage.

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Extended LaMer Synthesis of Nonstoichiometric Ferrites with Enhanced Magnetic Properties for Magnetic Hyperthermia

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In magnetic field hyperthermia, the energy produced during this time period is related to both the frequency (number of cycles) and the field magnitude as each field flip can be seen as a minor hysteresis loop with the area of the loop being the energy released in a single magnetization event.[1] To maximize the area in these minor hysteresis loops, two main materials properties can be manipulated. The first being the effective anisotropy which is an intrinsic value based on the material composition and the second being particle volume. The ability to control and change effective anisotropy and particle volume present a unique opportunity to produce materials that can be optimized for maximum power output at a given field and frequency.

Recently, to improve upon the material properties of iron oxides, researchers have begun to consider nonstoichiometric ferrites. These complex materials show a wide range of magnetic properties, including tunable magnetic saturation, magnetocrystalline anisotropy, and blocking temperature. Coupled with recent advancements in synthesis, and increasing control over both size and morphology of nanoscale colloids, these new materials have been shown to exhibit properties that are greatly improved form those of Fe-ferrites. One of these select materials is cobalt ferrite ($CoFe_2O_4$), which we have recently demonstrated methodology to be produced via the extended LaMer method first described by Vreeland et al.[2-3] By maximizing the effective anisotropy through Co doping, as well as identifying optimal volume for energy release using a novel drip synthesis the optimization of non-stoichiometric ferrite based materials for application in MagMED is possible.

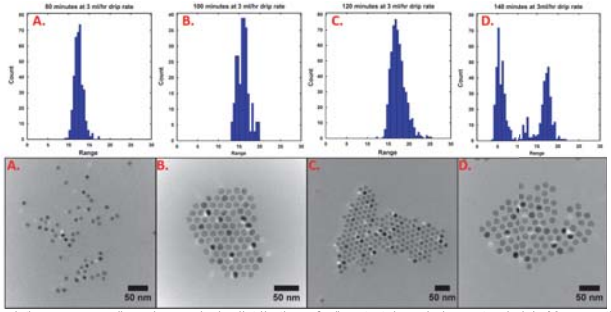

Figure 1. TEM and the corresponding size analysis distribution of aliquots taken during extended LaMer reaction synthesizing $Fe_{2.5}Co_{0.5}O_4$. A) 80 minutes diameter=12.4 nm, standard deviation=1.1 nm. B) 100 minutes, diameter= 16.0 nm, standard deviation= 1.4 nm. C) 120 minutes diameter= 17.4 nm, standard deviation= 1.8 nm. D) 140 minutes, diameter small= 6.54 nm, standard deviation small= 2.3 nm, diameter large= 17.4 nm, standard deviation large= 1.2 nm. All scale bars are 50 nm.

1. Carrey, J.; Mehdaoui, B.; Respaud, M., *Journal of Applied Physics* **2011,** *109* (8), 083921.
2. Fellows, B. D.; Sandler, S.; Livingston, J.; Fuller, K.; Nwandu, L.; Timmins, S.; Lantz, K. A.; Stefik, M.; Mefford, O. T., *IEEE Magnetics Letters* **2018,** *9*, 1-5.
3. Vreeland, E. C.; Watt, J.; Schober, G. B.; Hance, B. G.; Austin, M. J.; Price, A. D.; Fellows, B. D.; Monson, T. C.; Hudak, N. S.; Maldonado-Camargo, L.; Bohorquez, A. C.; Rinaldi, C.; Huber, D. L., *Chemistry of Materials* **2015,** *27* (17), 6059-6066.

Design and Synthesis of Magnetic Nanocomposites to Probe Sub-Cellular Behaviour

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All eukaryotic cells undergo mitosis, where cells duplicate and segregate their chromosomes, resulting in two identical cells. Segregation is dependent upon a self-assembled array of dynamic microtubules called the mitotic spindle. Chromosomes are attached to this *via* a structure known as a kinetochore. As microtubules grow and shrink, pulling and pushing forces are generated by the kinetochore in order to move chromosomes during mitosis. Experiments from the 1980s measured the stall force for chromosomes during anaphase. Recently, magnetic tweezers have displaced the position of the mitotic spindle in single celled nematode worm embryos, allowing some information about forces to be inferred, however this area, critical for understanding cell behaviour, remains poorly understood [1][2]. This research aims to address this, through the production and application of precision-engineered nanoparticles to human cell lines in conjunction with magnetic tweezer manipulation.

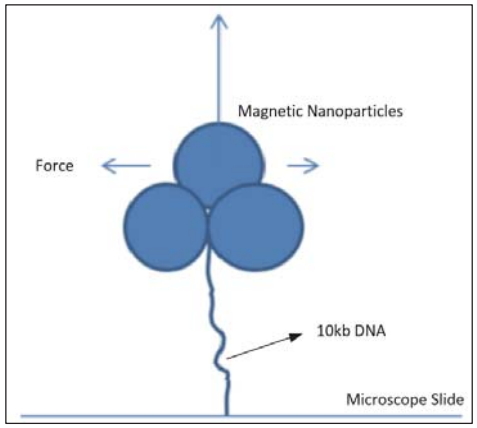

Figure 1 : Schematic illustration of DNA tethering assay explored to measure forces associated with chromosome movement during mitosis.

Superparamagnetic iron-oxide nanoparticles (SPIONs) exhibit size dependent magnetic properties. They have an established role in biomedicine and cell biology and are used as contrast agents for magnetic resonance imaging as well as for drug delivery, hypothermia treatment and as biosensors [4][5]. Here we develop magnetic nanoparticles which can be used in magnetic trapping methodologies to measure the forces involved in chromosome movement during mitosis using a multidisciplinary approach. Multifunctional superparamagnetic nanoparticles have been synthesised considering the final composite size, cytotoxicity, magnetic properties and surface functionality as parameters to optimise. Particles have been characterised using transmission electron microscopy, dynamic light scattering, zeta potential measurements, vibrating sample magnetometry (VSM), and infrared spectroscopy. Incorporation of these functional magnetic nanoparticles *in vivo* in human cell lines by both micro-injection and endocytosis is evaluated [3]. *In vitro* evaluation of the magnetic force of the nanoparticles in a biological system using a variation of a tethered particle motion (TPM) assay as seen in Figure 1. is considered. This work has enabled optimisation of the design of multifunctional particles and analysis of their subcellular behaviour towards magnetic tweezer and force studies.

References

[1] C. Garzon-Coral, H. Fantana and J. Howard, *Science*, 2016, 352, 1124-1127. [2] R. Nicklas, *Annual Review of Biophysics and Biomolecular Structure*, 1988, 17, 431-449. [3] W. Huang, G. Davies and J. Davis, *Chemistry - A European Journal*, 2013, 19, 17891-17898. [4] J. Mohapatra, A. Mitra, H. Tyagi, D. Bahadur and M. Aslam, *Nanoscale*, 2015, 7, 9174-9184. [5] E. Wang and A. Wang, *Integr. Biol.*, 2014, 6, 9-26.

Synthesis of ferrite nanoparticles for biomedical applications.

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Iron oxide-based nanoparticles (IONPs) have been extensively studied as contrast agents for magnetic resonance imaging (MRI) and heat mediators for magnetic hyperthermia cancer treatment. In this work we have evaluated the effect of doping iron oxide nanoparticles with different amounts of manganese in their potential use for these two biomedical applications.

We have synthesized $Mn_xFe_{3-x}O_4$ nanoparticles, by a thermal decomposition method,[1] changing systematically the Mn/Fe ratio of the metal precursors. In all cases, nanoparticles with average sizes between 6 and 9 nm were obtained. The so-obtained hydrophobic nanoparticles have been then transferred to water by surface modification with dimercaptosuccinic acid and dopamine. Thus, we have studied the influences of changes in the composition and the surface coating on physico-chemical properties like the blocking temperature, heating properties and relaxivity values.

Regarding their heating capacities, particles with the smallest amount of Mn show the lowest SAR values. Interestingly, increasing the Mn amount (up to x = 0.5) produces higher SAR values reaching levels similar to pure magnetite. It has been found that doping ferrites with different amounts of transition metals can serve to modulate their relaxivity values r_2 and r_1 [2,3] with a non-linear increase in r_2 and in the r_2/r_1 ratio if compared to magnetite. Some of the prepared materials show promising properties for their use in MRI and this work may serve to prepare highly efficient contrast agents in the future.

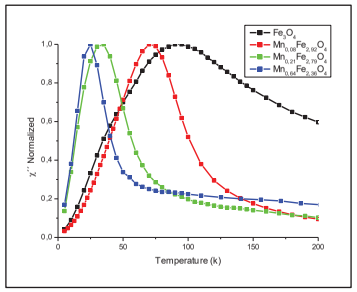

Figure 1 AC measurements changing the x value in the ferrite nanoparticles.

Table 1 Absorption rates values obtained for ferrite nanoparticles increasing Mn doping.

Stoichiometry	SAR (W/g_{Fe})	ILP ($nH \cdot m^2/kg_{Fe}$)
Fe_3O_4	238	0,72
$Fe_{2,92}Mn_{0,08}O_4$	70,1	0,21
$Fe_{2,79}Mn_{0,21}O_4$	84,1	0,25
$Fe_{2,36}Mn_{0,64}O_4$	143	0,43

[1] Sun, S. et al, "Size controlled synthesis of magnetite nanoparticles", J. Am. Chem. Soc., **124**, 8204 (2002).
[2] Jang, J. et al, "Critical enhancements of MRI contrast and hyperthermic effects by dopant-controlled magnetic nanoparticles ", Angew. Chem. Int. Ed., **48**, 1234 (2009).
[3] Yang, L. et al, "Composition Tunable Manganese Ferrite Nanoparticles for Optimized T2 Contrast Ability", *Chemistry of materials*, **29**, 3038 (2017).

NOVEL LINKERS FOR ADVANCED MAGNETIC MATERIALS TO BE USED IN MEDICAL THERANOSTICS

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Nanotechnology offers new pathways for the development of hybrid inorganic/organic materials with broad range of applications in medicine. Nanoparticulate materials, containing magnetic core, can be used as imaging agents for the cell and biomolecule labeling, contrast agents for MRI, or therapies as delivery or hyperthermia agents. Performance of these materials as imaging or therapeutic agents relies heavily on the organic shell which helps to stabilize them in colloidal form, alleviates toxicity, facilitates bioconjugation and provides the desired pharmacokinetic properties. The component of a hybrid material, which helps with its assembling, is the linker which is usually an organic molecule with several reactive functionalities and tunable geometry. One side of the linker molecule is responsible for the binding to inorganic core such as metal oxide, usually contains multiple oxygen, nitrogen or sulfur donor atoms. Another side of it has a reactive group responsible for bioconjugation with the targeting vector, drug or dye molecules.

Novel linkers based on 2-hydroxyisophthalic acid with demonstrated high affinity to inorganic surfaces and colloid stabilizing efficiency (Figs. 1 and 2) have been developed. The following acid derivatives with X = OH, NH_2, NO_2, N_2aryl, Br, OCH_2CO_2H, OC_2H_4OH, OC_3H_5, $OC_3H_5(OH)Cl$, $OC_3H_5(OH)_2$, $OC_3H_5(OH)NH_2$ and $OC_3H_5(OH)OC_3H_5$ have been synthesized and fully characterized. The linkers are redox stable, easily derivatized and offer an excellent linkage with various components, including biomolecules. HeLa cell viability assay on X = $OC_3H_5(OH)_2$ and $OC_3H_5(OH)OC_3H_5$ derivatives showed no toxicity.

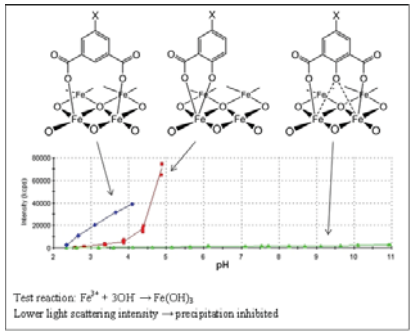

Fig.1

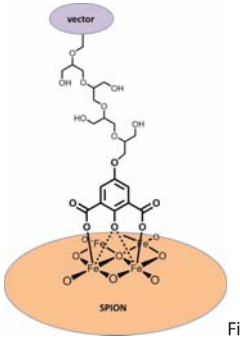

Fig.2

References: 1. Kolesnichenko, Vladimir; Goloverda, Galina; Komati, Rajesh. Preparation of 2-hydroxyisophthalic acid and derivatives thereof. PCT Int. Appl. (2017), WO 2017222921 A1 20171228.

Synthesis and characterization of magnetite with a biopolymer and gold nanoparticles for cancer treatment by hyperthermia.

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The development of nanoparticles (NPs) for cancer detection and therapy has shown great progress in the last two decades. NPs have been used as enhancement contrast agents for imaging, drug delivery and as therapeutic components in the promotion of tumor cell death in magnetic and photonic ablation therapies. Iron oxide NPs with nanocrystalline magnetite nuclei (Fe_3O_4) have great potential for use in oncological medicine due to their biocompatibility, biodegradability along with the ease of adjusting and functionalizing them.

In this work, we present the synthesis of NPs of magnetite with the polymeric shell in a single step carried out by the co-precipitation method in a temperature range of 50-80°C, besides the addition of gold nanoparticles to the magnetite with the polymeric enveloped by the method of nucleation. For the characterization of the NPs FTIR was used to determine the integration of the magnetite with the polymer , observing an intense band at approximately 600 cm^{-1} which indicates the union of the magnetite nanoparticles and Scanning Electron Microscopy (SEM) to know the morphology.

By the obtaining of magnetite nanoparticles in a size range of 50 nm to 100 nm, we suggest that these NPs have the potential to be applied in the treatment of tumor destruction by hyperthermia and signal amplification of Raman spectroscopy by SERS technique.

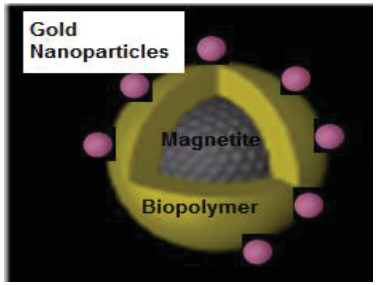

Figure 1. Schematic illustration polymer coating and functionalized with gold nanoparticles.

Properties of Superparamagnetic $ZnFe_2O_4$ Nanoparticles Synthesized by Hydrothermal Method

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Superparamagnetic zinc ferrite ($ZnFe_2O_4$) nanoparticles have found a high potential for use in biological applications. There are a lot of studies related to potential applications of $ZnFe_2O_4$ nanoparticles such as; MRI contrast agents, drug delivery and magnetic hyperthermia [1-3].

$ZnFe_2O_4$ nanoparticles were successfully synthesized by hydrothermal method at 110^0C. The effect of synthesis time changed from 2 hours to 24 hours on the structural and magnetic properties of $ZnFe_2O_4$ nanoparticles was investigated. The crystal structure and particle diameter were determined by X-ray Diffraction peaks. And, the peak between 395-404 cm^{-1} can be assigned to the Zn–O bond, while the peak around 554 cm^{-1} is related to the Fe–O bond in the Fourier Transmission Infrared Spectroscopy spectrums. Particle diameter distributions were also confirmed using Transmission Electron Microscope images. Figure 1a shows a good magnetic response of the nanoparticles. Further magnetic measurements by a Vibrating Sample Magnetometer exhibited that all samples were superparamagnetic (see Figure 1b). In Figure 1c, as the duration of hydrothermal process increased, the maximum magnetization of the nanoparticles is first increased and then stabilized at 30.8 emu/g, which were synthesized through hydrothermal method for 24 hours at 110 °C.

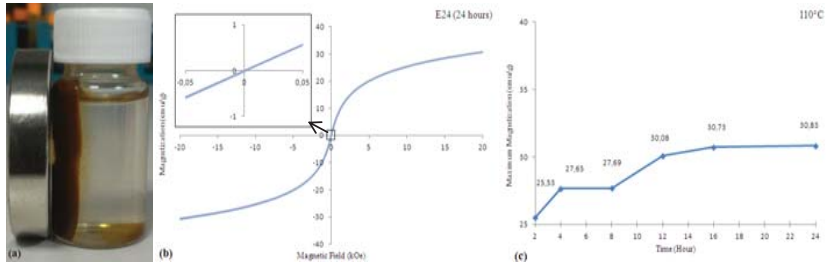

Figure 1. (a) A good magnetic response of $ZnFe_2O_4$ nanoparticles. (b) Magnetization loops for 24 hours at 110^oC (Inset shows the superparamagnetic curve at ±50 Oe). (c) The maximum magnetization versus synthesis time.

References

[1] S. Manjura Hoque , C. Srivastava , N. Venkatesha , P.S. Anil Kumar & K. Chattopadhyay, Philosophical Magazine 93 (14) (2013) 1771–1783

[2] V.J. Sawant, S.R.Bamane, R.V.Shejwal, S.B.Patil, Journal of Magnetism and Magnetic Materials 417(2016) 222–229

[3] O.V. Yelenich, S.O. Solopan, T.V. Kolodiazhnyi, V.V. Dzyublyuk, A.I. Tovstolytkin, A.G. Belous, Materials Chemistry and Physics 146 (2014) 129-135

Synthesis and characterization of anisotropic magnetic nanoparticles for incorporation into thermoresponsive hydrogels

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The subject of this work is to create multi-stimuli responsive hydrogels. The first stimulus is the temperature. Therefore we are using a thermoresponsive polymer, namely poly-N-Isopropylacrylamide (pNIPAM), with the ability to undergo a coil-to-globule transition at a critical temperature of about 34°C, the so called lower critical solution temperature (LCST). Below the LCST the pNIPAM chains are hydrophilic and elongated. Once the temperature rises above the LCST the chains collapse and form globules in aqueous solution due to their now hydrophobic character.[1]

The second stimulus is provided by anisotropic magnetic particles. Anisotropic magnetic nanoparticles present new possibilities compared to isotropic particles. They offer heightened coercivity field strength compared to isotropic particles and align to an applied field.[2] Loaded with magnetic particles the hydrogel is responsive to two different stimuli, temperature and applied magnetic fields. The gel responds to a magnetic stimulus in two ways: First by aligning the particles and second the particles are able to emanate heat when brought into an alternating current field inducing the temperature transition in the polymer network.

The magnetic particles are synthesized mostly by reduction in a hydrothermal reactor. This approach has the advantage of an aqueous system with controlled temperature and pressure. The synthesis happens in a two-step process that starts with the synthesis of an iron(III) oxide-hydroxide precursor species which is partly reduced in the second step to magnetite.[3] The resulting magnetite particles are shaped like depicted in figure 1. The reaction mixture stays in the hydrothermal reactor at 160 °C for 24 hours. Kinetic studies allowed us to determine the phase and shape evolution of the particles during the hydrothermal process. We observed that the phase changes during the reaction from an initial goethite phase to the final magnetite phase. The transition into magnetite is completed within the first 10 hours reaction time, after this time there are no traces of phase impurities visible in the XRD pattern. The changes in particle shape on the other hand occur during the entire reaction time. In the beginning there is a huge number of rods, typically observed in samples while goethite is still present. Later in the reaction timeframe the amount of rod-like shapes declines, some large particles made up of magnetite persist but are decomposed to form rhombohedral and cube-like magnetite particles with a diameter of about 22 ± 6 nm. During the stabilization process, larger particles precipitate naturally which provides a homogenous gel.

The presented system provides unique properties, tunable by temperature and applied magnetic fields.

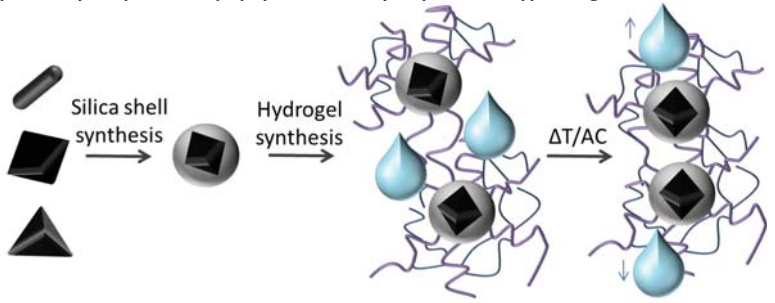

Figure 1: General reaction scheme from the synthesis of anisotropic magnetic particles (left), the coating with silica (second from left) to the transition at the lower critical solution temperature (right). The magnetic particles in different shapes are depicted in black, the surrounding silica shell is shown in grey and the polymer network consists of pNIPAM in purple and a crosslinker in dark blue.

[1] Heskins M. et al. *J. Macromol. Sci. A* 2:8 **(1968)**: 1441-1455
[2] Nemati Z. et al. *J.Alloy. Compd.*714 **(2017)**: 709-714
[3] Hinrichs S. et al *J Magn. Magn. Mater.*431 **(2017)**: 237-240

NOCANTHER

Design, development, optimization and scaling of MNPs for cancer therapy

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Abstract: BIOPRAXIS faces drug development for rare cancers, including glioma and pancreatic cancer, using nanotechnology and biological molecules like proteins and peptides. Moreover, is also working in controlled release systems through nanoparticles (Solid Lipid Nanoparticles, Magnetic Nanoparticles, Lipid Nanovesicles), which improve therapeutic action of APIS and allow protection of biological molecules and targeting, imaging and future theragnostic of different diseases including cancer. BIOPRAXIS and some European Entities have worked previously in this area in protects as THERAGLIO project (Contract n FP-602923), MULTIFUN a finished FP7 project and HEATDELIVER: Heat and and Drug Delivery nanosystem with active tumortargeting features (Eurotransbio project).

Based on the knowledge obtained, project **NoCanTher** (H2020-685795) aims at translating one of these nanoformulations to early clinical development for pancreatic cancer. The therapy is based on iron oxide nanoparticles with the effect of hyperthermia generated by an external alternate magnetic field. To successfully reach this objective, we will concentrate our efforts in two main group of activities:

• Nanomedicine up-scaling under GMP conditions: NoCanTher will scale up the manufacturing of the proposed nanoformulation, which is currently being optimized, from milligram-scale laboratory synthesis up to multigram-scale production to generate sufficient material for clinical and regulatory assays. To this aim, a GMP production line will be optimized and the relevant quality control will be conducted at the different stages of the up-scaling process.

• Clinical trial: NoCanTher will include late preclinical parameter testing to raise a clinical treatment protocol, regulatory assays, as well as the design of the clinical trial and the preparation of the Investigational Medicinal Product Dossier (IMPD). This strategy will allow us to apply for Clinical Trial Authorisation (CTA) then, we will carry out a Phase I clinical trial. NoCanTher involves the participation of institutions from three different sectors (academia, industry, clinical) and from five different countries (Ireland, France, Germany, Spain and the UK).

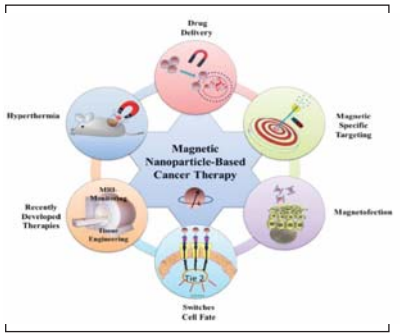

Figure 1: Figure illustrating the process.

References:

[1] Aires Antonio, et al. Multifunctionalized iron oxide nanoparticles for selective drug delivery to CD44-positive cancer cells. Nanotechnology 27 (2016) 065103 (10pp)
[2] Kossatz Susanne, et al. Efficient treatment of breast cancer xenografts with multifunctionalized iron oxide nanoparticles combining magnetic hyperthermia and anti-cancer drug delivery. Breast Cancer Research (2015) 17:66
[3] Salas Gorka, et al. Controlled synthesis of uniform magnetite nanocrystals with high-quality properties for biomedical applications. J. Mater. Chem., 2012, 22, 21065
[4] Kossatz Susanne, et al. High Therapeutic Efficiency of Magnetic Hyperthermia in Xenograft Models Achieved with Moderate Temperature Dosages in the Tumor Area. Pharm Res (2014) 31:3274-3288.

Pd-Fe Nanoparticles: Correlation between Magnetic Behaviour and Structural Composition

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The application of Palladium-Iron alloys on diverse areas, such as phase transformation, chemical catalysis, shape memory alloys or biomedicine has yield the preparation of $Pd_{1-x}Fe_x$ bulk alloys of different compositions. In the case of Pd-rich Fe bulk alloys, the most remarkable magnetic property is the onset of giant magnetic moments at Fe sites if the magnetization is thought as originated only by the Fe atoms. Nevertheless, the impact of the finite size effects, short range order, the composition or the internal magnetic coupling of particles over the properties is still controversial. In this work, we present three samples of Palladium rich Iron nanoparticles, representative of different synthesis conditions, to correlate their magnetic behaviour with the structural properties and chemical homogeneity of the inorganic cores.

PdFe nanoparticles have been prepared by a synthetic route based on the reduction and thermal decomposition of metallic precursors in the presence of surfactants. The influence of the temperature, heating rate, type of capping ligands and molar ratio of metallic precursors play a crucial role on the magnetic properties of the resulting PdFe FCC nanoparticles. Electron Magnetic Resonance technique has been employed in addition to the standard magnetization measurements as a function of field and temperature, to obtain a more direct insight on the magnetic coupling and relaxation phenomena characteristic of these bimetallic nanoparticles. EMR also will help determining the role of the hypothetical Palladium spin polarization in the magnetic properties of these nanoalloys and to identify different magnetic contribution.

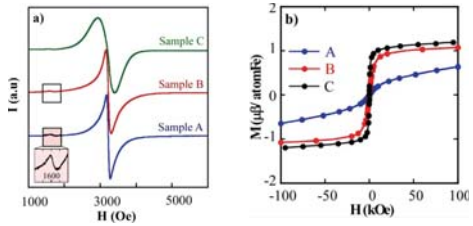

Figure 1. (a) EMR spectra of samples A, B and C in colloidal dispersion at room temperature and (b) Hysteresis loops of powder samples A, B and C at room temperature.

Properties of Superparamagnetic Cobalt Ferrite Nanoparticles

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Magnetic nanoparticles have recently attracted quite interest and became the subject of the investigations. Different properties of the nanoparticles compared to their bulk counterparts make them suitable for many applications. Especially superparamagnetic nanoparticles have a potential to be used for biomedical applications. Cobalt ferrite ($CoFe_2O_4$) is an encouraging material with high specific absorption rates (SAR) for magnetic hyperthermia and high relaxation time ratios for magnetic resonance imaging (MRI) [1]. The applications of $CoFe_2O_4$ nanoparticles strongly depend on their magnetic characteristics.

In this study superparamagnetic cobalt ferrite nanoparticles were synthesized by co-precipitation and the effects of synthesis parameters on the properties of the nanoparticles were investigated. The structural analysis of the nanoparticles done by X-ray diffraction technique (XRD) and Fourier transform infrared spectroscopy (FTIR) indicated that the samples are $CoFe_2O_4$. Particle sizes were determined from XRD patterns and transmission electron microscope (TEM) images. Magnetic properties of the nanoparticles were measured with a vibrating sample magnetometer. Magnetic particle sizes were also calculated by using magnetic data. It was observed that the saturation magnetization, M_s of $CoFe_2O_4$ nanoparticles increased from 12.7 emu/g to 35.5 emu/g as the reaction time increased. Further increase was also seen with the decrease of stirring rate, and maximum M_s was obtained as 41.0 emu/g for superparamagnetic $CoFe_2O_4$ nanoparticles. $CoFe_2O_4$ nanoparticles with higher M_s values showed coercivity. The critical superparamagnetic size limit of $CoFe_2O_4$ nanoparticles was found to be around 8 nm. The figure shows the magnetization curve, TEM image and FTIR spectrum of the sample with the M_s=35.5 emu/g. In the FTIR spectrum, the peak at 590 cm^{-1} can be assigned to Fe-O bond and the peak around 400 cm^{-1} is related to Co-O bond. Particle sizes of the sample calculated using TEM image, XRD pattern and magnetic data are 5.5±2.3 nm, 7.3 nm and 6.4±0.7 nm, respectively.

For further work, to obtain biocompatible $CoFe_2O_4$ nanoparticles, the core ($CoFe_2O_4$ nanoparticles) is going to be coated with biocompatible polymers and the uptake and cytotoxicity effects are going to be investigated.

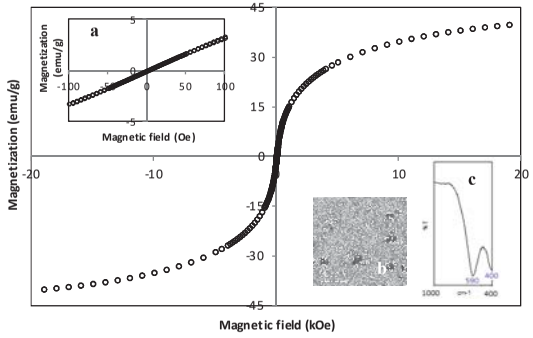

Figure: Magnetization curve of the sample with M_s=35.5 emu/g. Insets show a) Magnetization curve (at ±100 Oe), b) TEM image, c) FTIR spectrum of the sample.

References

[1] Amiri, S., Shokrollahi, H., Materials Science and Engineering, C 33, 1-8 (2013).

Chitosan stabilized iron oxide nanoparticles for MRI

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The rapid development of such fields of science as nanotechnology, molecular biology, and imaging techniques offers wide opportunities for the application of nanoparticles in medicine. In particular, it concerns early detection of cancer, which is a prerequisite for successful treatment. Among a large number of existing nanomaterials, iron oxide nanoparticles occupy a special place. It is because of their unique properties, involving low toxicity, and specific magnetic properties. These nanoparticles are therefore suitable for a lot of applications such as contrast agents for magnetic resonance imaging (MRI) and for targeted drug delivery. Iron oxide nanoparticles are usually coated with natural or synthesized polymer that provides the stability of the suspension. In our work, we have focused on the preparation of magnetite nanoparticles by the coprecipitation method, where particles were stabilized by chitosan coating. Since it is known that magnetic iron oxide nanoparticles have a large magnetic moment, which primarily shortens transverse relaxation time, we have tried to enhance T_2 shortening (to increase the contrast effect) by magnetite stabilization process using chitosan. The prepared samples have been subsequently characterized in terms of particle size, zeta potential, saturation magnetization (M_s), and T_1 and T_2 relaxation times. The obtained data showed that the hydrodynamic diameter is equal to 136 nm, and the zeta potential to 48 mV. For MRI analysis the relaxation rate (R) and relaxivity (r) have been calculated: r_1 = 0.713 $mM^{-1}s^{-1}$, r_2 = 238.16 $mM^{-1}s^{-1}$, and r_2^* = 276.1 $mM^{-1}s^{-1}$ (Fig.1). An acquired high r_2/r_1 ratio (334) indicates that the prepared nanoparticles have significantly prevailing effect on the transversal relaxation time (T_2) in comparison to longitudinal relaxation time T_1. These results demonstrate the potential usefulness of our nanoparticles as contrast agent for MRI.

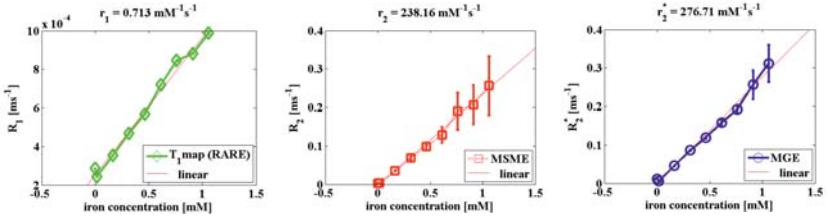

Fig.1 The relaxation rate (R) and relaxivity (r) values of chitosan coated magnetite nanoparticles.

Acknowledgements This work was supported by Slovak Research and Development Agency under the contracts No. APVV-14-0120 and APVV-14-0932, and by the "Biomedical Center Martin" project (ITMS code: 26220220187, project co-financed from EU sources).

WORKING OUT THE METHOD TO OBTAIN MONODISPERSE SMALL-SIZE RANGE MAGNETITE NANOPARTICLES FOR BIOMEDICAL PURPOSES

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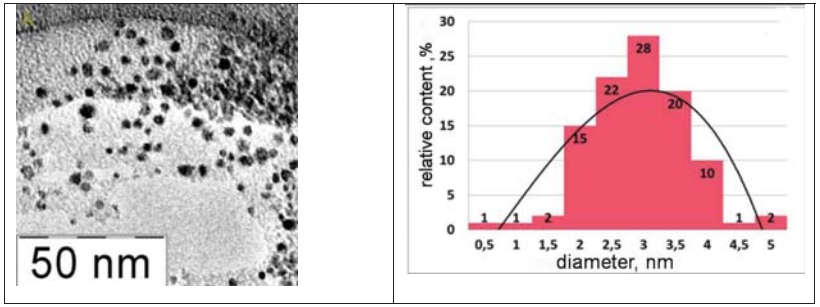
The work presents the results of working out the technology to obtain monodisperse small-size range magnetite nanoparticles by thermal decomposing of iron (III) acetylacetonate in an excess of triethylene glycol for biomedical purposes. The size and structure of the particles were studied by transmission electron microscopy method. The chemical content of particles was confirmed by electronic diffraction method by comparing with magnetite standard sample. Depending on the temperature (250°C - 290°C) and process duration (30 min-10 h) we have obtained magnetite monodisperse nanoparticles sized: 2.94±0.75 nm, 4.54±0.8 nm, 5.97±0.92 nm, 5.53±0.94 nm. It has been established that the size of the synthesized nanopartices depend both on the temperature and duration of reaction. The methods of nanoparticles magnetic separation from their suspension on Sm-Co magnet and resuspending of the particles in water were developed.

Electronic microphotographs of the magnetite nanoparticles sized 2.94 nm synthesized at 250°C during 1 h and histograms of their distribution by sizes are represented on the figures.



It has been established figure LD50 by investigating acute toxicity of the obtained nanoparticles by intravenously injection to mice with Spirmen-Karber method. Considering the low toxicity of the obtained magnetite nanoparticles they can be perspective for biomediical purposes, in particular, as magnetic carriers of anti-cancer preparations and as MRT-contrasting agents of organs and tissues. At present the method of obtaining magnitoliposom with incorporated anti-cancer compounds on the basis of magnetite nanoparticles synthesized by thermal decomposing of iron (III) acetylacetonate is being worked out.

SYNTHESIS AND FUNCTIONALIZATION OF ROD-LIKE IRON OXIDE NANOPARTICLES

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Abstract

Magnetic iron oxide nanoparticles have been recognized for use in various promising biomedical applications, such as detection of biological molecules, contrast agents in magnetic resonance imaging (MRI), vectors in drug delivery and mediators to convert electromagnetic energy to heat (hyperthermia). We reproduce a simple two-step reaction strategy for the synthesis of uniform magnetic iron oxide nanorods with ~50 nm in length and ~5 nm in diameter (Figure 1) and their colloidal stabilization with three different polymers (bisphosphonate polyoxyethylene - Optima 100, polymethacrylate polyoxyethylene - PCP45 and polyacrylic acid sodium salt - PAA) in water. Two-step reaction consists on synthesis of akaganeite followed by its transformation by reduction using hydrazine in microwave to obtain magnetic iron oxide nanorods [1]. The nanorods present the saturation magnetization value of ~64 kA/m and residual magnetization of ~15 kA/m, thus this material has ferro- or ferrimagnetic behavior. To estimate the iron oxide composition we use the technique of Mössbauer spectroscopy and a mixture of maghemite (strongly magnetic phase) with a quasi-amorphous intermediate phase (weakly magnetic phase) was detected, explaining a relatively low magnetization saturation. The suspensions of MNPs were probed by dynamic light scattering (DLS) and the distribution curve provides the Z-average hydrodynamic diameter equal 70±5 nm for Optima 100, 82±8 nm for PCP45 and 99±8 nm for PAA. We also study the effect of the polymer concentration and of the solution pH on the suspension stability.

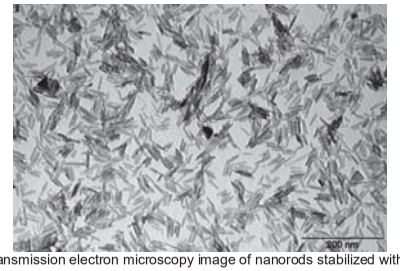

Figure 1: Transmission electron microscopy image of nanorods stabilized with Optima100

[1] Milosevic, I., Jouni, H., David, C., Warmont, F., Bonnin, D., and Motte, L. Facile microwave process in water for the fabrication of magnetic nanorods. J. Phys. Chem. C 115, 18999-19004 (2011).

Morphological control of Hematite magnetic nano-carries using chelating agents

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The intensive use of nanostructured materials as magnetic liquids for drug delivery has driven systematic studies to tailor their structural and morphological properties for specific physical and/or chemical properties. To this end great interest is focused in controlling the growth habit of α-Fe_2O_3. For this purpose, chemical methods based on adding chelating agents are widely applied. Using this approach, we report on the anisotropic growth of α-Fe_2O_3 nanoslabs produced by co-precipitation method with the addition of sucrose characterized using X-ray diffraction (XRD), high-resolution transmission electronic microscopy (HRTEM) and magnetization measurements as a function of temperature and applied magnetic field.

The resulting HRTEM images of samples prepared with 10 mmol/l of sucrose consist of faceted-like nanoslabs, while those from samples prepared without sucrose exhibits particles with a non-uniform shape. This observation is further confirmed by the XRD patterns, allowing us to conclude that the sample prepared with the chelating agent (sucrose in this case) shows clearly a preferential growth of the [110] crystallographic direction. To strengthen our assumption, we turn to the *T*- and field-dependence magnetization data, which are consistent with a superparamagnetic behavior. Besides, the fit of the ZFC-FC curves (zero field cooling and field cooling) for the samples grown with 10 mmol/l of sucrose presents a strong increase of the effective anisotropy constant, K_{eff}. This observation arises from magnetic anisotropy and suggests a wide range of potential applications for these samples e.g., in cancer therapies and medical diagnosis.

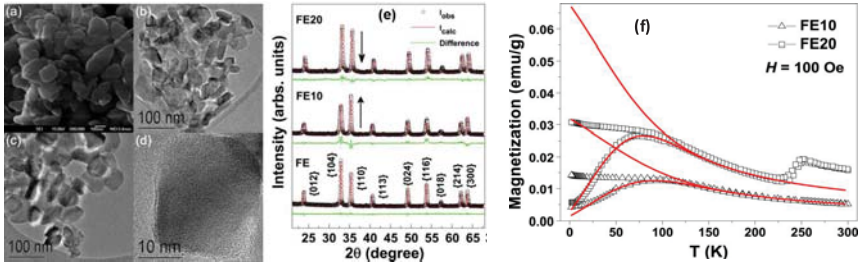

Fig. 1. (a) SEM image for FE sample and TEM and HRTEM images, respectively, for FE10 (b) and for FE20 (c and d) samples. (e) XRD patterns for Fe_2O_3 nanostructures grown with 0, 10 and 20 mmol/l of sucrose taken at room temperature[1]. (f) ZFC-FC magnetization curves measured with H = 100 Oe for FE10 and FE20. Solid lines are the best fits.

[1] Jesus, J. R., Lima, R.J.S. et al. "Anisotropic growth of α-Fe2O3 nanostructures". Ceramics International 44 (2018) 3585–3589

Targeting of carbonic anhydrase IX-positive cancer cells by glycine coated superparamagnetic nanoparticles

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In our work we describe preparation, characterization and biological activity of magnetite nanoparticles functionalized with biocompatible glycine (Gly). This amino acid was chosen as a molecule with carboxylate groups, which adsorbed onto the iron oxide surface, and NH_2 groups on surface of Fe_3O_4 could interact with immunoglobulin molecule – the antibody (Ab) which specifically recognizes carbonic anhydrase IX (CA IX). CA IX is a cell surface protein frequently present in human tumors but not in the corresponding normal tissues. This almost exclusive expression on cancer cell surface makes CA IX a suitable target for anticancer treatment.

The characterization of MNPs with bound glycine (Gly-MNPs) was detected by X-ray photoelectron and Fourier Transform Infrared (FTIR) spectroscopies. The optimal Gly/Fe_3O_4 weight ratio for the MNPs modification of 5 was confirmed by UV/Vis spectroscopy, DLS and TGA techniques. The average hydrodynamic size of Gly-MNPs particles determined by DLS was in range from 30 to 50 nm in dependence of Gly loading. SEM image showed near-spherical shape of the nanoparticles (Fig.1a) and TEM confirmed MNPs diameter in the range of 8-10 nm. Measuring magnetic properties of naked and functionalized magnetic nanoparticles using VSM showed superparamagnetism. MNPs with the optimal Gly content were successfully conjugated with monoclonal antibody VII/20 directed to the conformational epitope localized in the catalytic domain of CA IX. Moreover, this Ab can trigger the Ab-mediated endocytosis. Immunofluorescence assay demonstrated that the Ab-conjugated MNPs, i.e. Gly-MNPs, bound specifically to the CA IX antigen localized on the cell surface of the transfected B16 CA IX cells. Moreover, the Ab-Gly-MNPs were capable of efficient internalization as demonstrated by the dotted intracellular immunofluorescence signal (Fig.1b) but did not bind to B16 CA IX negative cells (Fig.1c). Cell viability was measured by the CellTiter-Blue method to estimate the cytotoxic or cytostatic properties of the unconjugated MNPs and VII/20 Ab-conjugated nanoparticles Gly-MNPs. Specific binding and penetration of monoclonal Ab conjugated nanoparticles into multicellular 3D colorectal carcinoma HT29 spheroids have been studied by immunohistochemistry.

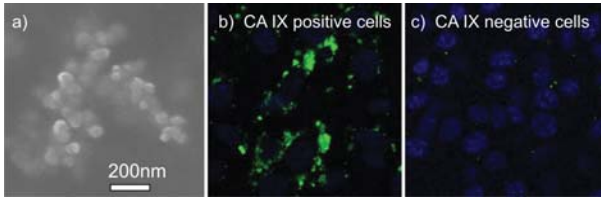

Fig. 1 SEM image of Gly functionalized MNPs (a). Analysis of the Ab-Gly-MNPs for their specific binding to CA IX and their capacity to internalize. B16 CA IX cells grown on glass coverslips were incubated with Ab-Gly-MNPs (100 μg/ml) at 37°C for 1 h to allow for internalization, washed and fixed with methanol (b). Ab-Gly-MNPs did not bind to B16 CA IX negative cells, which were used as a control (c). After incubation with the anti-mouse Alexa Fluor 488 antibody for 1 h at 37°C and washing, the cells were analyzed by confocal laser-scanning microscope Zeiss LSM 510 Meta.

Acknowledgements This work was supported by Slovak Research and Development Agency under the contracts No. APVV-14-0120 and APVV-14-0932 and by the Slovak Scientific Grant Agency projects VEGA 2/0108/16, VEGA 2/0064/18, VEGA 2/0016/17, VEGA 2/0141/16 and VEGA 2/0133/16.

Magnetic Hydrogels and the Exploitation of the Silica Shell: A Novel Approach to Tune Magnetic Hydrogels

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The creation of composite materials consisting of organic soft matter and anorganic hard matter is an extensive field in material science. In our working group we combine hard magnetic cobaltous ferrite with a crosslinked gel matrix consisting of the thermoresponsive poly(*N*-isopropylacrylamide). This composite material is soluble in water and reacts to temperature change with a rapid collapse of the polymer chains, turning the solution turbid. While the gel is collapsed it is still stable in solution due to the charged residues of the polymerization initiator. The magnetic particles have only little effect on the mechanical properties of the polymer solution or the solid polymer when crosslinked respectively. The introduction of the ferric particles enables us to abuse the magnetic properties to influence the mechanical properties of the soft gel. When synthesizing these composite materials the general strategy is to coat the particles with a passive silica shell using the Stoeber process to prevent the particles from agglomerating in solution. The silica shell is then functionalized with a methacrylate to conduct a grafting from radical polymerization in the next step. The finished so-called microgels can optionally be crosslinked to form a solid macrogel. Both are able to react to temperature change with a rapid switch from hydrophilic to hydrophobic. While these effects are a very fascinating topic in itself this project focusses on the manipulation of the silica shell.
Since the silica shell is usually only utilized as a stabilizer prior to the polymerization process there is no inherent reason as to why a post synthetic modification should not be considered. In this project we present a post synthetic route that dissolves the silica shell after the polymerization process. In theory the particles should be more susceptible to the application of an alternating current magnetic field due to their increased mobility, offering the opportunity to conduct hyperthermia. First experiments show that the basic structure of the gel stays intact after the etching process while the swelling behavior changes. The gel is still stable in solution after the process and retains its thermoresponsive characteristics. First experiments insinuate a faster heating rate of the etched gels compared to the corresponding gel prior to etching. Further studies will be systematically conducted to consolidate the first observations.

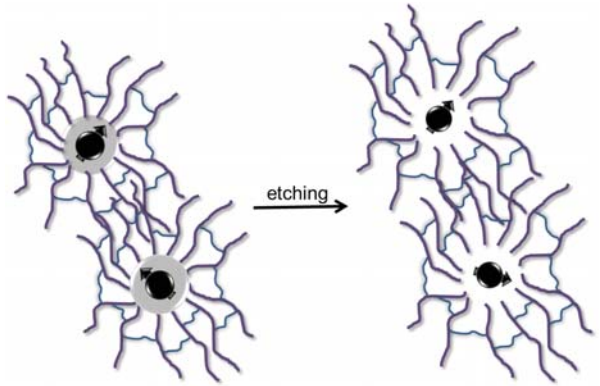

Figure 1: Schematic of a thermoresponsive microgel with embedded cobaltous ferrite particles with a stabilizing silica shell prior to (left) and after postsynthetic etching of the shell (right). The magnetic moment of the particles is depicted as black arrows which are randomly aligned without an external magnetic field. The gel is slightly more swollen after etching and the particles can align themselves to an external magnetic field without mechanical stress on the gel matrix.

[1] Stoeber, W. *et al. J Colloid Interf Sci* **(1968)**: 62-69
[2] Chen, Y. *et al. ACS Nano* **(2010)**: 529-539
[3] Suzuki, K. *et al. J. Control. Release* **(2001)**: 183-189

Continuous process for the synthesis of magnetite (Fe_3O_4) nanoparticles via thermal decomposition

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` The research in this paper demonstrates a novel continuous flow production method for magnetite (Fe_3O_4) nanoparticles as an alternative to the traditional batch method. Magnetite nanoparticles are becoming increasingly important in modern day technology. Specifically, magnetite has become of great interest in the medical field for its potential use in biomagnetic imaging and as an alternative cancer treatment using hypothermia.[1,2] Specific particle characteristics and properties are required before the particles are compatible with the human body. Therefore, nanoparticles with size monodispersity are crucial for proper particle response in biomedical applications. The size affects magnetic field response, stability, miscibility, etc.

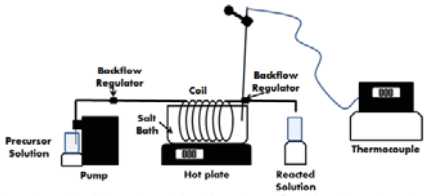

Figure 1: Illustrates the diagram for the reactor used in the continuous flow synthesis

Advantages of the continuous flow reactor include the consistent formation of uniformly spherical particles, thorough mixing of reactants resulting in monodispersity, and the capacity for high volume production of nanoparticles (**Figure 1**). In this study, a reaction mechanism was proposed in which the size of the final particles produced is primarily determined by the initial number of nucleates. The iron oleate/ligand ratio in the precursor directly affects the number of initial nucleates; therefore precursor composition displayed the highest size control in the reactions. Smaller concentrations of iron oleate in the precursor solution resulted in greater size of particles produced. Reaction temperature affected the growth rate of particles, and consequently, higher reaction temperatures were found to produce larger particles. Flow was determined to produce uniformity of spherical shaped particles and to control particle growth on the walls of the reactor.

1. Pankhurst, Q. A.; Connolly, J.; Jones, S. K.; Dobson, J., Applications of magnetic nanoparticles in biomedicine. *J. Phys. D: Appl. Phys.* 2003, *36* (Copyright (C) 2012 American Chemical Society (ACS). All Rights Reserved.), R167-R181.
2. Yu, J.; Huang, D.-Y.; Yousaf, M. Z.; Hou, Y.-L.; Gao, S., Magnetic nanoparticle-based cancer therapy. Chinese Physics B 2013, 22 (2), 027506.

Heating properties of poly-L-lysine modified magnetic nanoparticles

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Iron oxide magnetic nanoparticles (MNPs) have been extensively used in biomedical research with the aim of wide range of applications. Among them, the magnetic hyperthermia uses the fact, that MNPs can deposit heat when they are exposed to alternating magnetic field. Numerous *in vitro* experiments were conducted with promising results. The issue making difficult the *in vivo* applications of MNPs is biocompatibility and stability under physiological conditions. In terms of magnetic hyperthermia enhanced cellular uptake in tumour cells could be of great advantage which can make more effective the process of tumour cells death.

We have synthetized magnetic iron oxide (Fe_3O_4) nanoparticles of core diameter ~10 nm and modified with poly-L-lysine (PLL) to stabilize the particles and improve their biocompatibility. These modified MNPs (MFPLL) were tested for magnetic hyperthermia suitability by calorimetric measurements. Based on the estimated heating rates the specific absorption rates (SAR) for non-modified and MFPLL particles (as it is presented in Fig. 1) were calculated. The SAR values of MFPLL particles were about 14-15 $W.g^{-1}$ at frequency 190 kHz and applied field ~ 8 $kA.m^{-1}$. In the paper [1] we have shown that MFPLL exhibits cytotoxic activities in a cell type-dependent manner and bind to cells expressing CA IX when conjugated with the CA IX-specific antibody. The combination of the SAR values with the cytotoxic activities of MFPPL holds great promise for the future development of targeted synergistic cancer treatments.

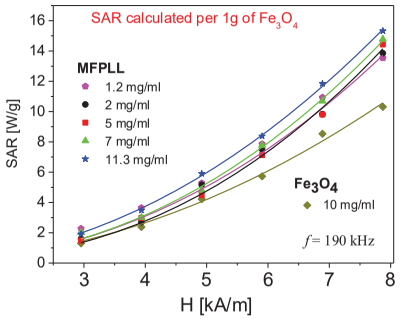

Fig. 1 SAR dependence of non-covered Fe_3O_4 and MFPLL particles measured for various concentrations and recalculated per 1g of Fe_3O_4 at the frequency f = 190 kHz and applied field H up to ~ 8 $kA.m^{-1}$.

Acknowledgements This work was supported by Slovak Research and Development Agency under the contracts No. APVV-14-0120 and APVV-14-0932 the Scientific Grant Agency contracts No. 2/0141/16 and 2/0016/17.

[1] I. Khmara, M. Koneracka, M. Kubovcikova, V. Zavisova, I. Antal, K. Csach, P. Kopcansky, I. Vidlickova, L. Csaderova, S. Pastorekova, M. Zatovicova: Preparation of poly-L-lysine functionalized magnetic nanoparticles and their influence on viability of cancer cells, J. Magn. Magn. Mater. 427 (2017) 114–121.

Top-down fabrication routes of biocompatible non-isotropic nanoparticles in a magnetic vortex state

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Non-isotropic magnetic nanoparticles respond in a different way depending on the direction of the external magnetic field. This asymmetric behavior allows them to rotate in a liquid solution or exert a mechanical torque when attached to a surface. Top-down lithography techniques can be used to fabricate such non-isotropic nanoparticles with large volume and null remanence, i.e. suitable for biomedical applications but presenting high magnetic moments. This is an advantage for sensor devices, magnetic manipulation and magneto-mechanical actuation processes.

This work evaluates advantages and disadvantages of two high throughput fabrication routes of planar nanoparticles using interference lithography. Disk-shaped particles of NiFe with diameters between 400 – 1000 nm and thicknesses of 30 – 60 nm were designed to exhibit a magnetic vortex state, a spin configuration with a negligible magnetic moments in the absent of external fields (Fig. 1). The active magnetic material was protected by a Ti layer. Thus, no toxicity effects were observed in cell viability assays in vitro skin cancer cultures, demonstrating the feasibility of these non-isotropic particles in biomedical applications.[1]

Work supported by FIS2016-76058 (AEI/FEDER, UE), H2020-MSCA-RISE-2016-734801, GV-IT970-16.

[1] B. Mora, et al. ACS Appl. Mater. & Interfaces, DOI: 10.1021/acsami.7b16779

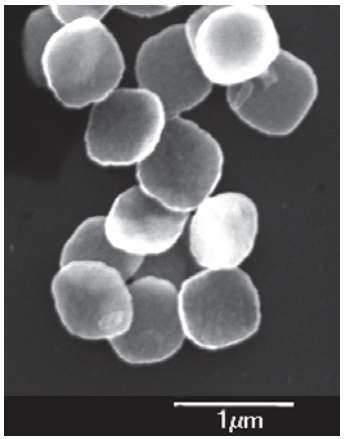

Fig. 1. SEM image of disk-shaped nanoparticles in a magnetic vortex state.

Development and use of iron oxide nanoclusters in biomedicine

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Magnetic nanoparticles with strongly marked anisotropy represent a special interest for biomedical applications. In particular colloidal clusters with controlled size and shape have been an area of great interest for researchers coming from a wide range of disciplines. The controlled assembly of initial small magnetic nanoparticles into cluster structures with defined shape and size opens horizons for materials, which combines properties of individual nanocrystals as well as collective properties due to interactions between the single units.

This work presents, for the first time, a modified thermal decomposition method that involves self-assembly of magnetite nanoparticles and results in highly ordered flowerlike or cubic magnetite nanoclusters (MNCs) with a controlled size in a suitable for biomedical application size ranges. Influence of the different aliphatic and aromatic organic acids as surfactants on a shape and size of nanoclusters was investigated. By this method, MNCs with size from 23 to 41 nm were obtained. Structure and properties of samples were determined by HRTEM, XRD, Mössbauer spectroscopy, magnetic measurements, TGA, FTIR and MRI. Magnetic measurements showed that obtained MNCs have relatively high saturation magnetization values (65.1 – 81.5 emu/g). XRD analysis and Mössbauer spectroscopy showed that all samples are pure magnetite. For determination of T_2-relaxivity values as well as *in vitro* and *in vivo* testing MNCs were modified by Pluronic F-127 (MNCs@Pluronic F-127). *In vitro* experiments on five cell cultures (4T1, B16-F10, CT-26, SC-1 and HFF) showed that IC_{50} value for MNCs@Pluronic F-127 is above 250 μg/ml. MRI investigations showed high T_2-relaxivity values of MNCs@Pluronic F-127 (~ 100 – 170 $mM^{-1}s^{-1}$). *In vivo* MRI was performed on three tumor types: 4T1, B16-F10 and CT-26. Accumulation of MNCs@Pluronic F-127 in tumors was observed in 96% (25/26) of cases. In the case of pronounced accumulation, this value was 62% (16/26). Biodistribution analysis in BALB/c mice was performed to evaluate the accumulation of MNCs@Pluronic F-127 in organs. In addition, the biodistribution of MNCs@Pluronic F-127 24 hours after intravenous injection was studied by magnetometry.

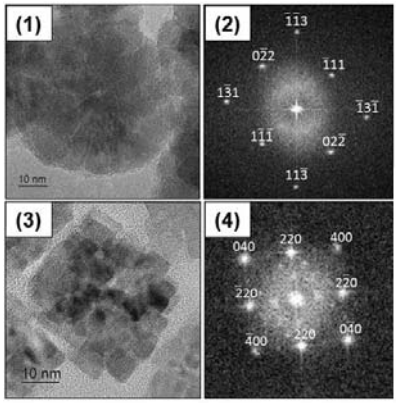

Figure: HRTEM images of: (1) spherical MNCs and (3) cubic MNCs (3) Fourier transform images of (2) spherical MNCs and (4) cubic MNCs

The reported study was funded by RFBR according to the research project № 17-00-00442.

Ascorbic acid and tartaric acid coated iron oxide nanoparticles obtained by hydrothermal method

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Magnetic nanoparticles have found various important applications in biomedicine such as drug delivery, magnetic resonance imaging (MRI), magnetic seperation and magnetic hyperthermia due to their interesting properties [1]. Magnetite and maghemite are the most convenient and highly preferred materials because they are easily synthesized at low cost and their properties can be adjusted by changing the synthesis technique and parameters. Various synthesis routes have been employed to produce magnetic nanoparticles such as co precipitation, microemulsion, hydrothermal method and thermal decomposition. The hydrothermal method is a useful method to obtain highly crystalline nanoparticles. It is also important to select a non-toxic stabilizer which is effective in adjusting the properties of the nanoparticles for biomedical applications. Ascorbic acid and tartaric acid are both water dispersible and biocompatible coating materials. The coated nanoparticles are thought to have potential to be used for biomedical applications such as MRI and magnetic hyperthermia.

In this study, ascorbic acid and tartaric acid coated iron oxide nanoparticles were synthesized via hydrothermal method at 160°C for 12 h. The samples coated with tartaric acid, ascorbic acid and both of them were labelled as TA, AA and TA+AA, respectively. The samples which have the same core structure have the characteristic (220), (311), (400), (422), (511), (440) peaks of cubic spinel structure of magnetite (JCPDS no.019-0629) or maghemite (JCPDS no.039-1346) phase. The effect of the surfactants on the magnetic properties and particle sizes of the nanoparticles were investigated. Transmission electron microscope (TEM) images are presented in Fig. 1. The sizes of the sample TA, AA and TA+AA were 7.4±15, 7.8±1.7 and 6.8±2 nm, respectively. The small size of the nanoparticles were maintained because of the surfactants despite the hydrotermal conditions. Tartaric acid coated iron oxide nanoparticles show a better dispersion among the other samples. Magnetic measurements indicate that all samples are superparamagnetic at room temperature. The saturation magnetization of the samples TA, AA and TA+AA are 66.8 emu/g, 58.8 emu/g and 48.0 emu/g, respectively. The decrease in saturation magnetization results from the small size effect and the amount of surfactant on the surface of the nanoparticles observed from the FTIR and TGA results.

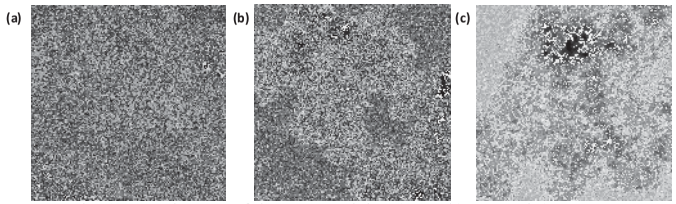

Fig. 1. TEM images of the nanoparticles a) TA, b) AA and c) TA+AA.

[1] Lu AH, Salabas EL, Schüth F (2007) Magnetic nanoparticles: synthesis, protection, functionalization, and application. Angew Chem 46(8):1222–1244.

Hierarchical clustering involving stellate mesoporous silica, iron oxide and quantum dots towards biocompatible theranostic magneto-luminescent nanoprobes

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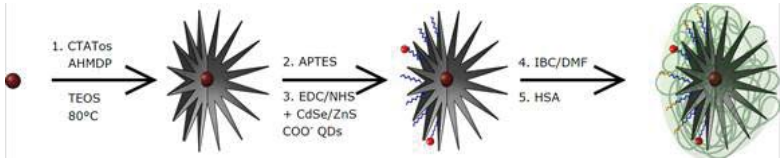
There is currently a crucial need to design innovative multifunctional nanoparticles (NPs) combining multimodal imaging capacities for precision nanomedecine applications. Indeed the combination of imaging modalities and targeting capacities in a unique formulation through nanotechnological approaches bears an enormous potential for the early diagnosis of cancers, evaluation of malignancy, therapy follow up by imaging. Multimodality imaging with two or more imaging modalities allows integration of the strengths of individual modalities. Adding therapeutic fonctions would so provide a smart theranostic nanoplateform. However, key challenges in this field are the design of probes with a great control over the structure and composition and which can be produced in large scale.

In that context, we have designed new magneto-fluorescent nanocomposites made of large pore stellate mesoporous silica (STMS) for bimodal fluorescence and MRI bioimaging. They consist in magnetic and fluorescent NPs which have been hierachically embedded in a porous silica matrix. The magnetic iron oxide NPs bring the remote magnetic features ensuring imaging by magnetic resonance imaging (MRI) and/or therapy by magnetic hyperthermia (MH) and magnetic manipulation, quantum dots (QDs) provide fluorescence imaging and the porous silica shell brings: a high colloidal stability in aqueous solution, a high degree of surface functionalization and capacity of therapy by drug delivery.

Thus the magnetic core (*ca.* 18 nm size), synthesized by thermal decomposition, is shelled by a stellate large pore STMS shell deposited through a surfactant-templated method. This method allows to form uniform individual magnetic cores covered with a STMS shell denoted IO@STMS NPs. To confer fluorescence property visible in cellular conditions, CdSe/ZnS quantum dot (QDs) NPs are grafted to the large pores of the IO@STMS NPs nanocomposites. The immobilization of a protein layer onto them has provided biocompatibility and a resulting good colloidal stability in water (pH = 7). Regarding interactions with cells, the bimodal NPs used in a low range concentration ($\mu g.mL^{-1}$) were shown to have low non-specific interactions with epithelial cells, low cytotoxicity, conserved fluorescence properties and resistance to degradation in the cytosolic and endosomal microenvironments over 48h. Thereby such nanoconstructs may offer a promising platform not only for bimodal diagnostics but also for theranostics applications.



Structural characterization of magnetic nanoparticles coated with dextran

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Nanotechnology and nanoparticle research is rapidly increasing with growing interest and new technological applications in drug delivery, biomedical sciences, therapeutics and data storage. The main advantage of magnetic nanoparticles is in their high surface-to-volume ratio, which contributes to a higher surface energy and to excellent magnetic properties.

Magnetic nanoparticles were successfully synthesized by co-precipitation of ferric and ferrous ions. Solutions of dextran were prepared with different concentrations of dextran (2.5 mg/mL, 4 mg/mL and 5 mg/mL) and used as coating material on magnetic nanoparticles. Obtained dextran coated magnetic nanoparticles Dex1-MNPs (2.5 mg/mL), Dex2-MNPs (4 mg/mL) and Dex3-MNPs (5 mg/mL) were characterized using various techniques including FT-IR, TGA-DSC, SEM and DLS. FT-IR analysis of all Dex-MNPs confirmed the presence of dextran with characteristic vibrational modes at 1010 cm^{-1} and at 3408 cm^{-1}, which correspond to dextran νC–O vibrations and to characteristic νO–H stretching and δO–H deformation modes of dextran hydroxyl groups. TGA-DSC analysis revealed that all Dex-MNPs display a single drop in mass in the temperature range of 150-350 °C, which indicates a single layer of dextran on the nanoparticle surface. The SEM analysis (Figure 1) revealed that Dex1-MNPs and Dex3-MNPs give the smallest sizes of 27.31 nm and 28.00 nm, respectively. DLS analysis confirmed the most consistent sizes of Dex3-MNPs with the lowest PdI (0.31). In addition, ζ-potential was measured that confirmed the negatively charged hydroxyl groups of dextran on the surface of MNPs.

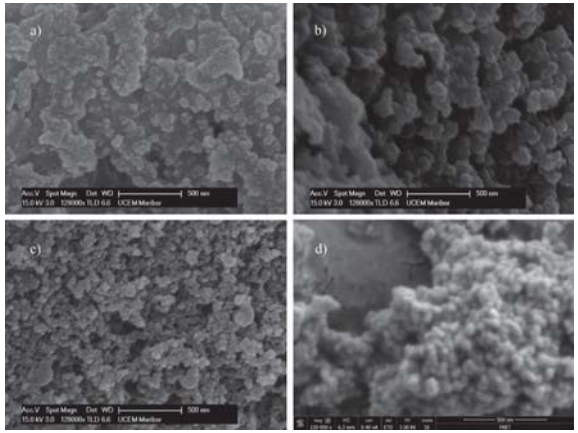

Figure 1: SEM of Dex-MNPs coated with CM-dextran concentrations of (a) 2.5 mg/mL (Dex1-MNPs), (b) 4 mg/mL (Dex2-MNPs), (c) 5 mg/mL (Dex3-MNPs) and (d) uncoated MNPs.

Magnetorheological effect using iron-oxide nano-hollow spheres

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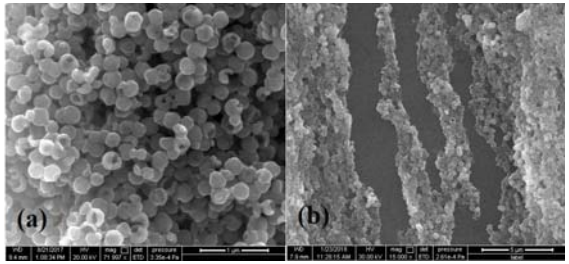
Abstract

Magnetorheological fluids (MR fluid) are a kind of smart material, composed of magnetic particles dispersed in carrier fluid, which can change their viscosity in millisecond under the application of external magnetic field [1]. The magnetic particles form chain like structure under the applied magnetic field, thus enhancing their viscosity to a solid like state, and on removing the field they return back to the initial state.

In this work silicon oil based Magnetorheological fluids (MR fluid) are prepared using Fe_3O_4 nano-hollowspheres (NHSs) of three different diameters 250nm, 350nm and 600nm and Fe_3O_4 nano particles (NPs) of 100nm diameter. Ideally larger particles are expected to show higher shear stress. In our present work the effect of particle size as well as the morphology on shear stress is investigated. The magnetic NPs and NHSs are synthesized via solvothermal technique [2]. All the samples are characterized using X-Ray Diffraction (XRD), Field Emission Scanning Electron Microscope (FESEM) and Vibrating Sample Magnetometer (VSM). Same concentration (15% by weight) of each sample is used to prepare the MR fluids to exactly know the effect of particle size and morphology on MR effect. Shear stress of all the MR Fluid samples is measured using Anton Paar rheometer MCR-301. The NHSs based MR fluid showed higher MR effect and better stability over NPs based one.

Fig.1 shows the FESEM image of the (a) bare Fe_3O_4 NHSs and (b) NHS-based MR fluid in presence of an magnetic field ~1T. Fig.1(b) clearly shows the formation of NHS-chain under the influence of a magnetic field.



References:

[1] "Magnetorheological fluids: a review", Soft Matter, 2011, 7, 3701.

[2] "Domain controlled magnetic and electric properties of variable sized magnetite nano-hollow spheres", J. Appl. Phys. 112, 064318 (2012).

MagnoTher: A Fully Inorganic Drug-loaded Magnetic Hyperthermia Agent

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This work describes the preparation of a magnetic nanocomposite fluid oriented for cancer multimodal therapy based on the combination of local magnetic hyperthermia and thermally-triggered drug delivery. Particularly, our nanocomposite consists of Fe_3O_4 nanoparticles embedded into a Mg-Al layered double hydroxide (LDH) matrix. The synthesis procedure involves the sequential hydrolysis of iron salts (Fe^{2+}, Fe^{3+}) under strongly alkaline conditions (pH>11) and the coprecipitation of Mg^{2+}/Al^{3+} nitrates in a carbonate-rich mild alkaline environment. Magnetite nanoparticles with a diameter around 30 nm represent the hyperthermia-active phase able to generate a specific loss power above 700 W/g when a 765 kHz AC magnetic field is applied. On the other hand, the LDH structure is able to host anionic anticancer agents, such as fluorouracil, by substituting carbonate ions and controllably release them upon temperature increase. Evaluation experiments include the drug adsorption/release efficiency, the recording of AC hysteresis loops and the calorimetric determination of the specific loss power under alternating magnetic fields (up to 24 kA/m and 765 kHz). Cell internalization and toxicity studies assess the potential of the Fe_3O_4/LDH nanocomposite for cancer treatment. Overall, the ultimate product of this methodology is beneficiary for cancer research since it combines multiple modalities such as magnetic targeting of tissues, sufficient heating efficiency, on-site heat-assisted release of drugs and the lower side-effects owed to the exclusive presence of inorganic phases.

Acknowledgments
The project is financially supported by Stavros Niarchos Foundation and Eastern Macedonia and Thrace Institute of Technology fellowships for assisting young scientists in prototyping innovative products by using cutting-edge technology.

Upscaling the Production of Fe_3O_4 Nanoparticles in a Continuous-flow Setup

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In an attempt to improve the conditions of synthesizing magnetite (Fe_3O_4) nanoparticles and promote a process for industrial-scale production, this work introduces a continuous-flow system able to operate under time-independent conditions by adopting the optimum parameters of a relevant batch approach. More specifically, the method of Fe^{2+} oxidative precipitation through the formation of green rust, developed to prepare uniform aqueous-dispersible Fe_3O_4 nanocrystals at variable sizes [1], was transferred in a sequence of two stirring reactors in which the formation of the hydroxide gel and its ageing under mild oxidizing conditions are separately carried out (Figure 1). The main advantages of the proposed approach are (i) the complete separation of green rust's nucleation with Fe_3O_4 formation, (ii) the achievement of constant concentrations in all ionic and solid forms throughout the production line when steady-state is reached, (iii) the possibility to control critical parameters (e.g. OH^- excess) through quantitative and on-line measurable magnitudes such as the reactor's pH and redox potential, and, (iv) the infinite operation which enables high producing capacities and low energy consumption. The obtained nanoparticles were evaluated according to their magnetic response as potential magnetic hyperthermia agents.

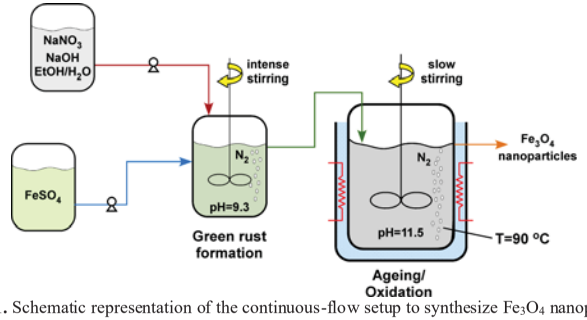

Figure 1. Schematic representation of the continuous-flow setup to synthesize Fe_3O_4 nanoparticles.

References

[1] Y. Luengo, M. P. Morales, L. Gutiérrez, S. Veintemillas-Verdaguer, Counterion and Solvent Effects on the Size of Magnetite Nanocrystals Obtained by Oxidative Precipitation. J. Mater. Chem. C, 4 (2016) 9482–9488.

Acknowledgments

The project is financially supported by Stavros Niarchos Foundation and Eastern Macedonia and Thrace Institute of Technology fellowships for assisting young scientists in prototyping innovative products by using cutting-edge technology. Funding by the Spanish Ministry of Economy and Competitiveness, COMANCHE project, N◦ MAT2017-88148-R, is also acknowledged.

Magnetic Metal-Organic Framework Nanoparticles and Ferrihydrite Nanoagents for MRI-Contrasting and Drug Delivery

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MRI-contrasting agents based on paramagnetic and superparamagnetic nanoparticles substantially enhance the resolution and stimulate developments of new modalities of MRI bioimaging. Furthermore, these nanomaterials may be further modified into multifunctional theranostic agents that are useful not just for imaging, but for drug delivery. In this study, we synthesized a series of new magnetic nanomaterials promising for theranostic applications and investigated their in vivo behavior.

First, we developed a novel method of fabrication of superparamagnetic nanoparticles composed of magnetite core within porous metal organic framework shell (MP-MOF) [1]. These particles can be used both for MRI contrasting and carrying small molecule payload for drug delivery. Moreover, we demonstrated their functionalization with bioreceptors with further application in lateral flow assays. Beside MRI, we used the original real-time magnetic particle quantification method (MPQ) for investigating their in vivo behavior. The blood circulation of the particles was recorded non-invasively by placing mouse tail into the measurement coil of MPQ reader (Fig. 1a). Biodistribution of the particles in the organs was registered by MRI (Fig. 1b) and MPQ.

We also developed a method of chemical synthesis of paramagnetic ferrihydrite nanoparticles stabilized by a polymer shell (FHN) [2]. These are paramagnetic nanoagents that can be used as a "non-magnetic" control in a variety of studies devoted to magnetic materials as well as a stand-alone MRI-contrast agent. Importantly, the colloidal stability of FHN is combined with good biocompatibility and low toxicity. The particles exhibited notable MRI-contrasting properties (Fig. 1c). However, the optimal modes for manifestation of the contrasting properties by the ferrihydrite particles differed from those of the traditionally used ferro- and superparamagnetic iron oxides and were similar to the endogenous magnetic materials of the organism (such as ferritin). This fact makes FHN a good model of some natural metalloproteins for studying its MRI-related properties.

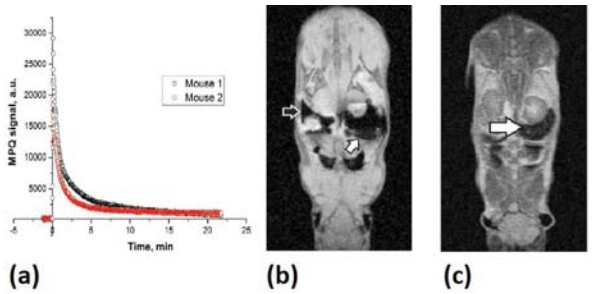

Fig. 1. **(a)** Blood circulation kinetics of the MP-MOF, **(b)** MRI-images showing MP-MOF biodistribution, **(c)** MRI-images showing FHN biodistribution.

[1] A.A. Tregubov, et al., J. Magn. Magn. Mater., vol. 449, pp. 590–596, 2018.
[2] I.L. Sokolov, et al., Colloids Surfaces A Physicochem. Eng. Asp., vol. 539, pp. 46–52, 2018.

Preparation of Graphene Oxide/Magnetite Nanocomposites: Mild Electrostatic vs. Harsh Chemical Routes

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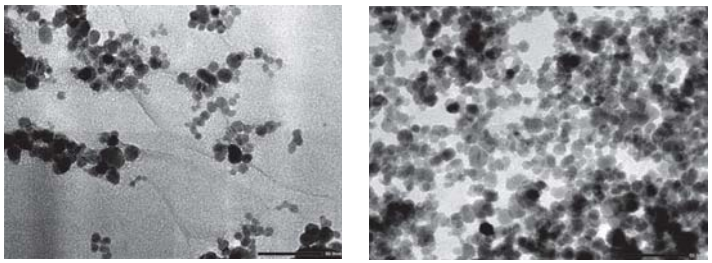
Recently, magnetite (MNP)/graphene oxide (GO) nanocomposites have been extensively studied for potential applications in the fields of medicine as hyperthermic material, diagnostics as MRI contrast agent, molecular biology, and environmental technology [1, 2].

Co-precipitation of magnetic nanoparticles from the mixed Fe(II) and Fe(III) salt solutions under very alkaline conditions is certainly the most popular among the synthesis routes, superior to the prevalence of direct pyrolysis or solvothermal processes for magnetic GO/MNP nanocomposite preparation. In these processes, the in situ crystallization of MNPs takes place on the carbonaceous GO layers and usually lead to the simultaneous structural transformation of GO. We have worked out a one-pot scalable method for the combination of magnetic nanoparticles with graphene oxide nanosheets exploiting the electrostatic interaction between their colloidal particles in aqueous solutions as provided by the development of their opposite surface charge [3]. We will present here a comparison of GO/MNP nanocomposites prepared via one-pot scalable method under mild conditions and a low-cost in situ chemical precipitation method. In both processes, the GO/MNP ratio was varied from the low to the high coverage as seen in the TEM images (mass ratios of (left) 1 to 5 and (right) 1 to 50):



Electrokinetic potentials and Z-average particle diameters of the GO/MNP nanocomposites in aqueous dispersions as a function of the GO/MNP mass ratio, and magnetization measurements and heating curves in AC magnetic fields of nanocomposite dispersions were compared as well. The heat production of nanocomposites with the same magnetite contents (5 mg/ml) was 36 and 46 % higher for 1/5 and 1/50 GO/MNP, respectively, than that of pure MNPs after 5 minutes of AC magnetic field exposure (109.4 kHz, 24.7 mT).

Acknowledgement: Authors gratefully acknowledge the support of grant FK-17/124851 NKFI.

References

[1] B. Zhang, Y. Li, T. Wu, D. Sun, W. Chen, X. Zhou, Materials Chemistry and Physics 205 (2018) 240-252

[2] A.R.K. Sasikala, R.G. Thomas, A.R. Unnithan, et al. Scientific Reports 6 (2016) 20543

[3] T. Szabó, L. Nánai, D. Nesztor, et al. Advances in Materials Science and Engineering, 2018:1390651

Heteroaggregation Approach for Depositing Magnetite Nanoparticles onto Silica-Overcoated Gold Nanorods

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Hydrophobic, oleylamine-stabilized magnetite nanoparticles (Fe_3O_4 NPs) dispersed in hexanes can assemble into dense coatings on the surface of silica-overcoated gold nanorods (SiO_2-GNRs) dispersed in ethanol by mixing. In this non-aqueous heteroaggregation process, Fe_3O_4 NPs are destabilized when ethanol is added, resulting in core/satellite Fe_3O_4-SiO_2-GNRs within a few minutes. The composition of the solvent mixture allows tuning of the polarity and driving forces toward aggregation. At the optimal 2:1 volume ratio of hexanes:ethanol, heteroaggregation to form Fe_3O_4-SiO_2-GNRs occurs quickly, while avoiding homoaggregation of Fe_3O_4 NPs or SiO_2-GNRs. Fe_3O_4-SiO_2-GNRs retain the longitudinal surface plasmon resonance of the gold nanorod cores and are magnetically responsive and separable. The Fe_3O_4 NPs remain bound on the surface of the Fe_3O_4-SiO_2-GNRs during multiple cycles of magnetic extraction and redispersion. Oleylamine ligands on the Fe_3O_4 NPs render the Fe_3O_4-SiO_2-GNRs dispersible in non-polar solvents. Functionalization of the outer Fe_3O_4 surface with poly(ethylene glycol) catechol (PEG-catechol) for PEGylation results in PEG-Fe_3O_4-SiO_2-GNRs that disperse in water. In comparison with seeded growth or use of molecular crosslinkers to form multifunctional nanoparticles, heteroaggregation approaches are potentially quite general, simple, and efficient. The ability to continuously adjust the solvent polarity is expected to allow tuning of the heteroaggregation process for many different types and sizes of NPs.

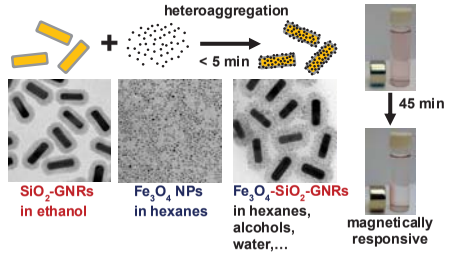

Figure 1. Mixing SiO_2-GNRs (left) and Fe_3O_4 NPs (center) yields Fe_3O_4-SiO_2-GNRs (right), which are magnetically responsive. Reprinted with permission from B.S. Chapman, W.-C. Wu, Q. Li, N. Holten-Andersen, J.B. Tracy, *Chemistry of Materials*, **2017**, *29*, 10362-10368. © 2017 American Chemical Society.

Effects of process parameters on ADH immobilized dextran coated magnetic nanoparticles

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In modern green chemistry, enzymes are valuable natural biocatalysts that bear some excellent properties, such as high activity, high selectivity and specificity. Alcohol dehydrogenase (ADH) is an important biological catalyst that catalyzes the oxidation of alcohols and reduction of aldehydes or ketones, providing many applications in chemical industry. Nevertheless, ADH has low stability, which limits its use in many industrial applications. Therefore, we investigated effects of process parameters, where single-factor experiments were applied into studying the factors influencing ADH immobilized onto dextran coated nanoparticles (Dex-MNPs).

Concentration of cross-linking agent is directly involved in the system enzyme-carrier. Therefore, different concentrations of epoxy cross-linker (epichlorohydrine, ECH) were studied, ranging from 0.5 % to 10 % (v/v) of ECH. Temperature of the immobilization system is an important factor, as well affecting enzyme activity (Figure 1). Temperature range was investigated at 4, 20, 30 and 40 °C. Rotation speed plays an important role influencing the binding capacity of Dex-MNPs to ADH. Different rotation speeds were investigated (200, 300, 400 and 500 rpm) to determine the most suitable rotation speed for successful attachment of ADH to Dex-MNPs.

The micro-environment around the enzyme strongly affects enzyme's properties and performance, resulting in the optimum pH of each enzyme. For this reason effect of pH on ADH immobilized Dex-MNPs was investigated. Additionally, two different enzyme concentrations, as well as immobilization time were investigated, which affects the activity of immobilized enzyme significantly.

When investigating different ECH concentrations, 4 % (v/v) of ECH resulted in the highest residual activity of ADH-Dex-MNPs (88.44 %). The residual activity of ADH-Dex-MNPs was decreasing with increasing temperature, but the highest residual activity was achieved with immobilization temperature of 4 °C, resulting in 52.04 %. When optimizing the rotation speed of immobilization, the highest residual activity was achieved with 400 rpm, resulting in 72.32 % of residual activity. Optimizing other process parameters, the highest residual activities were achieved with pH 7.5, 2 hours of immobilization time and with enzyme concentration of 0.02 mg/mL, resulting in 87.46 % of residual activity.

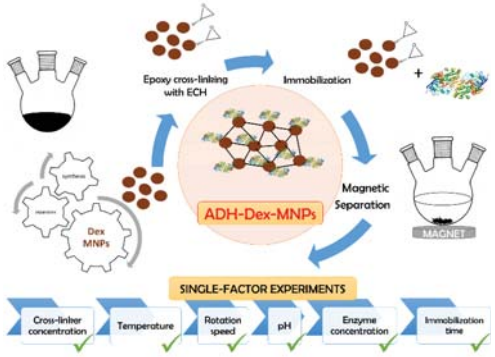

Figure 1: Effect of different process parameters on activity of immobilized ADH onto Dex-MNPs.

Effect of the sodium polyacrylate on the magnetite nanoparticles produced by oxidative precipitation

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Keywords: Magnetite nanoparticles, sodium polyacrylate, oxidative precipitation, draw solutes.

ABSTRACT

The industrial application of magnetic draw electrolytes in forward osmosis heavily depends on the feasibility for mass production by simple green chemical processes of the magnetic nanocomposites. One possible approach for the production nanocomposites based on magnetite nanoparticles and sodium polyacrylate is the aqueous precipitation of an Fe(II) salt in excess of sodium polyacrylate. By changing the polymer proportion in the reaction media, a broad spectrum of nanocomposites with variable particle size, magnetic response and polyacrylate content have been obtained. In this work we will present the characterization of these materials by means of chemical analysis, thermogravimetry, X-ray diffraction, transmission electron microscopy and magnetometry. The magnetic nanoparticle size decreases from 50 to 3 nm along with the saturation magnetization, going from 120 to 19 $Am^2/KgFe$ at room temperature, as increasing the sodium polyacrylate (Mw 2100 g/mol) in the reaction media form 0 to 50 g (figure1) (1.67g $FeSO_4.7H_2O$, 1.12 g $NaNO_3$ with 0.7 g NaOH in 225 mL of water). Finally the possibilities of using these materials as draw electrolytes in forward osmosis will be discussed.

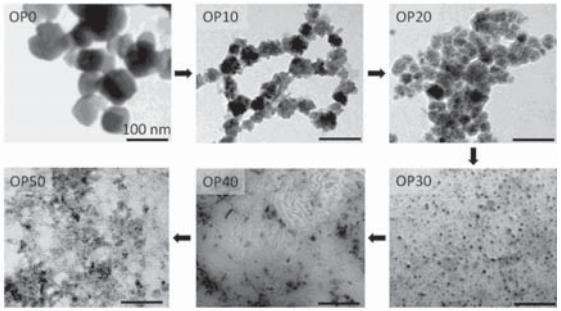

Figure 1

Rod-like particles of silica-coated magnetite: synthesis via akaganeite, characterization and biological properties

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Plethora of methods have been reported for synthesis of spherical or cubic nanoparticles of magnetite, maghemite and spinel ferrites but the preparation of highly anisotropic shapes, like nanorods, and well-defined nanostructured systems remains challenging. The availability of such materials is of great interest for fundamental research and might offer novel properties for applications, e.g. shape-dependent interactions with cells and enhanced contrast agent in imaging techniques.

Multistep procedure was developed to prepare rod-shape magnetite particles. At first, akaganeite (ß-$FeOCl_xOH_{1-x}$) nanorods were synthesized by hydrolysis of $FeCl_3$ under hydrothermal conditions, and the nanorods were coated with mesoporous silica by using a surfactant-assisted procedure. The silica shell allowed to maintain the original shape of rods during transformation to magnetite and prevented the particles to growth together during the subsequent thermal treatment. The transformation was carried at 240 °C under solvothermal conditions in ethylene glycol with sodium acetate [1]. The product was further heated to 700 °C in argon atmosphere to enhance the crystallinity and increase magnetization. Thereafter, the original silica coating was removed by alkaline leaching, and bare rod-shape particles of magnetite were isolated. Eventually, the particles were coated by silica, and mild size fractionation was applied.

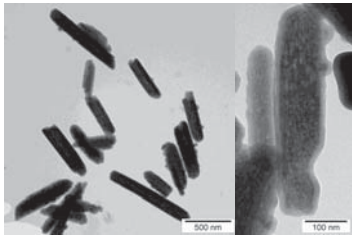

Fig. 1. TEM images of rod-like magnetite particles coated with ~14 nm thick silica shell.

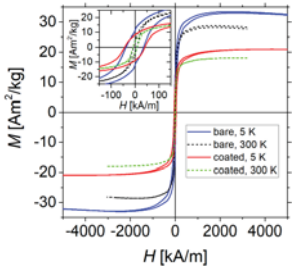

Fig. 2. Hysteresis loops of bare and silica-coated rod-like magnetite particles at temperatures of 5 K and 300 K.

XRD measurements evidenced the phase transformation from akaganeite to the spinel structure of the $Fd\bar{3}m$ symmetry. TEM inspection (see Fig. 1) confirmed that the shape of akaganeite nanorods (diameter of 100–150 nm, length up to few hundreds) was preserved, but the individual rods were actually polycrystalline (mean size of crystallites <10 nm according to XRD). Magnetic measurements demonstrated significant increase in magnetization of particles upon the additional thermal treatment (magnetization of treated magnetite particles was 29 Am^2/kg at 1 T and 300 K compared to 17 Am^2/kg observed for untreated cores). The hysteresis loops of both the bare and final silica-coated particles are shown in Fig. 2.

The evaluation of cytotoxicity properties of the silica-coated product was performed on two cell lines, namely A549 and MCF-7 cells that were incubated with particles at the concentration of 0.416, 0.208, 0.104 mmol(Fe)/L. The viability of cells was determined after 24 h and 48 h of incubation by the trypan blue exclusion test, and the viabilities normalized to the viability of controls were generally higher than 95 %.

References: [1] J. Choi, J. Cha and J.-K. Lee, *RSC Advances*, 2013, **3**, 8365-8371.

Effect of magnetic nanoparticle coating on cell proliferation and uptake

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Magnetic nanoparticles (MNPs) are being utilized for a plethora of biotechnological and biomedical applications, e.g. as heating mediators in magnetic hyperthermia, nanocarriers in targeted gene and drug delivery, magnetite cell labelling, antibody-conjugated magnetoliposome cell labelling, or as contrast agent in MRI. Although the benefit of MNPs is obvious, a thorough investigation of the nanoparticle biosafety and understanding of the nanoparticle-cell interactions is an urgent need.

The objective of this study was to prepare MNPs with different surface modification and evaluate their biocompatibility and internalisation efficacy in human lung A549 cells. Magnetic nanoparticles were prepared by coprecipitation method and their surface was covered by sodium oleate (MFSO) and consequently by bovine serum albumin (MFBSA) or polyethylene glycol (MFPEG). PEGylated MNPs were also embedded into copolymer poly(lactic-*co*-glycolic acid) nanospheres (PLGA-MFPEG). The nanoparticle properties of all studied magnetic fluids (MFs) were characterized in-depth using several techniques. A hydrodynamic diameter of the samples was obtained by Dynamic Light Scattering (DLS) measurement and Differential Sedimentation using CPS Disc Centrifuge. The results from both methods were in good correlation. A zeta potential of all samples had a negative value. Core of magnetic particles was observed by transmission electron microscopy (TEM) and morphology of modified nanoparticles by scanning electron microscopy (SEM, see Fig. 1a-d). Magnetic measurements confirmed superparamagnetic behaviour of all types of studied MFs. Cytotoxicity of surface modified MNPs was determined by MTT assay and the internalized amount of MNPs was quantified by atomic absorption spectrometry (AAS). Our results showed that the surface chemistry influenced both the cell viability as well as MNPs uptake. We found that PLGA-MFPEG NPs exerted the highest cytotoxic effect on A549 cancer cell line. The lowest cytotoxicity was observed after cell treatment with MFSO while the highest internalized amount of MNPs was determined in cells exposed to MFPEG. In contrast, MFBSA were less efficiently internalized into A549 cells although their cytotoxicity was comparable with MFSO.

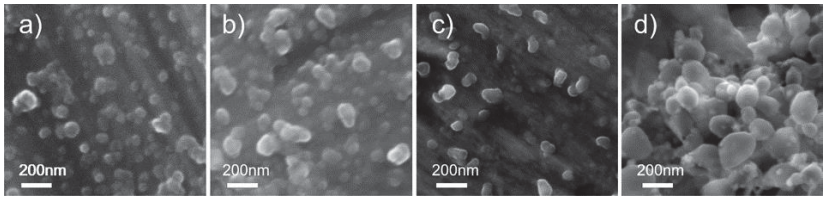

Fig. 1 SEM images of MFSO (a), MFBSA (b), MFPEG (c), PLGA-MFPEG (d) samples.

Acknowledgements This work was supported by Slovak Research and Development Agency under the contracts No. APVV-14-0120 and APVV-14-0932, the Scientific Grant Agency contracts No. 2/0056/17, 2/0113/15, 2/0016/17 and 2/0141/16, and by the H2020 (H2020/2014-2020) funding under Grant agreement 685817 - HISENTS and by the grant EEA FM and the NFM (Project SK0020).

Manipulation of Magnetically Coated Swimmers Inside a Magnetic Particle Imaging Scanner

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Magnetic manipulation of medical devices such as catheters, small cameras or drug filled capsules enables to improve the precision of minimally invasive surgery. Catheters can be steered towards tissue regions difficult to access. Drugs can be delivered directly to cancerous tissue or inflammatory regions, which allows lower dosages and healthy tissue is less affected. In-vitro experiments can be easily visualized with video and microscopy methods, but the manipulation process in-vivo needs to be imaged with a tomographic real-time imaging technique to facilitate image guided interventions. Here, Magnetic Particle Imaging (MPI) is a promising method. [1,2] MPI images the spatial distribution of superparamagnetic nanoparticles. It is based on the nonlinear response of the particles to alternating magnetic fields. A gradient field forming a field free point encodes the signal spatially. A commercially available preclinical MPI scanner (Bruker Biospin MPI 25/20 FF) features homogeneous offset fields, called focus fields, applicable in three dimensions to enlarge the field of view. [3]

By applying an alternating current to the focus field coils, a rotating magnetic field of up to 10Hz can be generated. These rotating focus fields can be used to manipulate magnetic material inside the MPI scanner, since the materials' magnetization and the rotating fields induce a torque. This enables dual use of existing MPI scanners for both imaging and manipulation. [4,5]

The presented swimmers are 3D printed (Projet 3510 HD+) using the printing material VisiJet®X. The swimmers have a savonius rotor shape (see figure 1) and are produced in two sizes. The large swimmer measures 5.6x14.0mm and the small one 1.8x4.5mm. The swimmers are coated with a lacquer containing two kinds of particles: First, Sigma Aldrich Iron (II, III) oxide nanopowder without coating, which are highly magnetic and perform very well for manipulation and second, KLB94m (water-soluble Iron (II,III) oxide with dextran coating), which perform very well for imaging. Both swimmers are put in a water filled phantom, a glass cylinder of 5cm length. Rotating magnetic fields of 18mT have been applied in the frequency range between 1 and 10Hz. It has been observed that the small swimmer rotates very well at all frequencies and fulfills a lateral movement when applying a frequency between 4 and 10Hz, e.g. at 10Hz it travels with a speed of approximately 0.4cm/s (see figure 2). The large swimmer rotates at frequencies between 1 and 4Hz, however it fulfills nearly no lateral movement.

Fig. 1: CAD model of the savonius shaped swimmer.

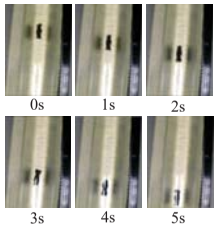

Fig. 2: Lateral movement of the small swimmer within 5s at a frequency of 10Hz.

In order to proof the suitability of the coating for MPI, amplitude spectra were measured with the preclinical MPI Scanner (see figure 3). It can be seen that the KLB94m particles and the lacquer provide a good signal. Also the spectrum of the large swimmer can be clearly distinguished from the background signal. However, the amount of painting is not sufficient in case of the small swimmer. Nevertheless, the technique seems to be promising in order to fabricate small swimmers suitable for e.g. vascular applications, which can be steered by using the magnetic fields inside an MPI scanner and visualized simultaneously with MPI.

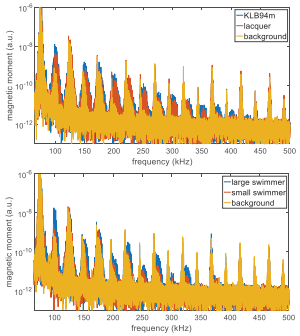

Fig. 3: Amplitude spectrum of the particles and the lacquer (top) and the magnetically coated swimmers (bottom).

References
[1] Rahmer et al., IEEE Trans. Med. Imag., 36(7), 2017.
[2] Mahmood et al., IEEE Trans. Magn. 51(2), 2015.
[3] Gleich, Weizenecker, Nature 435(7046), 2005.
[4] Rahmer et al., Scie. Robot. 2(3), 2017.
[5] Nothnagel et al., IEEE Trans. Biomed. Eng. 63(11), 2016.

Microreaction technology as powerful synthesis platform (not only) for MPI tracer development

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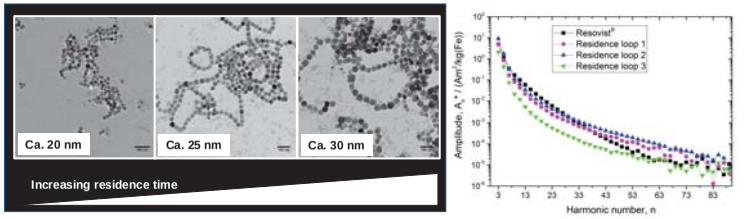
Magnetic nanoparticles hold enormous potential for innovation in a wide variety of technical and biomedical applications. Of course, each of these specific applications has its certain requirements on the particle characteristics as e.g. their core size, hydrodynamic size, surface functionality and in particular the magnetic properties.

Magnetic Particle Imaging (MPI) is an example for a new powerful imaging technology that operates without harmful radiation. MPI strongly relies on suitable tracer materials to exploit its full potential. In literature a large number of different approaches to synthesize iron oxide nanoparticles for MPI are reported. Those include several sophisticated and time-consuming multistep synthesis strategies as e.g. thermal decomposition from organic precursors or biotechnological mineralization with microorganisms. Even if a good size control can be achieved with these methods, scalability and reproducibility stay a big issue. Thus, the reproducible production of larger amounts of tracer material with optimal characteristics in an economic and save production process is still a challenging task.

In our contribution we would like to present how our microtechnological approach enables the production of a broad range of well-defined single-core as well as clustered iron oxide particles in a reproducible and scalable process. Fast and easy screening of synthesis parameters leads to a broad range of particle species, thus effective adjustment of particle characteristics to the specific application requirements becomes practicable. Representative experiments have shown that already by variation of one parameter - the retention time in the reactor - a broad range of core sizes are accessible. Moreover the micromixer based set-up also allows a controlled clustering of iron oxide cores to obtain more complex composite materials. Measurements by magnetic particle spectroscopy (0-dimensional MPI) confirm a good reproducibility as well as the adjustability of magnetic properties by specific synthesis parameters.



TEM images and MPS signal spectra of continuously manufactured magnetic nanoparticles obtained at different residence times.

Magnetic nanoparticles: investigation of the effects of coating on the ^{1}H-NMR relaxation properties

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Magnetic nanoparticles (MNPs) are useful for many applications, as e.g. biomedical imaging and storage media. Iron-oxide-based MNPs (IONPs) are preferred for their stability and biocompatibility and are, so far, the most commonly employed for biomedical applications.
Each IONP consists of a magnetic core and a coating based on organic moieties like polymers, sugars and so on. The efficiency of such particles for diagnostics (e.g. MRI) and therapy (e.g drug release and/or Magnetic Fluid Hyperthermia, MFH) have been demonstrated to depend on many parameters, (e.g kind of magnetic ion, core diameter, shape and also coating).
We present an experimental study, by means of NMR relaxometry, of the possible dependence of the MRI contrast efficiency (i.e. the nuclear relaxivity) on the chemical compound in charge of coating the particle. In particular, we studied maghemite-based nanoparticles in water with two different diameters and five different coatings. The polymers used are specifically polyelectrolytes, i.e. molecules that have many repeating units containing an electrolyte group that dissociates in aqueous solution, making the polymers charged. In particular PAA, PAA-PEG, PAA-PEG$_R$ with hydrophobic residue (JP09), PAA-PEG$_R$ with hydrophilic residue (JP10), PAmA-PEG$_R$ with hydrophilic residue (JP12), were used.
Nuclear magnetic resonance (NMR) relaxometry consists in the measurement of the longitudinal and transverse relaxation times, T_1 and T_2 respectively, of a nucleus (generally the hydrogen nucleus ^{1}H) as a function of the magnetic field, i.e. vs. frequency; one obtains the so-called nuclear magnetic relaxation dispersion NMRD profiles. The results (see Fig.1) allowed us, although not predicted by any theory, to highlight that the polarity of the coating seems to play an important role in the nuclear relaxation of ^{1}H, probably because it can influence the diffusion of the protons in proximity of the nanoparticles and/or acts on the surface spins dynamics.
The NMRD profiles of magnetic nanoparticles coated with PAmA-PEGR (sample JP12, that has positive polarity due to the presence of a quaternary amine in the polymer structure) present an enhancement (r=4nm) or a decrease (r=8-10 nm) of the transversal relaxivity r_2 depending on the size; on the other hand for both sizes the longitudinal relaxivity r_1 is reduced.
Further studies are in progress to confirm these experimental evidences.

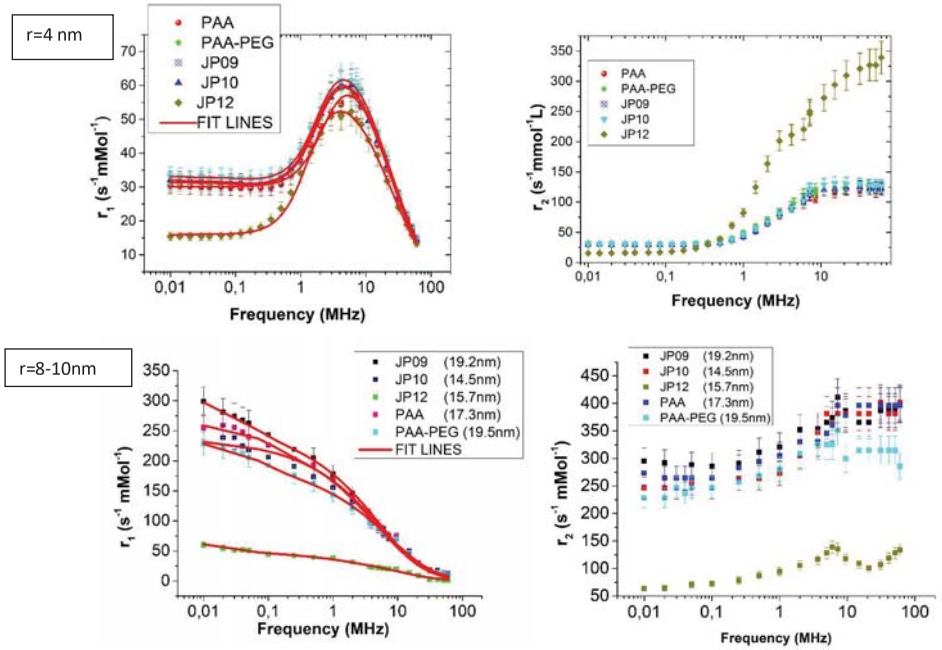

Fig. 1: Longitudinal r_1 and transverse r_2 relaxivities as a function of the magnetic field. Maghemite-based nanoparticles, r=3.5 nm and r=8-10 nm, with five different coating: PAA, PAA-PEG, PAA-PEG$_R$ with hydrophobic residue (JP09), PAA-PEG$_R$ with hydrophilic residue (JP10), PAmA-PEG$_R$ with hydrophilic residue (JP12).

Magnetic nanoparticles spying on their environment in magnetorelaxometry imaging

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Magnetic nanoparticles (MNP) are widely employed in diagnostic and therapeutic applications in medicine. For example, in magnetic hyperthermia the particles are used as heat sources to increase the temperature of the surrounding tissue, deliberately damaging tumor cells. Another application is the detection of diseases by tracking MNP attached to biomolecular targets. Research has focused on developing an optimized MNP type for each application, to achieve e.g. a fine resolution in disease detection, or high heating rates in magnetic hyperthermia.

From a clinical viewpoint it would be highly desirable and cost-effective to have an imaging tool that is capable of reconstructing the spatial distribution of multiple MNP types simultaneously, allowing to combine different MNP applications. Additionally, it would be beneficial if environmental parameters such as MNP clustering, tissue viscosity and tissue temperature could be retrieved to enhance the performance of the biomedical applications. Indeed, the performance of even well-optimized MNP often decreases when the MNP are immersed in biological environments [1]. A promising MNP imaging technique for such a platform is magnetorelaxometry (MRX) imaging, because information about the MNP types, tissue viscosity, tissue temperature and particle interactions is encoded in the MRX relaxation curves. In a previous study we successfully employed MRX imaging to distinguish between various particle types and to allow their simultaneous imaging [2]. In the current study, the MRX imaging procedure was adapted to include tissue information from other imaging modalities, significantly increasing image quality. Additionally, by using Kaczmarz' algorithm, the prerequisite of *a priori* MRX reference curves for different MNP types and environments could be omitted. Finally, the feasibility of extracting temperature information, tissue properties (Fig. 1), particle binding states and possible MNP interactions using MRX imaging was investigated. Thus, we advanced MRX imaging to a powerful technique using MNP to spy out relevant parameters of their (biological) environment.

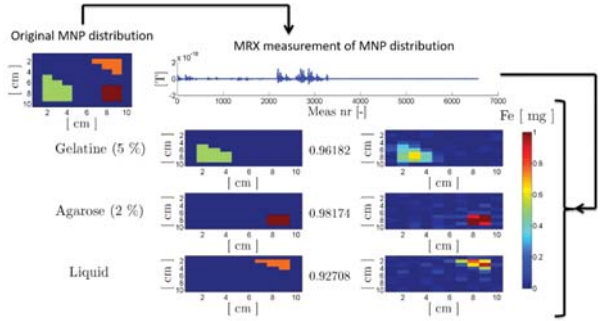

***Figure** 1 Reconstruction of the spatial distribution of MNP suspended in different environments using MRX imaging. The three numbers indicate the correspondence between the actual MNP distribution in a given environment and the reconstructed MNP distribution over the complete sample with 1 representing a 'perfect' reconstruction.*

[1] D. Cabrera et al. ACS Nano. (2018) DOI : 10.1021/acsnano.7b08995
[2] A. Coene et al. Phys. Med. Biol. 62 (2017) 3139–3157

Using Heparin-mimics to Produce High Performance Stabilised Negative MRI Contrast Agents

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Magnetic resonance imaging (MRI) is a powerful non-invasive imaging technique, the effectiveness of which can be improved through the introduction of contrast agents (CAs).[1] Magnetic iron oxide nanoparticles have demonstrated excellent potential in biomedicine and have been used as clinical MRI contrast agents (*e.g.* Feridex, Resovist), though many have been withdrawn recently due to poor performance and biocompatibility issues. There is therefore a real need for new negative CAs which possess both excellent MRI contrast capabilities and good biocompatibility.

Biopolymers have previously been exploited to improve the stability of iron oxide nanoparticles, concurrently improving their biocompatibility and MRI contrast signal, due to strong interparticle interactions resulting from the templating behaviour of the biopolymer.[2–4] Their natively high polydispersity, however, means that precise control over interparticle interactions are lacking. In this work, synthetic analogues of the naturally occurring anti-coagulant heparin, with controlled numbers of templating backbone sulfonate sites, are used as a templating stabiliser (Figure 1).[5] This well-defined and low polydispersity polymer, whose chain length can be carefully tuned synthetically, facilitates greater control over nanoparticle templating and growth, as well as the unique magnetic assembly behaviour of the produced iron oxide particles. This approach thus offers fine tuning of interparticle interactions vital to strong protic relaxation enhancement. A family of polymer-stabilised iron oxide nanoparticles with varying polymer chain lengths and iron oxide:polymer ratios have been prepared and fully characterised by a number of analytical methods. The MRI contrast agent capability of these stabilised particles have been determined at the clinically relevant field strength of 23 MHz, achieving relaxivity values (r_2, the quantitative measure of the efficacy of negative contrast agents) of up to 322 $mM^{-1}s^{-1}$, greatly outperforming existing clinical standards (*e.g.* Feridex®, r_2 = 108 $mM^{-1}s^{-1}$).[1] This improved performance is due to the templating action of the synthetic polymer resulting in strong anisotropic behaviour (Figure 1c and d). Most importantly, this crucial behaviour can be precisely tuned thanks to the low polydispersity and well controlled chain lengths of the synthetic heparin analogues. These families stabilised iron oxide particles display excellent potential as the next generation of negative MRI contrast agents.

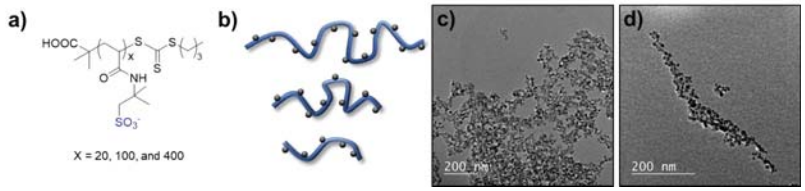

Figure 1. a) Hydrophilic synthetic heparin polymer analogue utilised for particle stabilisation[5]; b) schematic of polymers of differing chain lengths stabilising the magnetite nanoparticles at varying iron oxide:polymer ratios; c) transmission electron microscope images of stabilised iron oxide nanoparticles in the absence and (d) presence of an externally applied magnetic field.

1. *Chem. Soc. Rev.*, 2015, **44**, 4501–4516.
2. *J. Mater. Chem. B*, 2016, **4**, 12–15.
3. *J. Am. Chem. Soc.*, 2008, **130**, 4214–4215.
4. *Chem. Commun.*, 2004, **22**, 2560.
5. *Polym. Chem.*, 2017, **8,** 5513

Magnetic Particle Imaging for the Imaging and Treatment of Stroke

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Ischemic stroke is a devastating disease and a leading cause of disability and death worldwide. Thrombolysis of cerebral blood clots with tissue-type plasminogen activator (rt-Pa) is the only evidence-based medical treatment for stroke. Despite 20 years of experience with rt-PA, fifty percent of treated patients remain disabled for life. A narrow therapeutic time window, insufficient thrombolysis rates, serious side effects of this therapy, and time-consuming imaging techniques decrease the efficacy of stroke treatment. Our project aims to develop a new dual approach by combining therapy and monitoring of stroke patients with Magnetic Particle Imaging (MPI). This new imaging technique enables the rapid assessment of cerebral perfusion (Real-time MPI)[1], as well as the steering of magnetic nanoparticles (MNPs) by magnetic fields (Force-MPI)[2]. Therefore perimag® particles with improved MPI signal intensities compared to Resovist® will be applied as tracer particle candidate (Figure 1)[3] beside new interesting "nanoflower"-shaped synomag®-D particles[4].

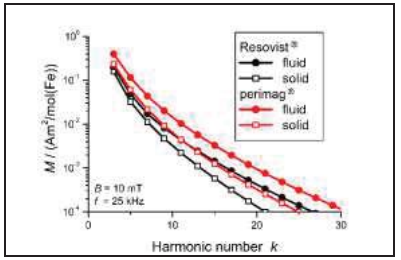

Figure 1. Magnetic particle spectra (MPS) of suspended and immobilized perimag® and Resovist® (left)[3], TEM image of perimag® particles (scale: 50 nm) (right)

We will develop strategies for continuous bedside cerebral perfusion monitoring by using red blood cells (RBC) as a biomimetic tracer-delivery system for the MNPs, which otherwise would be quickly eliminated. This method will enable the rapid diagnosis of stroke or bleeding and facilitate faster treatment and better patient outcomes. Additionally, we will conjugate therapeutics, such as rt-PA, on the surface of MNPs. Using the magnetic fields of the MPI system, we will trap the coupled nanoparticles in the occluded vessel. Through this approach, we will locally increase the amount of active enzyme, resulting in an increased rate of successful revascularization while decreasing systemic side effects. MPI has the potential to substantially improve stroke therapy and the benefits of nanomedicine by combining targeted therapies with ultrafast imaging.[1]

1. Ludewig, P., et al., Magnetic particle imaging in a mouse model of acute ischemic stroke. Proceedings of 2016 International Workshop on Magnetic Particle Imaging (IWMPI), 2016.
2. Nothnagel, N., et al., Steering of Magnetic Devices with a Magnetic Particle Imaging System. IEEE Trans Biomed Eng, 2016. 63(11): p. 2286-2293.
3. Eberbeck, D., et al., Multicore magnetic nanoparticles for magnetic particle imaging. IEEE Trans Magn, 2013. **49**(1): p. 269-274.
4. Bender, P., et al., Relating Magnetic Properties and High Hyperthermia Performance of Iron Oxide Nanoflowers. J. Phys. Chem. C, 2018. **122**(5): p. 3068-3077.

Evaluation of a separate-receive coil by magnetic particle imaging of a solid phantom

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Magnetic particle imaging (MPI) is an emerging tomographic imaging technique capable of quantitatively determining the 3D distribution of a magnetic nanoparticle (MNP) based tracer material. The MPI technology is still under development, and requires significant improvements, especially of the signal to noise ratio (SNR).

The preclinical MPI system (Bruker MPI 25/20 FF) installed at Charité University Hospital Berlin has recently been extended by the addition of a prototype gradiometric coil system as a separate signal-receive unit [1] (Bruker in cooperation with PTB). This differs from the established scanner design, in which excitation field and signal collection are conducted using a single coil. The new separate-receive coil offers an increase in sensitivity of our system by an approximate factor of 10. In addition, we have evaluated this coil by a solid phantom measurement, to demonstrate the benefit in terms of resolving structure details in the image reconstruction. The phantom (Fig. 1 a)) has a structure width of 2 mm and is filled with four times 45 µL Perimag + Manitol, freeze-dried (c(Fe) = 0.22 M/L), to produce a long-term stable phantom employable for comparison of different MPI scanners.

The imaging measurements were performed using a drive field of 12 mT, and a selection gradient field of 2.5 T/m in the z-direction (1.25 T/m in x- and y-directions). We compare the image reconstruction quality of data obtained using the original signal receiver, and the prototype separate-receive coil. The same system function was used to reconstruct the data from each channel measured with a freeze-dried reference sample of 3*3*1.5 mm^3. We performed the reconstructions on the two data sets using identic parameters: Kaczmarz algorithm with 20 iterations and a regularization factor of 10^{-5} and SNR threshold of 5.

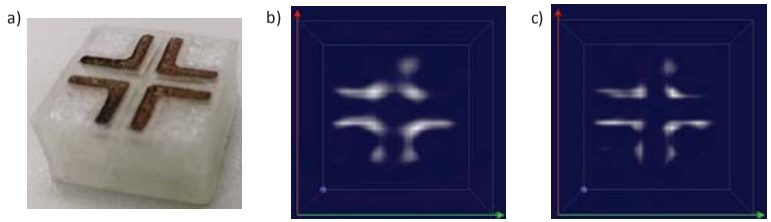

Figure 1: a) Perimag filled phantom, freeze dried **b)** reconstructed image of the original x-channel data; **c)** reconstruction result of the separate receive coil data

The phantom was placed in the xy-plane of the scanner, where we have a lower resolution due to the lower gradient field in x- and y-direction. The four parts in the reconstruction from the original x-channel (Fig. 1 b)) are not clearly separated, and the edges are blurred. For the data from the separate-receive coil, we achieve four separate parts with straight lined edges. This is because the separate-receive coil offers higher sensitivity, especially at the higher harmonics, enabling increased resolution. We conclude that the separate-receive coil enables not only a higher sensitivity for low concentration particle distributions, but also improved structural resolution in image reconstructions. The long-term stability of the phantom is currently being tested and developed.

Reference:

[1] J. Wells *et al.*, "Characterizing a Preclinical Magnetic Particle Imaging System With Separate Pickup Coil," *IEEE Trans. Magn.*, vol. 53, no. 11, 2017.

Characterizing the Magnetic Particle Imaging Performance of Magnetic nanoparticles by Magnetic Particle Spectroscopy

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Magnetic particle imaging (MPI) is a promising tomographic technique that specifically detects super-paramagnetic iron oxide nanoparticles (SPION) with excellent temporal (milliseconds) and good spatial (millimeter) resolution. The MPI technology is still under development and requires significant improvements in both the highly complex instrumentation and in optimizing the dynamic magnetic properties of the SPION used as MPI tracer. To this end, a rapid analysis of the imaging properties of MPI tracer is highly desirable. However, the conventional analysis of tracer performance using the whole imaging process in MPI is very time consuming due to the mandatory recording of the so-called system function (recording time 1-2 days). Therefore, we determined the capability of magnetic particle spectroscopy (MPS), which constitutes a zero-dimensional MPI without spatial encoding, as a time saving alternative to quantify the tracer performance in MPI.

We analyzed deliberately synthesized multi core (MC-SPION, Bayer AG) and single core (SC-SPION, Bayer AG) SPION for MPI seeking for as large as possible amplitudes (normalized to iron amount) and only smoothly decreasing higher harmonics (higher frequencies). The obtained results were compared with those from Resovist®, the accepted Gold standard MPI tracer. The corresponding MPS and MPI spectra of the three systems are shown in Fig. 1 a) and b) for 12 mT drive field amplitude.

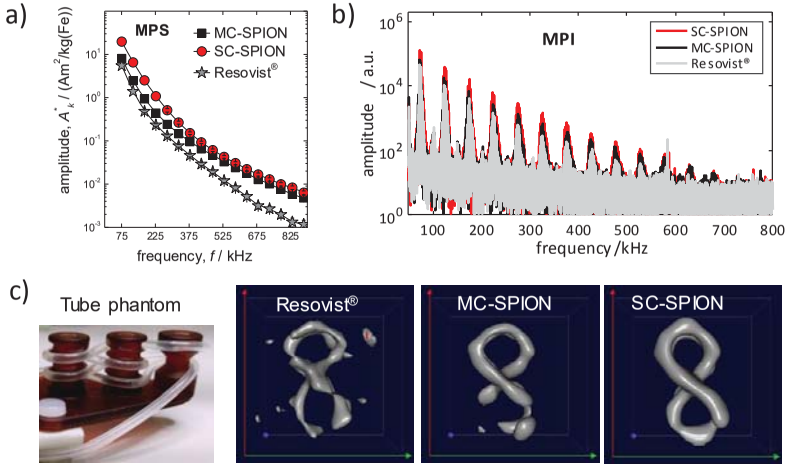

Figure 1: a) MPS spectra and **b)** MPI spectra of the three SPION systems measured at 12 mT drive field amplitude; **c)** Tube phantom and MPI images of the SPION (concentration of 1 mM/L).

We compared MPS and MPI spectra and related the results to the reconstructed MPI images for the three different particle systems. Generally, the better MPI performance is already visible in the amplitude A_3* of the MPS (which only took 10 s compared to more than 38 h duration to acquire the SF used here for reconstructions). But the flatter MPS and MPI spectra observed for MC-SPION (black squares in Fig. 1 a)) are not visible as an improved image quality, which could be attributed to the missing magnetic gradient fields in MPS. Concluding, the MPS spectra provide us a fast but rather raw characterization of the imaging properties of a tracer.

Nanomagnets for MRI: Transverse relaxivity of ε-Fe_2O_3

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Iron oxide nanoparticles attract considerable attention due to their prospective applications in medicine, in particular as negative contrast agents for T_2-weighted magnetic resonance imaging (MRI), cellular and molecular imaging. The ability of a contrast agent to enhance the relaxation rate of hydrogen nuclei in its vicinity is quantified by its relaxivity. In the case of magnetic nanoparticles, the transverse relaxivity $r_2 = (R_2 - R_{H2O})/c$ is more significant, with R_2 and R_{H2O} being relaxation rates of ^{1}H in the examined suspension and in pure water, respectively, and c the molar concentration of the contrast agent in the suspension. While most iron oxide nanoparticles are superparamagnetic at room temperature, nanosized ε-Fe_2O_3 is ferrimagnetic due to its high crystalline anisotropy resulting in giant coercivity of ~ 2 T at room temperature [1-3].

We studied relaxivities of ε-Fe_2O_3 nanoparticles coated with amorphous silica, particularly with the aim to determine their dependences on the external magnetic field, temperature and thickness of the silica coating. The size of magnetic cores (i.e. uncoated particles) derived from the transmission electron micrographs was ~ 20 nm, and the thicknesses of the silica coating ~ 8, 13 and 19 nm in three samples subjected to the relaxometric study. For each sample, we prepared aqueous suspensions of different concentrations of coated nanoparticles, and measured the relaxivities in various external magnetic fields. The temperature dependence of relaxivity of a chosen sample was studied in fields 0.47 T and 11.75 T. In general, the relaxivities of all samples increased with the rising external magnetic field up to a certain saturation value, together with the increase of particle magnetization. Interestingly, the highest relaxivity for all external fields was found for nanoparticles with the thickness of silica coating 13 nm. The decrease in relaxivities with rising temperature is related to the decrease of magnetization and the increase of self-diffusion coefficient of water. The absolute values of r_2 relaxivity of examined samples were comparable to commercial superparamagnetic iron oxide nanoparticle contrast agents (SPIOs and USPIOs) or those under clinical investigation, whose relaxivity ranges between roughly 20 and 200 s^{-1} $mmol^{-1}$ L per Fe at 1.5 T and 37°C [4]. Finally, we interpreted the experimental data in the context of relevant theoretical models describing the influence of magnetic particles on R_2 of ^{1}H (see e.g. [5]).

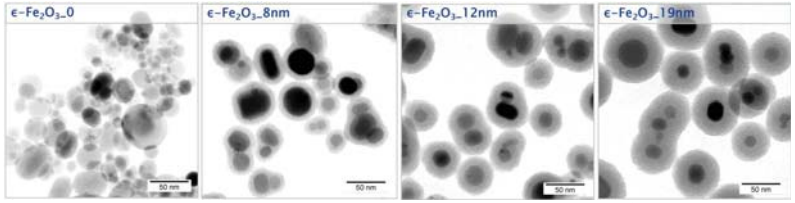

Figure 1: TEM images of examined samples, labelled according to the thickness of the silica coating

Acknowledgment
The support by the project GA CR 16-04340S is gratefully acknowledged.

References

[1] J. Jin, S. Ohkoshi and K. Hashimoto, *Advanced Materials* 16, 1 (2004) 48–51
[2] J. Tuček, R. Zbořil, A. Namai and S. Ohkoshi, *Chemistry of Materials* 22, 24 (2010) 6483–6505
[3] J. Kohout, P. Brázda, K. Závěta, D. Kubániová, T.Kmječ, L. Kubíčková, M. Klementová, E. Šantavá and A. Lančok, *Journal of Applied Physics* 117, 17 (2015) 17D505
[4] S. Laurent, D. Forge, M. Port, A. Roch, C. Robic, L. Vander Elst, and R.N. Muller, *Chemical Reviews* 108, 6 (2008) 2064–2110
[5] M.R.J. Carroll, R.C. Woodward, M.J. House, W.Y. Teoh, R. Amal, T.L. Hanley, and T.G. St Pierre, *Nanotechnology* 21, 035103 (2010)

Speeding up magnetorelaxometry imaging of magnetic nanoparticle distributions using advanced excitation schemes

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Novel cancer therapies based on magnetic nanoparticles (MNP) share the need for a quantitative MNP imaging within tolerable measurement times. In magnetorelaxometry imaging (MRXI) [1], different sub-regions of a spatial MNP distribution are consecutively magnetized and their magnetization decay is recorded by a sensor array. The magnetizing fields are generated by a number of small excitation coils placed around the body. In conventional MRXI (cMRXI), single coils are activated consecutively to partially magnetize the MNP distribution. Hence, the total measurement duration is determined by the total number of coils. Here, we present experimental results of a simultaneous excitation approach "sMRXI" where a proper choice of multiple simultaneously activated excitation coils shall reduce the total measurement duration while the reconstruction quality should ideally be preserved.

MRXI signals were recorded by the PTB 304 vector magnetometer system. Inside a sample support, 15 MNP filled cubes (l=12 mm, X_{MNP}=3.7 mg/cm^3) are assembled to form the letter "P". A total of 30 excitation coils (d=36 mm) with 15 coils above and 15 coils below the support were controlled by a multiplexer supplying an excitation current of I_{mag}=800 mA to one (cMRXI) or multiple coils simultaneously (sMRXI). In each measurement of an MRXI scan the MNP distribution was magnetized for t_{mag}=1 s and MRX signals were recorded for t_{meas}=2 s at 250 Hz sampling frequency. A settling time between two consecutive MRX measurements needed for the multiplexer was set to 500 ms. Hence, the total measurement duration for the N_{MRX}=30 individual MRX measurements in cMRXI summed up to 105 s. We randomly generated excitation sequences for a fixed number of coils N_{SP}=[2, 3, 4, 5] within each sMRXT scan. Accordingly, N_{MRX}=30/N_{SP}=[15, 10, 8, 6] MRX measurements were needed to use each coil once in a complete scan, respectively. From the set of measurements, the MNP distribution was reconstructed using minimum-norm estimation within a FOV of about 600 cm^3 discretized by a voxel grid of k=(10N_x×10N_y×5N_z) with single voxel volume of V=1.72 cm^3.

Examples of quantitative images reconstructed by cMRXI (1) and sMRXI (2-5) are depicted in figure 1. Even for N_{SP}=4 (using 4 coils at the same time for magnetizing) with a total measurement duration of only 28 s, a reasonable reconstruction quality similar to cMRXI was achieved. For N_{SP}=5 with a total measurement duration of only 21 s the deviation of the total reconstructed iron amount was below 10%, though a slight smearing of the "P" was observed.

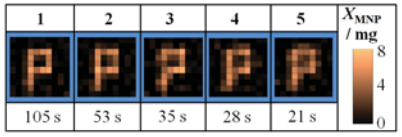

Figure 1: Reconstructed MNP distributions of a "P" shaped phantom for cMRXI (row1) and sMRXI (row2-5). The row number (1-5) indicates the number of excitation coils N_{SP} simultaneously used for magnetizing. The resulting total measurement duration is given in the bottom line.

In conclusion, we demonstrated that by simultaneous excitation of multiple excitation coils the total measurement duration of quantitative MRXI can be considerably reduced. Hence, sMRXI enables fast and flexible imaging of large FOVs, an advantage particularly for in-vivo imaging. Future work will focus on finding sensitivity optimized coil combinations for magnetizing coil arrangements in a given MRXI setup.

Acknowledgement: This work was supported by Deutsche Forschungsgemeinschaft (DFG) in the framework of the priority programs SPP1681 (WI4230/1-2) and SPP1798 (BA 4858/2-1) as well as by the EMPIR program grant no. 16NRM04 "MagNaStand".

[1] M. Liebl, F. Wiekhorst et al. (2015), *Biomed Eng/Biomed Tech*, 60(5):427-43.
[2] D. Baumgarten, J. Haueisen et al. (2013), *EMBS Proc*, 2013:3258-60.

Effect of PEGylated Coating of Magnetic Nanoparticles on Renal Perfusion: Evaluation Using 7T DSC-MRI in Rats

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Magnetic nanoparticles (MNPs) can be used as magnetic resonance imaging (MRI) contrast agent due to their ability to alter the magnetic susceptibility of the surrounding water protons. Polyethylene (PEG)-coating of MNPs may alter biodistribution and behaviors of MNPs in the organ microenvironments. Our preliminary results demonstrated a transient hypotensive effect induced by administration of PEG-MNPs in rats. In this study, we aim to evaluate the effect of PEGylation of MNPs on rat renal perfusion parameters using dynamic susceptibility contrast (DSC)-MRI.

Anesthetized Sprague Dawley rats were cannulated for intravenous injection of dextran-coated MNPs (250 nm) with or without PEGylation, i.e., PEG-MNPs *vs.* MNPs (nanomag®-D, micromod), at a dosage of 5 mg/kg. DSC-MRI images were scanned continuously for 300 seconds on the axial section with the largest cross-sectional area of both kidneys using a 7T Bruker scanner. Six cortical regions of interest (ROIs), two medullary ROIs on bilateral kidneys, and one aortic ROI were analyzed to derive tissue response functions using Matlab software (Figure 1).

None of the perfusion parameters between the two kidneys of the same rat were significantly different. However, longer time to 25% down from the peak of the tissue response functions was observed in the PEG-MNP group (n=3) comparing to that without PEG (n=3) in both renal cortex (42.3 ± 2.7 *v.s.* 28 ± 1.5 sec) and medulla (42.3 ± 3.7 *v.s.* 28.3 ± 1.8 sec) (Figure 2). Nevertheless, no significant difference of time to peak, time to 50% down from the peak, or mean transit time of the tissue response functions was observed between the two groups. In conclusion, PEGylation of MNPs induced a transient delay early in the washout phase of renal perfusion in both cortex and medulla of the rat kidneys, which may potentially alter renal hemodynamics.

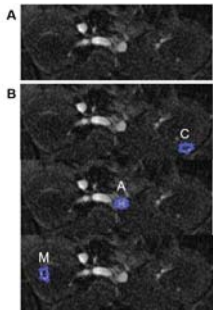

Figure 1. (A) Representative DSC-MRI image of abdominal axial section of a rat. (B) ROIs of renal cortex, aorta, and renal medulla.

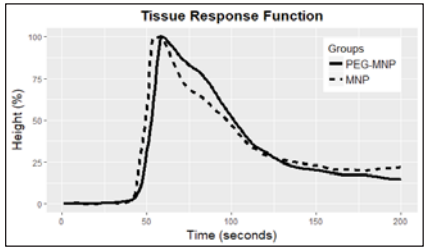

Figure 2. Averaged tissue response functions of PEG-MNP and MNP groups.

Hypotonic Swelling: A Method of Encapsulating Fluorescence-Labeled SPIONs into Red Blood Cells for Magnetic Particle Imaging

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Superparamagnetic iron oxide nanoparticles (SPIONs) are used, for example, as tracer material or contrast agent for medical imaging like MRI or magnetic particle imaging (MPI), a new imaging modality [1]. To use the SPIONs as a tracer for biomedical imaging the material needs to be injected interstitially (for example in case of a lymphatic uptake study) or intravenously as blood-pool contrast agent. Since the half-life time of the SPIONs in the blood stream is quite short and they are quickly absorbed by the reticuloendothelial system (RES), the particles are introduced into red blood cells (RBCs) to increase their half-life time in the blood circulation. The distribution of SPIONs in the tissue is strongly influenced by surface properties and particle size. The nanoparticles used here consist of a magnetite core with a coating of dextran and the hydrodynamic diameter varies between 80 nm and 120 nm. Particles with a diameter of more than 50 nm are quickly trapped by macrophages in the blood stream and transported to the liver. Therefore, SPIONs have been used by many groups particularly for liver imaging. However, the SPIONs quickly disappear from the blood stream.

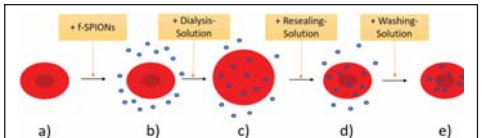

Fig. 1: Procedure of hypotonic swelling to introduce SPIONs in RBCs

Unfortunately, the large particles – which are predominantly contributing to the MPI signal quality, are detected faster than smaller particles as foreign bodies by the RES [2]. To increase the half-life time of the SPIONs in the blood stream, the dextran coated nano-particles need to be camouflaged. Here, we introduce the particles ex-vivo into erythrocytes by hypotonic cell swelling [3,4] and consecutively reintroduce the particle-loaded RBCs into the blood stream.

For the evaluation of the SPION cell uptake three methods are used, transmission electron microscopy (TEM), magnetic particle spectroscopy (MPS), and fluorescence microscopy. For the latter, in this contribution, the SPIONs are labeled with fluorescein isothiocyanate (FITC) and rose bengal, because the biocompatibility of these dyes has been proved [5]. The fluorescence labeling is a cross check with TEM, if the SPION uptake was successful.

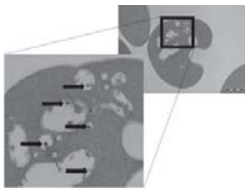

Fig. 2: TEM image of encapsulated SPIONs.

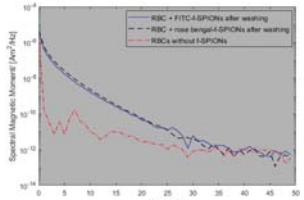

Fig. 3: MPS measurement of the loaded RBCs encapsulated SPIONs.

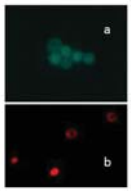

Fig. 4: Fluoresces image of RBCs encapsulated SPIONs. a) SPIONs labeled with FITC. b) SPIONs labeled with rose bengal.

References

[1] B.Gleich, J. Weizenecker, Nature. 2005, 435(7046):1214–1217.
[2] D.E. Owens, N.A. Peppas, International Journal of Pharmaceutics, 2006, 307(1):93-102.
[3] J. Rahmer et al., Phys Med Biol. 2013; 58(12):3965-77.
[4] A. Antonelli, et al. Journal of Nanoscience and Nanotechnology 2008, 8: 1-9.
[5] Carl Roth GmbH: SDB_3162_DE_DE.pdf; SDB_5283_BE_DE.pdf

Surface modified fluorescent transition metal oxide nanostructures for biomedical applications

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Surface plays a very effective role in determining the properties of nanomaterials. We observed many novel and enhanced properties in Iron and other transition metal oxides nanostructures due to surface effects depending on their shape, size and surface functionalization [1, 2]. They can be functionalized with suitable ligands to get biocompatible and water soluble nanoparticles with interesting multifunctional properties. Intrinsic multicolour fluorescence (Fig.1) in ferrite nanoparticles from blue, cyan, and green to red is observed upon functionalization with organic ligands such as Na-tartrate, malate and citrate because of charge transfer from ligand to the lowest unoccupied energy level of transition metal ions of the NPs and Jahn−Teller distorted d−d transitions [3,4]. Surface functionalized ferrite NPs have been utilized for studying DNA binding interaction and nuclease activity for stimulating their beneficial activities toward diverse biomedical applications. The spectroscopic measurements indicate that T-$MnFe_2O_4$ NPs bind calf thymus DNA by intercalative mode. The ability of T-$MnFe_2O_4$ NPs to induce DNA cleavage was studied by gel electrophoresis technique where the complex is found to promote the cleavage of pBR322 plasmid DNA from the super coiled form I to linear coiled form II and nicked coiled form III with good efficiency. Many of the above oxide nanostructures show excellent photocatalytic activities and potential for various biomedical applications depending on their shape, size and surface functionalizations [3].

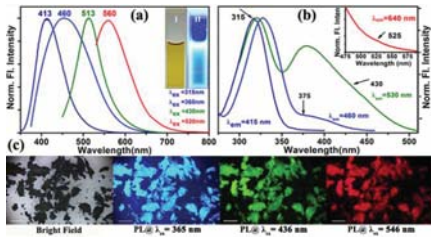

Fig.1: Fluorescence (a) emission (b) excitation spectra and (c) micrographs from Tartrate modified $MnFe_2O_4$ Nanoparticles

References

[1] R. Rakshit, M. Mandal, M. Pal, K. Mandal, Applied Physics Letters, **104**, (2014), 092412.
[2] S Mitra, S Das, K Mandal, S Chaudhuri, Nanotechnology **18** (2007), 275608.
[3] M Pal, R Rakshit, K Mandal, ACS applied materials & interfaces **6,** (2014), 4903-4910
[4] I Chakraborty, U Saha, R Rakshit, S Talukdar, G. S. Kumar, K Mandal, Materials Today Chemistry, **5**, (2017), 92-100.

MRI Investigation of Magnetic Hybridmaterials for Implant Engineering

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Magnetic nanoparticles (MNP) are used as additives for the fabrication of biodegradable implants in order to deliver information about their location as well as the postoperative degradation degree *via* magnetic resonance tomography (MRI). In this regard, MRI quantitative methods relying on the accurate analysis of contrast differences between T_1 and T_2 weighted images are used to determine the local MNP concentration. Further, MRI relaxometry is applied to determine the degradation state of the implant in which the MNP are embedded. For quantitative assessment of MNP concentration and implant degradation, reliable models must be developed which describe the relation between proton relaxivity and MNP immobilization state during the degradation process as well as MNP size and aggregation. Theoretical considerations classify the dependence of the transverse relaxivity on MNP size in three regimes: the motional averaging regime (MAR), the static dephasing regime (SDR) and the echo limiting regime (ELR) [1]. In this study, we experimentally confirm this regime classification of proton relaxivity and quantify the impact of the immobilization state of MNP on the relaxivity values. This impact varies according to the corresponding MNP relaxivity regimes.

For the investigations, we synthesized lauric acid stabilized iron oxide MNP with different hydrodynamic sizes between 22.7 nm and 187.7 nm. In order to obtain the transverse relaxivities r_2 using the linear relation between MNP concentration and proton relaxation rates R_2, iron concentration series up to 0.2 mM of each sample were prepared and the R_2 were measured with a Philips Achieva 3 T MRI Scanner. The results displayed in Fig. 1 show that the relaxivity depends on MNP hydrodynamic size as predicted by the MAR, SDR and ELR theory. In the MAR, the proton diffusion length is larger than the characteristic length of the magnetic field disturbance caused by the MNP. Water protons diffuse rapidly around the MNP, experiencing thus a broad range of fast changing magnetic fields, which are effectively time-averaged. As the MNP diameter increases, water protons have a small diffusion length in comparison to the MNP size. Then, they experience non-averaged variations in local magnetic fields causing a quicker relaxation of the proton magnetic moments and an increase of r_2. In the SDR, r_2 reaches multiple plateaus having different magnitudes which are attributed to different saturation magnetization values of the MNP. In the ELR, r_2 decreases with MNP size due to partial refocusing of proton magnetic moments.

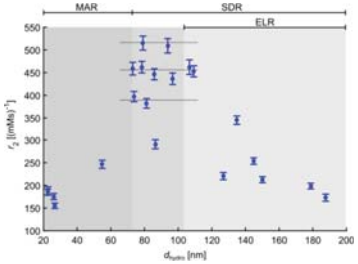

Fig. 1: Transversal relaxivity values r_2 for MNP with different hydrodynamic diameters between 22.7 nm and 187.7 nm. The measured values fit the theoretical predictions of the regimes MAR, SDR and ELR as indicated.

In order to investigate the relaxivity changes due to MNP immobilization, two particle systems corresponding to either the MAR or the SDR were embedded in gels with up to 3 %wt agarose. Higher agarose contents correspond to smaller pore sizes of the gel causing stronger immobilization of the MNP. In this way, different MNP immobilization states could be achieved which mimic the mobility of the MNP for different degradation states of implants. The relative difference between the relaxivity values of immobilized MNP and MNP dispersed in water shows a drastic increase in MAR and a moderate increase in SDR with increasing agarose content (Fig. 2). The relaxation behavior could be explained by the fact that the MNP immobilization results in a less pronounced fluctuation of the local magnetic fields leading to higher proton relaxivity values. To conclude, for a reliable quantification of implants with MRI, thorough investigations of the proton relaxivity behavior depending on MNP size and immobilization state must be performed. These will then be the basis for the development of suitable models to describe the relation between the degradation of the implant and the measured proton relaxivities.

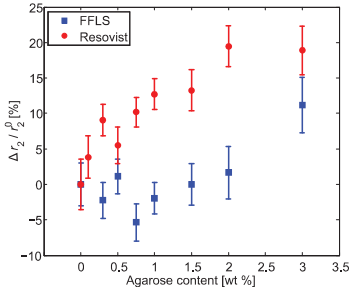

Fig. 2: Relative changes of r_2 for different agarose phantoms with incorporated MNP that fulfill the MAR condition (red) and the SDR condition (blue). The value r^0_2 denotes the relaxivity of the MNP dispersed in water.

[1] Alain Roch, Yves Gossuin, Robert N. Muller, and Pierre Gillis. Superparamagnetic colloid suspensions: Water magnetic relaxation and clustering. *Journal of Magnetism and Magnetic Materials*, 293(1):532–539, May 2005.

Effects of size and anisotropy of magnetic nanoparticles associated with dynamics of easy axis for magnetic particle imaging

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In order to advance the magnetic particle imaging (MPI) [1], magnetic nanoparticles (MNPs) possessing a high harmonic intensity were investigated. In our previous study, the effect of the core diameter and structure of MNPs on the harmonic intensity was examined [2]. In this study, the intensity and half bandwidth of the MPI signal were measured in terms of different core diameters and structures of the MNPs.

Measurements were performed using water-based maghemite nanoparticles (Samples I, II, III, and IV), which were supplied by Meito Sangyo Co. Ltd., Kiyosu, Japan. These MNPs were coated with carboxymethyl-diethylaminoethyl dextran, which enhanced their efficacy acting as a blood-pooling contrast agent [3]. Samples I, II, III, and IV exhibited core diameters of 4, 8, 5-6, and 6 nm, respectively. In order to measure the third harmonic magnetization as signal for 2D MPI, a set of permanent magnets, whose gradient was 1 and 2 T/m for the x- and y-axis, respectively, a drive coil, and a pick-up coil were prepared. The intensity and frequency of the AC drive field were 3.5 mT and 3 kHz, respectively.

Figure 1 shows the images of the measured MNPs for 2D MPI. The maximal intensities of the MPI signals were 1, 3.9, 3.1, and 6.1. The half bandwidths were 1, 0.86, 0.81, and 0.76. Both parameters were normalized according to the values of Sample I. The maximal signal intensity of Sample II was higher than those of Samples I and III, since its core diameter was larger than those of the other two samples. With respect to Sample IV, the MPI signal was higher than those of the other samples because the MNPs with large magnetization were collected by magnetic separation.

Figure 2 shows the numerically simulated third harmonic intensity dependence on the anisotropy in the solid and liquid states of the particle media using the Ladau-Lifshitz-Gilbert equation. The third harmonic intensity was significantly increased by the rotation of the easy axis in the liquid medium compared with that in the solid medium in the case of high anisotropy constants. This is in agreement with the increase of the anisotropy with the structural effect of the measured MNPs described in Ref. [2].

[1] Gleich and Weizenecker, *Nature*, **435**, 1217 (2005).
[2] Ota *et al.*, *Int. J. Magn. Part. Imaging*, **3**, 1703003 (2017).
[3] Nitta *et al.*, *Jpn. J. Radiol*, **30**, 832 (2012).

This work was partially supported by the JSPS KAKENHI Grant Numbers: 15H05764, 17H03275, and 17K14693.

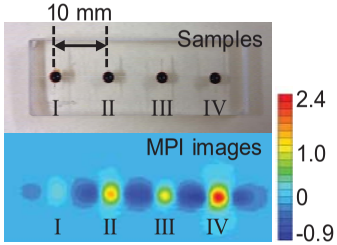

Fig. 1 2D MPI images of Samples I, II, III, and IV. The intensity and frequency of the applied field were 3.5 mT and 3 kHz, respectively.

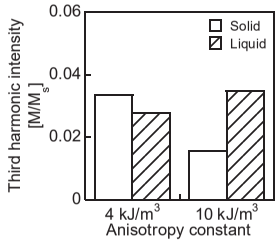

Fig. 2 Anisotropy dependence of the simulated third harmonic intensity in 12 nm of the particle core diameter at 10 mT and 10 kHz of field intensity and frequency, respectively.

Complementary magnetic particle imaging and magnetic resonance imaging: Finding the right magnetic nanoparticle concentration range

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Magnetic nanoparticles (MNP) comprise a unique smart nanomaterial class with promising prospects for biomedical applications [1]. The field of applications range from therapeutic purposes (e.g. hyperthermia) to diagnostic approaches (e.g. cell tracking). Most of these methods require a precise localization and quantification of the MNP amount in biological environment. Magnetic particle imaging is an upcoming imaging technology used for quantifying the 3D distributions of MNP in-vivo. This technique exploits the combination of static and dynamic magnetic fields to generate specific dynamic magnetization response of the MNPs proportional to the particle amount [2]. However, since signal is only generated from MNPs, biological tissue cannot be visualized directly. Therefore, a second imaging technique is needed to provide this information. Primarily magnetic resonance imaging (MRI) is used for this purpose. MRI is based on the measurement of the net magnetization of protons, which are probed by magnetic gradients and radiofrequency pulses. The presence of MNPs in the vicinity of the protons leads to a faster transversal signal decay proportional to the MNP concentration, lowering the measured image intensity. Since the MRI signal decreases with increasing MNP concentration while the MPI signal increases, the question arises: which concentration range is suitable for combined imaging using both modalities?

To answer this question, we performed measurements using a serial dilution of multicore MNP in water (MCP, Charité, mean core size 33 nm), optimized for both imaging systems [3], using 160 µL sample volumes at iron concentrations varying between 73 mmol/L and 4 µmol/L. First, we determined the MNP performance using the respective spectroscopic methods, nuclear magnetic resonance (NMR) and magnetic particle spectroscopy (MPS), both using the same principle as their corresponding imaging techniques but without magnetic gradients for spatial encoding. For MRI and NMR, a multi-spin-echo (MSE) sequence were used to record the signal decay over time. The total measurement time varied in the range of 10 s and 10 minutes to acquire the full signal decay. An exponential fit was used to determine the transversal relaxation time T_2 (Fig.1 a). MPI/MPS measurements were performed with an acquisition time of 10 s and quantitative information were extracted from the spectrum/image and are displayed in Fig 1b.

All measurement techniques showed linear scaling with the iron concentration for the T_2-relaxation time (NMR, MRI) or the signal intensity (MPS, MPI). Above an iron concentration of about 2 mmol/L the signal decay was too fast to acquire a signal in MRI using our MSE sequence. On the other hand, the limit of detection (LOD) for the MPI scanner was determined to be about 30 µmol/L. Therefore, the concentration regime for complementary MPI-MRI measurements lies between these values. However, this concentration range is only valid for the used MNP system in an aqueous solution. Further analysis of different MNP systems and surrounding media are needed and will be presented at the conference.

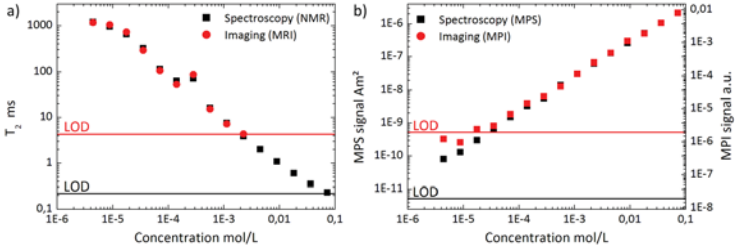

Figure 1: T_2-values extracted from NMR and MRI for samples at different iron concentrations (a). Below the respective limit of detections (LOD) the acquired signal was too low for a reliable fitting procedure. Signal intensities over the iron concentration determined by MPS and MPI (b). The LODs display the mean values of an empty scanner measurement.

[1] C. Alexiou *et al.*, *J. Nanosci. Nanotechnol.*, vol. 6, no. 9, pp. 2762–2768, Sep. 2006.
[2] B. Gleich *et al.*, *Nature*, vol. 435, no. 7046, pp. 1214–1217, 2005.
[3] H. Kratz *et al.*, *PLoS One*, vol. 13, no. 1, p. e0190214, Jan. 2018.

Study of an Anisotropy of Magnetic Noise, Generated by Magnetic Particles in Geomagnetic Field

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Any device for visualization of magnetic nanoparticles within a living organism has a group of receiving coils distributed in space near the object under investigation. The coils have to generate an electrical signal proportional to the magnetic field created by the magnetic nanoparticles at the sensor location. Faraday's law of induction states that the induced electromotive force in a coil is equal to the negative of the time rate of change of the magnetic flux enclosed by the coil. Therefore, all known designs of such devices require the imposing of external magnetic field on the sample. This field serves both for the preliminary orientation of the magnetic moments of all particles (with the aim of creation of the flux), and for changing the magnetic flux through the receiving coils. A significant drawback of this method of visualization is the fact that the magnetic fields, applied to the object in the process of measurement, effect on the distribution of magnetic particles in space.

There is an alternative approach that allows us to abandon the application of an external magnetic field. In this approach the geomagnetic field orients magnetic particles in one direction. The change in magnetic flux from these oriented magnetic particles occurs because of their natural fluctuation movement. The sources of such movement in the living body can be breath, heartbeat or Brownian motion of nanoparticles in the cytoplasm of cells. The possibility of such 3D imaging of magnetic nanoparticles in the ferrofluid using the 7-channel SQUIDs based magneto-encephalography (MEG) device without pre-magnetization and mechanical movement of the sample was demonstrated in [1]. The spatial distribution of elementary magnetic sources was reconstructed by the method of frequency-pattern analysis of multichannel time series [2]. This method decomposes the multichannel signal into the large set of elementary oscillations, which can be localized individually, providing the functional tomogram of the system. The localization of ferrofluid was performed based on the analysis of quasi-random time series in two cases of oscillation source. One of them was infrasound from outer urban noise, and another one was the human heartbeat.

The purpose of the present work was to study an angular distribution of the magnetic noise, generated by the magnetic nanoparticles in geomagnetic field. It was revealed that the ferrofluid generates spontaneous magnetic fields, which at certain frequencies have a strong spatial anisotropy. The detected effect can essentially increase the spatial resolution of the proposed method of visualization of magnetic nanoparticles.

The work was supported in part by the Russian Foundation for Basic Research under Grants 18-02-00629 and 16-07-00937.

References

1. Polikarpov M.A., Ustinin M.N., Rykunov S.D., Yurenya A.Y., Naurzakov S.P., Grebenkin A.P., Panchenko V.Y. 3D imaging of magnetic particles using the 7-channel magnetoencephalography device without pre-magnetization or displacement of the sample. Journal of Magnetism and Magnetic Materials 427, 2017, 139-143.
2. Llinás R.R., Ustinin M.N. Precise Frequency-Pattern Analysis to Decompose Complex Systems into Functionally Invariant Entities: U.S. Patent. US Patent App. Publ. 20160012011 A1. 01/14/2016.

Microfabricated Magnetic Gel Composites as pH Sensitive Contrast Agents

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Chemically synthesized, superparamagnetic iron oxide nanoparticles (SPIONs) and high concentrations of paramagnetic ions (Gd, Mn) are often used as agents for generation of T_2/T_2* contrast for MRI imaging. Here, we combine such nanoparticles and / or paramagnetic ions with a smart polymer to create environmentally sensitive, magnetic-hydrogel composite MRI agents. Polyethylene glycol dimethacrylate 200 (PEGDMA-200) is mixed with dimethacrylic acid and a UV-photoinitiator to produce a UV-curable, pH sensitive hydrogel. The cured hydrogel is pH sensitive, swelling at neutral-basic pH of 7-8 and contracting at an acidic pH of 3-4. Two types of magnetic materials, manganese acetylacetonate (III) / $(acac)_3$ and oleic acid coated SPIONs (10 nm dimeter), are dispersed in toluene and combined with the uncured PEGDMA. Evaporation of toluene from the mixture leaves the $Mn(acac)_3$ and SPIONs behind, forming a photo-curable magnetic gel precursor. Since the precursor is UV-curable, microstructure fabrication is compatible with traditional photolithography techniques. Discs ranging in diameter from 50-200 um (Figure 1) have been created by first sandwiching the PEGDMA precursor between a Si wafer and thin sheet of PVC plastic, then exposing the heterostructure with a contact aligner. Smaller structures (2-20 um) have been synthesized by a micro-molding approach. Wells of arbitrary shape are etched into a fused quartz or silicon wafers. The magnetic gel precursor liquid is added dropwise to the wells and sandwiched between the mold and an opposing substrate. After curing and removing the mold, hydrogel structures are left behind on the surface of the opposing wafer. In both cases, hydration of the hydrogel detaches the structures from the surface of the Si. SQUID magnetometry confirms that the bulk-like magnetic properties of the magnetic gel are tunable via the initial concentration of SPIONs or $Mn(acac)_3$. The addition of SPIONs increases the net moment/volume, while the addition of $Mn(acac)_3$ alters the paramagnetic susceptibility. Preliminary NMR measurements on thin films of the magnetogel show that it is an effective T_2 agent, especially with high concentrations of Mn-acac added to the original precursor (62.5 mM). Further, the effect on T_2 is strongly dependent on the pH of surrounding solution, showing a several fold decrease in T_2 from pH 7 to pH 4. While the concentration of Mn within the magnetic gel is quite high, the net concentration can be tuned by adjusting the number of composite particles. The top down approach for microstructure fabrication described here offers a route to engineer the magnetic and pH-responsive properties of lithographically designed MRI agents.

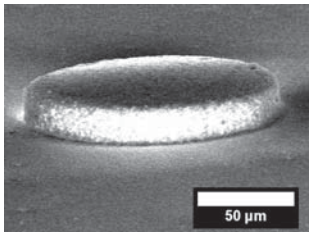

Figure 1. SEM image of microfabricated magnetic-hydrogel composite disc with a diameter of 100 um and a height of 10 um.

Assessing cell-nanoparticle interactions by high content imaging of biocompatible iron oxide nanoparticles as potential contrast agents for magnetic resonance imaging

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Stem cell tracking in cellular therapy and regenerative medicine is an urgent need, superparamagnetic iron oxide nanoparticles (IONPs) could be used as contrast agents in magnetic resonance imaging (MRI) that allows visualization of the implanted cells ensuring they reach the desired sites *in vivo*. Herein, we report the study of the interaction of 3,4-dihydroxyhydrocinnamic acid (DHCA) functionalized IONPs that have desirable properties for T_2 weighted MRI, with bone marrow-derived primary human mesenchymal stem cells (hMSCs). Using the multiparametric high-content imaging method, we evaluate cell viability, formation of reactive oxygen species, mitochondrial health, as well as cell morphology and determine that the hMSCs are minimally affected after labelling with IONPs. Their cellular uptake is visualized by transmission electron microscope (TEM) and Prussian Blue staining, and quantified using an iron specific colourimetric method. *In vitro* and *in vivo* studies demonstrate that these IONPs are biocompatible and can produce significant contrast enhancement in T_2-weighted MRI. IONPs are detected *in vivo* as hypointense regions in the liver up to two weeks post injection using 9.4 T MRI. These DHCA functionalized IONPs are promising contrast agents for stem cell tracking by T_2-weighted MRI as they are biocompatible and show no evidence of cytotoxic effects on hMSCs.

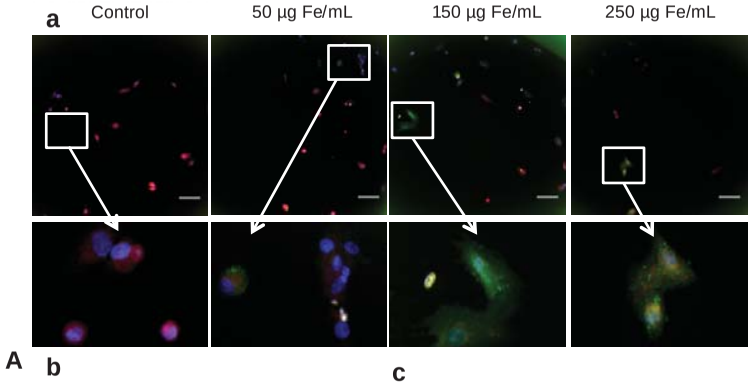

Figure 1. a) Cell viability (yellow), oxidative stress (green) and mitochondrial health (red) of hMSCs labelled with IONPs at various concentrations and determined by high-content imaging reveals significant induction of reactive oxygen species (ROS; green colour) at 10 and 50 µg Fe/ml. Scale bar 100 µm. b) Relative viability and c) production of reactive oxygen species determined by high-content imaging. Ref: ***Scientific Reports*. (2017) 7: 7850**

Highly biocompatible dextran-coated superparamagnetic iron oxide nanoparticles for magnetic resonance imaging

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Rising criticism of currently available gadolinium-based contrast agents for magnetic resonance imaging evoked the need for safer and more versatile agents. In the present study, we demonstrate the suitability of novel dextran-coated superparamagnetic iron oxide nanoparticles ($SPION^{Dex}$) for biomedical applications in terms of safety and biocompatibility. We developed a synthesis procedure to tune the particle size in the range between 30 and 130 nm. A crucial part of this study was to investigate the size-dependency of their imaging properties. For the latter purpose, we adopted a simple and easy-to-perform experiment to estimate the relaxivity of the particles by measuring their susceptibility (see figure 1). Furthermore, we performed an extensive analysis of particles' storage stability under different temperature conditions, showing their superb stability and the lack of any signs of agglomeration or sedimentation during 12 weeks. Furthermore, the particles showed a remarkable biocompatibility, independent of their size. For example, $SPION^{Dex}$ displayed no irritation potential in a chick chorioallantoic membrane assay. Cell uptake studies confirmed their internalization by macrophages, but not by non-phagocytic cells. Additionally, CARPA experiments in pigs treated with $SPION^{Dex}$ indicated the absence of hypersensitivity reactions. These results emphasize the exceptional safety of $SPION^{Dex}$, setting them apart from the existing SPION-based contrast agents, and making them a very promising candidate for further clinical development.

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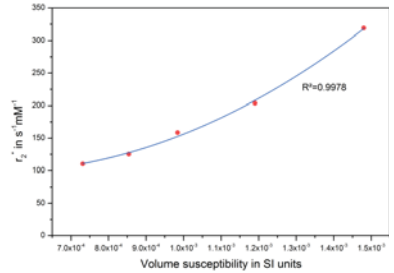

Figure 1: Good quadratic correlation between relaxivity and susceptibility of the particles.

Magnetic Nanoparticles in a gelatin matrix: A model system to study temperature dependent particle-matrix interactions in Magnetic Particle Imaging

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Magnetic particle imaging (MPI) is a novel biomedical imaging modality which maps the spatial distribution of a magnetic nanoparticle (MNP) tracer, by measuring the induced dynamic magnetisation when an AC magnetic excitation field (typically 25 kilohertz) is applied to the sample. One of the key assertions made about the technique is that it is non-invasive, as the superparamagnetic iron-oxide probes which are typically employed as a tracer material, as well as the magnetic field amplitudes and frequencies used, are deemed insufficient to impact upon the physiology of living tissue. Nevertheless, when magnetic nanoparticles dispersed in biological tissue or another medium are excited by an AC magnetic field, they may impact upon the surrounding matrix in two ways. The particles may exert mechanical stress on the surrounding matrix as they attempt to align with the field through Brownian rotation, and local heating may be generated depending on the frequency and amplitude of the excitation field used.

Here we study the behaviors of various MNP types dispersed within an immobilising gelatin matrix, which partially simulates the conditions within biological tissue. Temperature-controlled magnetic particle spectrometry (MPS) (in essence a simplified 0-dimensional, but highly-sensitive version of an MPI measurement), is used to demonstrate varying interactions between different MNP tracers and the gelatin matrix in which they are immobilized. The differing temperature and field dependences of the MPS signals in the immobilized state are used to probe the interactions between the particles and the matrix. We observe that the temperature at which the particles become mobile during melting of the gelatin matrix is dependent upon both the field amplitude and MNP system used. For the common MPI tracer Ferucarbotran in gelatin (iron concentration: 5 mmol/L), the onset of the melting transition in which the particles lose their immobilization is lowered by around 2 K when the field amplitudes are increased from 6 mT to 25 mT. The concentration of the particles is insufficient to generate any measurable heating within the bulk material, thus if the observed effects are the result of heating, then this is an indicator of local hotspots in the vicinity of the individual nanoparticles dispersed within the gelatin matrix.

These results demonstrate that we are capable of probing the interactions between the MNPs and the gelatin matrix, and that the impact of MNP probes on their local environment during MPI measurements is non-negligible. Additional work is underway to distinguish the contributions arising from mechanical strains placed on the gelatin matrix, and local heating generated by the nanoparticles. We will present analysis spanning various MNP systems and excitation fields. Our results are of relevance not only for MPI research, but also impact within the field of magnetic hyperthermia, where there is an ongoing debate about the contributions arising from the mechanical rupture of cell structures, and local hotspots close to individual nanoparticles.

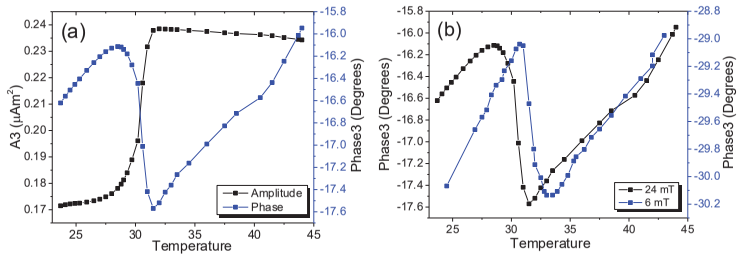

Figure1: Temperature-dependent MPS results from dilute FCT dispersed in gelatin. a) amplitude and phase of the 3rd harmonic at 25 mT, showing the melting transition of the gelatin. b) 3rd harmonic phase at 25 mT and 6 mT. The offset between the two melting events indicates a difference in particle-matrix interaction between the two measurements. This may be due to mechanical forces or local heating.

Acknowledgement: This work was supported by the DFG grant FKZ TR 408/9-1

Temperature effects in quantitative magnetic particle imaging of ferucarbotran nanoparticles

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Magnetic particle imaging (MPI) measures the response of a magnetic nanoparticle (MNP) tracer to an alternating (kilohertz frequency) magnetic drive-field. The nonlinear magnetisation curve of the particles produces an induced signal containing higher harmonics of the drive-field frequency. The spectral response of the MNP obtained by Fourier analysis provides a specific fingerprint which is used as a quantitative imaging signal. An additional field gradient for spatial encoding enables the reconstruction of images of the MNP distribution within the sample volume, without any tissue contribution.

The temperature dependence of MPI signals has been noted in recent publications [1-3], with remote thermometry, and even the reconstruction of temperature-resolved MPI images suggested as future applications. However, an in-depth study of the temperature dependence of MPI tracer spectra, and the impact of varying temperature on the quantitativity of MPI output is currently lacking.

First, we present results obtained using a magnetic particle spectrometer (MPS) (zero dimensional MPI scanner with exceptional sensitivity), which show significant and differing temperature dependences in the spectra produced by leading MPI tracer candidates. Next, temperature dependent measurements obtained using two different methods to control sample temperature within the preclinical MPI scanner at Charité University Hospital Berlin are presented. The first method measures the cooling behaviour of a tracer sample immersed in a small container of warm water, with no external temperature control. The second method uses a 3D printed sample holder, in which the tracer temperature is accurately controlled by heated water circulated from a pump located outside the MPI scanner's shielded room. We report strong agreement between the measurements using each method within the MPI scanner, and MPS. Furthermore, we present reconstructed images of MNP samples measured at different temperatures by MPI, in which the impact of varying temperature on the quantitativity of the image reconstruction is clearly demonstrated. A clear agreement is observed between data recorded using signal-receive coils located at different positions within the MPI scanner, as well as between the two methods for controlling sample temperature. This indicates that the observed effects are a result of the temperature induced changes in the MNP spectra, and not the result of heat leaking from the sample holder to the signal-receive coils. The alterations observed within reconstructed MPI images at varying temperatures suggest that temperature is an important parameter to be considered in the development of quantitative MPI. In addition, the presented measurements indicate sufficient sensitivity for developing temperature resolved MPI at the Berlin MPI scanner.

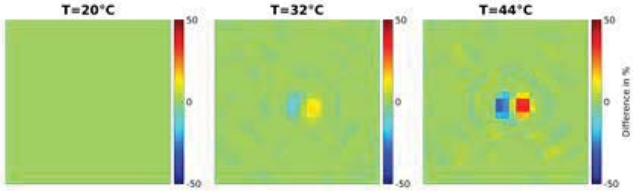

Figure 1: Difference images (subtracting T=20°C image values from each reconstructed image) using a point-like 30 µL sample of the common MPI tracer Ferucarbotran (FCT) measured at varying temperatures) non-zero values highlight alterations in image reconstruction at increasing temperatures, due to the temperature dependent dynamic magnetic behavior of FCT. The signals are scaled by the maximum intensity at 20°C.

[1] J. B. Weaver et al, Med Phys. 2009 May; 36(5): 1822–1829.
[2] C. Stehning et al, IJMPI, Vol 2, No 2, 1612001
[3] J.Wells et al. AIP Advances 8, 056703 (2018)

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Long-term stable measurement phantoms for multimodal imaging of magnetic nanoparticles

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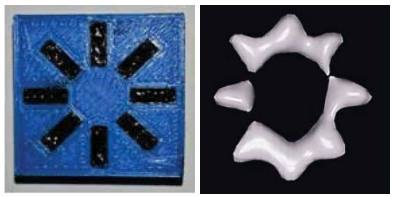
Magnetic particle imaging (MPI) is a promising imaging method to determine the spatial distribution of magnetic iron oxide nanoparticles (MNP) within a volume. To investigate the imaging capabilities of existing MPI scanners, their detection limits and image resolutions have to be evaluated. Hence, precisely defined magnetic structures (phantoms) are required to enable comparative studies between different MPI systems, to monitor the temporal stability and quantitative qualities of individual MPI scanners, but also for comparison with other imaging modalities, e.g. magnetic resonance imaging (MRI) or computed tomography (CT).

To help with the development of suitable MPI phantoms, we developed a method to incorporate different commercially available MNP into a long-term stable synthetic polymer with an iron concentration of up to 200 mmol/l. The properties of each type of polymer matrix embedded MNP were tested by magnetic particle spectroscopy (MPS) and MRI.

From all tested materials, the combination perimag®/ELASTOSIL® was the most promising with regard to long-term stability and signal performance. This material was then used in the preparation of phantoms for further studies. For shaping the particle loaded polymer, we used 3-D printed molds with a structure cross section of 2 x 2 mm^2, see figure. The particle loads within the phantoms were then imaged using MPI, MRI, and CT.



Photograph (left) and MPI reconstruction (right) of a phantom (20 x 20 mm edge length).

The MPS measurements after embedding the MNP into the polymer revealed a decrease of the higher harmonics, which is caused by inhibition of Brownian relaxation. Repeated MPS measurements (until now up to one year) showed no change in the magnetic properties of all samples. The particle-loaded polymer could be visualized by MPI, MRI, and CT.

In conclusion, we developed long-term stable measurement phantoms for multimodal imaging. Further work will focus on more detailed particle loaded structures in the phantoms and water based polymers with a homogeneous particle distribution.

Acknowledgements
The authors thank Sylvi Herzog (TU Ilmenau) for assistance during the CT scan. This work was supported by Deutsche Forschungsgemeinschaft (FKZ: DU1293/6-1, TR408/9-1, and AL552/8-1).

Excitation frequency dependence of temperature resolution in magnetic nanoparticle temperature imaging with a scanning magnetic particle spectrometer

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Non-invasive and in-vivo temperature imaging is crucial to biomedical applications, such as magnetic hyperthermia for cancer treatment and thermally-controlled drug delivery. Recently, magnetic nanoparticle (MNP) temperature imaging has been reported with a scanning magnetic particle spectrometer (SMPS). The 1st and 3rd harmonics of the MNP magnetization in an ac magnetic field are measured with the SMPS to realize MNP temperature imaging. The harmonic ratio, which is independent of MNP concentration but dependent on MNP temperature, is used to determine temperature.

This contribution reports on the simultaneous imaging of MNP concentration and temperature with a custom-built SMPS, in particular the dependence of temperature resolution on the frequency of the applied ac magnetic field is studied. Experiments on a multi-line phantom (see Figure 1a) are performed in different-frequency ac magnetic fields. A hot-water tube with cycling water is used to change the temperature profile of the phantom, as shown in Figure 1a. Figure 1b shows the temperature profile measured with the SMPS at 2004 Hz and 10 mT. As a consequence of the calibration of the harmonic ratio with an IR camera, the temperatures measured at the same y position but different x positions are the same - independent of frequency - due to heat dissipation from the hot-water tube to the MNP sample. Thus, the standard deviation of the temperatures measured at the same y position but different x positions is used to characterize the temperature resolution. Figure 1c shows the magnetic field frequency dependence of the temperature resolution. It indicates that the temperature resolution improves with increasing the frequency, which is mainly caused by a higher signal-to-noise ratio due to Faraday's law.

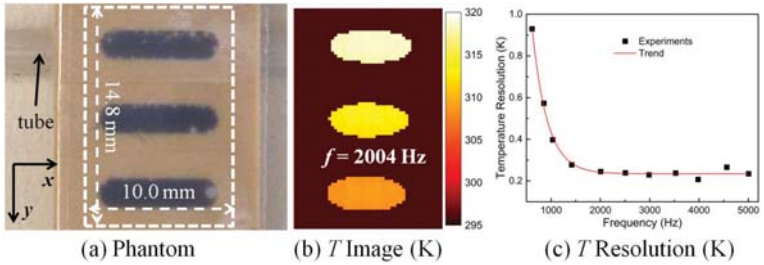

Figure 1. (a) the photo of the phantom. (b) Temperature imaging of the phantom in a 2004-Hz ac magnetic field. (c) Temperature resolution versus frequency curve. Symbols in (c) represent experimental data whereas solid line represents a guide to the eye.

Measurement of the magnetic movability of different superparamagnetic particles

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The movement of magnetic particles due to a magnetic field gradient is of great interest in different application fields. In this report, we present a technique based on a magnetic tweezers setup to measure the magnetic velocity factor of individual superparamagnetic particles in a fluidic environment. We show our results for different magnetic particles with hydrodynamic diameters between 100 and 5000 nm from diverse manufacturers.

The main part of the experimental setup is shown in Fig. 1. With the help of two electromagnets and soft-magnetic focusing tips, it is possible to generate almost parallel magnetic field lines in the centre area between the tips. The magnetic gradient can be set by applying different currents over these coils. With the help of optical image processing of the magnetic tweezers microscope videos, the trajectories of individual particles at different magnetic gradients were tracked. The particle speed is determined and the so-called velocity factor, a particle property independent of fluid viscosity and field gradient, is calculated. Care is taken that just individual particles are tracked in order to exclude cooperative effects like chain formation. From the tracking of multiple individual particles, statistics of one particle type is obtained within one measurement run. Particles which are smaller than the optical resolution limit were characterized by means of fluorescence microscopy.

The resulting velocity factors for different superparamagnetic particles from several manufacturers are shown in Fig. 2. These measurement data can, for example, be used to determine design parameters for a magnetic separation system, like maximum flow rate and minimum separation time, or to select suitable particles for fixed experimental requirements.

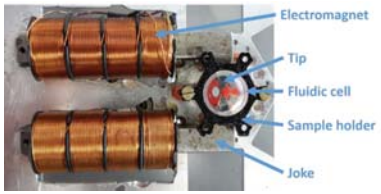

Fig. 1. Picture of the setup to measure the velocity of different magnetic particles by microscopic optical image tracking. The setup consists of two electromagnets fixed on a yoke, which directs the magnetic field towards the fluidic cell. In the fluidic cell, two tips are fixed which generate a magnetic field gradient with almost parallel field lines in the middle of the fluidic cell.

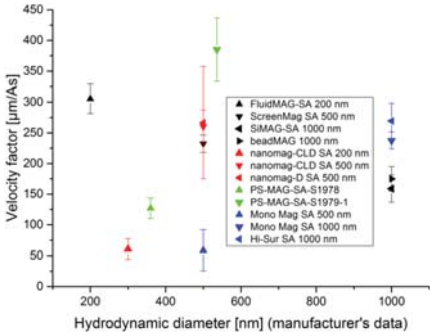

Fig. 2. Velocity factor as a function of the particles' hydrodynamic diameter provided by the manufacturers. The different manufacturers are color-coded while the different particle types of one manufacturer are marked with different symbols. For each particle type, also the error bars of the velocity factors are given. Companies: Chemicell (black), micromod (red), microParticles (green), and Ocean NanoTech (blue).

Phosphate modified magnetite nanoparticles for extraction and preconcentration of Zirconium in solution

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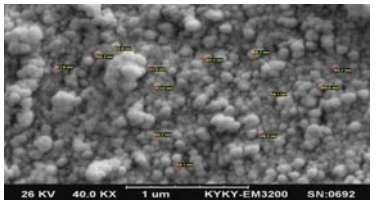
The solid phase extraction (SPE) is the most common method for sample preparation extraction and preconcentration. SPE has better isolation; lower detection limits and provides higher accuracy and precision than another method for extraction. This method has better repeatability. In this paper we have introduced a novel magnetic iron oxide (Fe_3O_4) nanoparticles Shell with SiO_2[1] and then modified with phosphate groups as adsorbent for zirconium preconcentration by SPE method. Absorbent nanoparticles were characterized by X-ray diffraction (XRD), scanning electron microscopy (SEM), and FT-IR.

The ability of modified magnetic nano-particles was investigated as a new adsorbent for preconcentration of Zirconium (as Zirconium- alizarin red S complex) in aqueous solution. The influences of the experimental parameters on Zirconium adsorption and desorption have been studied. Zirconium-alizarin complex after preparation were mixed with nano adsorbent and sonicated for a given time. Therefore was eluted with suitable elutant and Zirconium amount determined by spectrophotometry uv-vis at λ 525 nm .The effect of different parameters in elution such as pH, Concentration of the reagent, kinds of eluting reagent and volume of the eluted and sample were studied.

Precision determination ($n = 5$) for 0.25 mg L^{-1} of Zr was 0.0021 %RSD. The detection limit (3SD) of Zr was found to be 1.28 mg L^{-1}. And dynamic linear range for Zr determination was 0.2 to 1.2 mg L^{-1}.



TEM image of $Fe_3O_4@SiO_2.PO_3$

[1]Emadi, masoomeh: Shams Esmaeil AIP Conference Proceedings. 2010, vol 1311 . Pp 127-134.

In-situ orthogonal observation of particles deposition process on a ferromagnetic filter during high-gradient magnetic separation process

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In High Gradient Magnetic Separation (HGMS), magnetic particles suspended in a fluid are separated in a flow based on the magnetic force due to the steep magnetic field gradient generated by the magnetization of ferromagnetic fielter wires. In HGMS, the opening of the filter is much larger than the particle size, therefore, loss of pressure is samll enough. Furthermore, filter can be reused easily because collected particles detouched easily from the filter when the magnetic field removed. To optimize the condition for the magnetic separation process, simulations are often carried out, however, the effect of the deposited particles is difficult to consider in the simulation. Information about the way of particles deposition on the filter wire seems to contribute for the optimization of separation condition in practical processes. Therefore, we have been carried out *in-situ* observations of particles deposition process on the filter in HGMS. In previous meeting, we reported the result of *in-situ* observation from the direction perpendicular to the fluid flow. It was observed that the spike-like structure was formed toward the upper stream direction. As a results, the length of the spike structure tends to be long with lower applied magnetic field and lower flow velocity. In our recent study, we have succeeded to carried out simalteneous observation from two different direction. In addition to the conventional observation setup, a CCD camera was set on downstream of the flow. It gives us an orthogonal view of the ferromagnetic filter against the conventional observation. The magnet used in this study was cryocooler operated type of superconducting manget that can generate up to 13 T. The housing of the filter was made of acryl. A CCD camera, model QN42H of ELMO Co. Ltd., was utilized for the observation from the direction perpendiculat to the flow. The other CCD camera, model UN43H of ELMO Co. Ltd., was used for the observation along the flow direction. The filter used here was made of SUS430 whose whole diameter and wire diameter were 25 mm and 0.22 mm, respectively (30 mesh). Magnetite particles (Wako pure chemical Industries, Ltd.) were suspended in distilled water and was used for the experiment. Sample suspension was flowed from the above of the magnet and the behavior of particles near the filter set at the magnetic field center was observed. A series of experiments were carried out with changing the applied mangetic field and flow rates. We have succeeded to make simaltenous orthogonal observation of the particle deposition process in HGMS. Details of these observations will be reported in this presentation.

Synthesis of peroxy-functionalized magnetic mesoporous silica as recyclable oxidizing agents for epoxidation of alkenes

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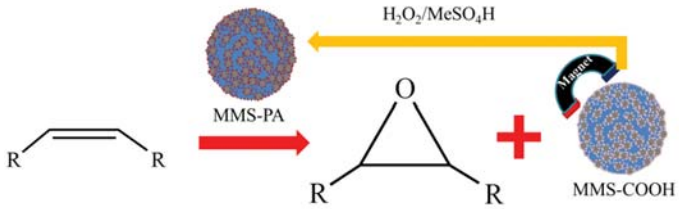
Epoxides, the products from epoxidation of alkenes, are versatile building blocks and intermediates in the synthesis of many valuable compounds such as pharmaceuticals, biotechnological products, adhesives or paints. Typically for the epoxidation reactions, homogeneous oxidants (epoxidants) such as tert-butyl hydroperoxide (t-BuOOH), m-choloperbenzoic acid (mCPBA) or other peroxy acids are commonly used. However, heterogeneous oxidants are remarkable in that they are less complicated for reusability and separation after reaction.

Magnetic nanoparticles (MNPs) have been widely utilized in separation process and heterogeneous catalyst system because of their unique superparamagnetic character that could facilitate separation and recovery of the materials. However, MNPs have some limitations such as low surface area and complicated functionalization compared to porous materials, leading to our interest in combining porous materials with magnetic nanoparticles. Mesoporous silica is a well-known catalyst and catalyst support due to their high surface area, chemical stability and ease in functionalization using silane coupling agents. Thus, in this work heterogeneous oxidants consisting of MNPs, mesoporous silica and peroxy acid as called peroxy-functionalized magnetic mesoporous silica (MMS-PA) composites were synthesized. The composites were confirmed that they contained peroxy acids, which act as a good oxidant for epoxidation, using fourier transform infrared spectroscopy (FTIR). Transmission electron microscope (TEM) revealed that the MMS-PA composites contain both magnetic nanoparticles and porous silica in the same particles of irregular shape. Surface area of the MMS-PA was 1048 m^2g^{-1}, similar to the base mesoporous silica. As measured using vibrating sample magnetometer (VSM), MMS-PA exhibit saturation magnetization of 0.12 emu/g, indicating that the heterogeneous oxidant could respond to an external magnet. For the investigation of oxidizing activity of the composites using nuclear magnetic resonance (NMR) spectroscopy, freshly prepared MMS-PA gave epoxide of vinyl acetate (VA) up to 51 % yield, and the yield decreased to 32 % after recycled by treating with concentrated H_2O_2 in acidic medium. In this work, we demonstrated that MNPs can be incorporated into mesoporous silica, and the composites can become heterogeneous oxidant with a magnetic response in the epoxidation of an alkene model.



Label-less separation of the magnetic biological entities through combination of numerical analysis and magnetic separator systems.

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Magnetic cell separation has found its place in the medical and life science field for various purposes; including isolation of cells for further research and clinical diagnosis/prognosis. The traditional method uses antibody, magnetic particle conjugation to target specific cells; but recent study of intrinsic magnetic property of various biological entities, including red blood cells (RBCs) in different chemical state and some cancer stem cells has led to investigation of their potential for label-less magnetic separation. Over the years, various methodologies have been used for this study, such as characterizing of the magnetic mobility of samples using cell tracking velocimetry (CTV), using powerful programming tools such as Maple or Matlab for numerical analysis of the magnetic separation systems, and many more.

Through incorporation of the finite element analysis (FEA) into the study, the possibility of developing a high-throughput magnetic separation system was explored. The Finite Element Methods Magnetics, a suite program that can solve electromagnetics problem using FEA methods, provided the magnetic gradient field of the developed magnetic separation system, and the extracted B data was further transferred to ANSYS Fluent, a computational fluid dynamics (CFD) program that can solve flow problems. With a combination of custom user defined function that incorporates the magnetic force balance of the CTV characterized RBCs, the trajectories of paramagnetic deoxygenated red blood cells were numerically modelled, which resulted in the deposition of the RBCs in the separation channel as shown in the **Figure** below. The result portrays that it is possible to change the deposition location for diagnostic purposes (such as deposition of target population with specific magnetic mobility) or for changing the throughput of the separation device with varying feeding points.

Numerical simulation of the pilot-scale magnetic separation system through CFD and FEA methods will provide results leading to the optimum condition for separation, which will eventually aid in design of a high throughput magnetic separation system good enough for clinical applications.

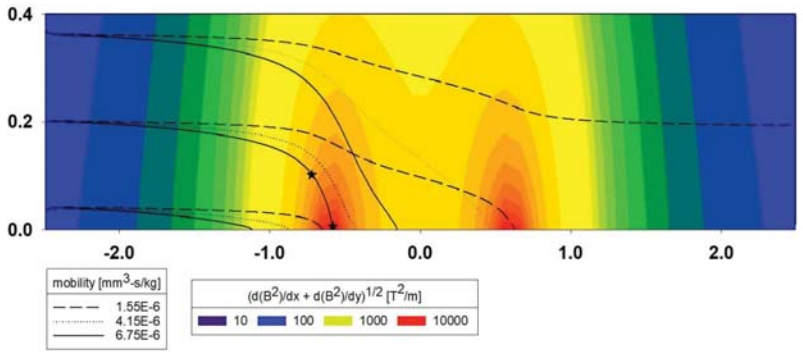

Figure. The trajectory of CTV characterized deoxygenated RBCs with three different mobilities (mean, -1 standard deviation and +1 standard deviation) at three different feeding points.

Equilibrium magnetization and magnetophoretic mobility of a spherical cluster of single-domain nanoparticles

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Magnetic properties of a rigid spherical cluster of uniaxial single-domain particles are investigated numerically using the stochastic Landau-Lifshitz-Gilbert equation. The spatial and orientation distribution of particles within the cluster is random and uniform. Dipole-dipole interactions between particles are taken into account. The particles are monodisperse. It is shown that the cluster equilibrium magnetization is generally lower than predicted by the classical Langevin model and that both anisotropy and interactions contribute to the magnetization decrease. For magnetically isotropic particles, the initial slope of the magnetization curve can be successfully described by the modified mean-field model, which was originally proposed for the description of concentrated ferrofluids (*A.O. Ivanov, O.B. Kuznetsova // PRE 2001*). In moderate and strong fields, the theory overestimates the cluster magnetization. However, the discrepancy can be minimized by adjusting the mean-field parameter (see Figure). The phenomenological generalization of the modified mean-field theory is proposed to take into account the effect of magnetic anisotropy on the cluster magnetization curve. Based on obtained results, the magnetophoretic mobility of the cluster in the gradient field is calculated as a function of the cluster size, the particle concentration and the magnetic moment of particles. The work was supported by Russian Science Foundation (Grant No. 17-72-10033).

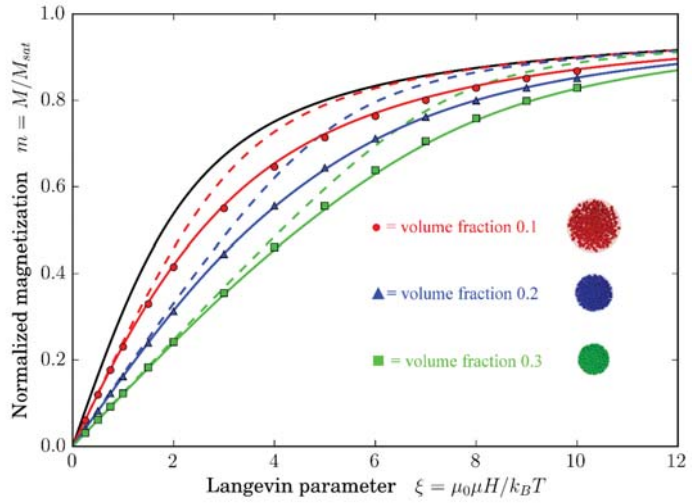

Figure. Equilibrium magnetization curves for a cluster of magnetically isotropic particles. Symbols – simulation results for different values of the particle volume fraction. Dashed lines – predictions of the standard modified mean-field theory for the same volume fractions. Uppermost solid curve is the Langevin magnetization, other solid curves correspond to the modified mean-field theory with the adjusted mean-field parameter.

Continuous magnetic depletion of erythrocytes from whole blood with a quadrupole magnet and annular flow channel

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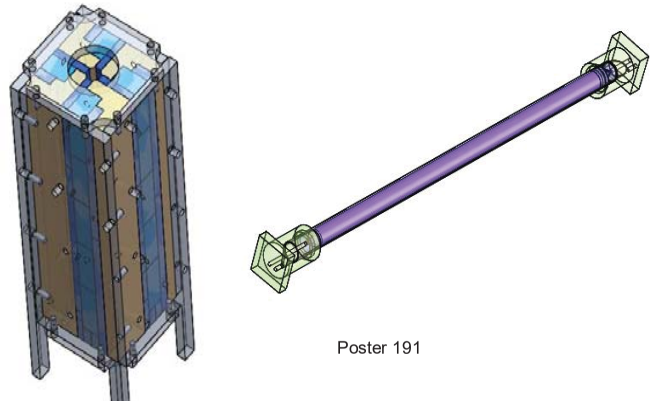
In biomedical research and in medicine, it is often desirable to remove red blood cells (RBCs) from a whole blood sample, thereby enriching the white cell fraction, which might contain stem cells or circulating tumor cells. The classical means or RBC removal includes centrifugation in a density gradient or exposure to hypotonic solution. Both techniques have the disadvantages of potential harm and significant losses to target cells, and integrate poorly with continuous processes, such as MEMS. An alternative approach takes advantage of the fact that unpaired electrons in deoxy hemoglobin make the overall RBC paramagnetic when oxygen is removed; otherwise, oxy hemoglobin is weakly diamagnetic, as is the overall RBC. Thus, we might consider deoxy RBCs to be biomagnetic microparticles. We have developed a Quadrupole Magnetic Sorter (QMS) comprising a magnet assembly and flow channel. The magnet assembly places off-the-shelf neodymium magnet blocks into the negative spaces of low carbon steel pieces for a maximum flux density of 1.22 T in the aperture. The magnet aperture has a diameter of 9.65 mm and length of 203 mm. The flow channel employs high-resolution 3D-printed manifolds, inlet and outlet, each with dual flows, while simultaneously supporting an axi-symmetric stainless steel rod and cylinder. The sort begins with dilute blood placed in a sample vessel while pure nitrogen is sparged over the stirred suspension. When the RBCs are fully deoxygenated the flows of sample, carrier, and enriched and depleted outlet fractions, begin. First, dilute whole blood was processed under variable flow rates to achieve optimal outcomes. More than 90% of RBCs were recovered in the enriched outlet at a total flow rate of 0.05 ml/min and inlet/outlet flow rate ratios of 0.5 and 0.55. Next, to mimic non-erythroid cells, 15.8-µm polystyrene microspheres (PS) were added to blood to yield a 90:10 RBC:PS number ratio. Again a sweep of the flow rates, showed an optimal total flow rate of 0.1 ml/min of 97% RBC recovery in the enriched outlet at 98% purity. These findings will be compared with modeling results.

Quadrupole magnet assembly and flow cell



Interferon gamma transcript detection on T cells by combining three types of magnetic separation

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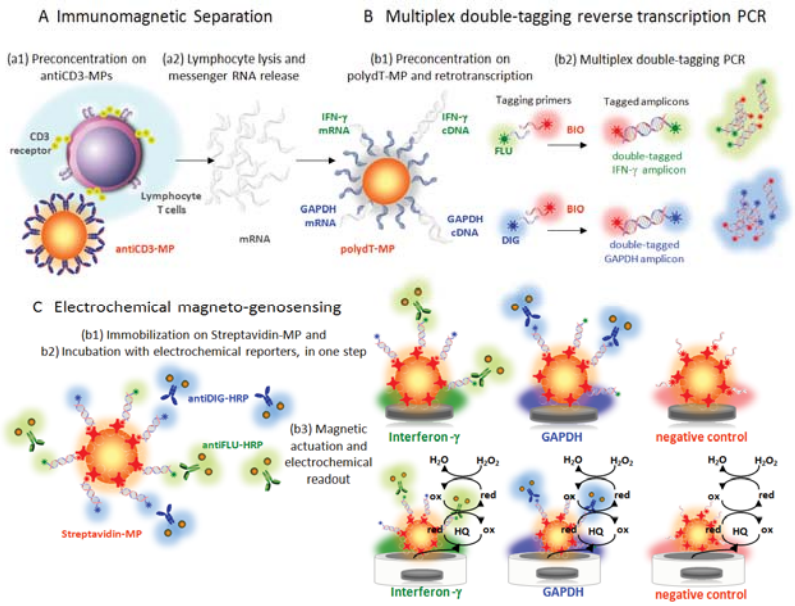
Interferon-γ is a proinflammatory cytokine, and its production is related with effective host defense against intracellular pathogens. Therefore, the level of interferon-γ is considered a good biomarker for intracellular infections [1]. Beside this, it is also useful for the assessment, treatment progression and follow-up of non-communicable diseases, including cancer and autoimmune disorders, among others. This work addresses the development of a new strategy to evaluate the expression of interferon-γ transcripts produced by stimulated T lymphocytes cells as biomarker. The method sequentially combined three different types of magnetic separation, including the immunomagnetic separation of the T lymphocytes performed with antiCD3 magnetic particles [2]. After that, the isolation and preconcentration of polyadenylated mRNA followed by the multiplex double-tagging RT-PCR amplification of the interferon-γ and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) genes (as a housekeeping control) were performed on polydT magnetic particles. Finally, the multiplex electrochemical genosensing is performed on streptavidin magnetic particles as a support [3]. This approach is able to quantify the levels of cellular interferon-γ produced by as low as 150 T cells with outstanding analytical features to be considered as a promising strategy for the quantification of this important biomarker for several clinical applications.



References

1. Van de Vosse, E., Hoeve, M. A., Ottenhoff, T. H., **2004**. Lancet Infect. Dis. 4(12), 739-749.
2. Carinelli, S., Ballesteros, C. X., Martí, M., Alegret, S., Pividori, M. I., 2015. Biosens. Bioelectron. 74, 974-980.
3. Ben Aissa, A., Jara, J. J., Sebastián, R. M., Vallribera, A., Campoy, S., Pividori, M. I., 2017. Biosens. Bioelectron. 88, 265-272.

Electrochemical biosensing of cancer exosomes in human serum based on magnetic separation

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The identification of novel biomarkers represents a worldwide challenge not only for the improvement of early diagnostics, but also for patient monitoring and for the evaluation of the efficiency of a therapeutic strategy. Exosomes are nano-sized and cup-shaped vesicles [1] (Figure 1), which are currently under intensive study as potential diagnostic biomarkers for many health disorders, including cancer [2]. Therefore, this is a growing need for sensitive methods capable of accurately and specifically determining the concentration of exosomes. This work addresses the study of different receptor by flow citometry (Figure 2) as well as the design of a quantitative and rapid method for total exosome counting based on magneto-actuated platforms with electrochemical readout. Two different strategies were explored for the magnetic separation of exosomes. Briefly, based on i) the direct covalent immobilization on tosyl-activated magnetic particles or, instead, by ii) immunomagnetic separation based on different receptors (Figure 2). The magneto electrochemical biosensor for the exosomes counting was successfully achieved in human serum. This proof-of-concept device represents a rapid, cost-effective, and high-sample-throughput detection of exosome and can be potentially established as promising approach for cancer diagnostics based on liquid biopsy.

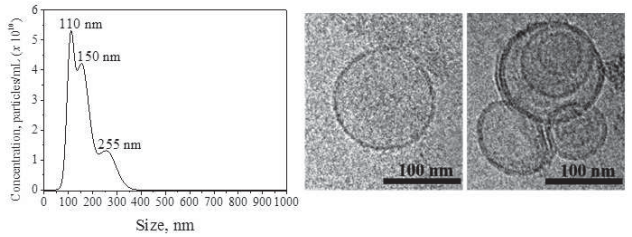

Figure 1. Nanoparticle tracking analysis (NTA) and Cryo TEM of purified exosomes from MCF-7 breast cancer cell line.

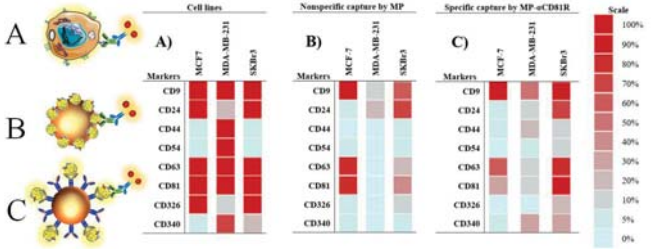

Figure 2. Flow cytometry study for (A) MCF7, MDA-MB-231 and SKBr3 breast cancer cell lines, and their respective exosomes, (B) directly immobilized on magnetic particles or (C) isolated by immunomagnetic separation on antiCD81-MP.

References

[1] Théry, L. Zitvogel, S. Amigorena, Nature Reviews Immunology, 2002, 2, 569.
[2] T. An, S. Qin, Y. Xu, Y. Tang, Y. Huang, B. Situ, et al. Journal of Extracellular Vesicles. 2015, 4, 1.

Magneto-actuated rapid test for the detection of circulating tumor cells

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According to WHO, breast cancer is the top cancer in women both in the developed and the developing world, and the number of new cases is expected to rise by about 70% over the next two decades. The early and accurate detection of breast cancer as well as the risk of metastasis in small healthcare centers remains as the cornerstone of breast cancer control. This work is intended to contribute in the development of Rapid Diagnostic Test (RDTs) for cancer diagnosis at point-of-care in low resource settings, taking breast cancer circulating tumor cells from MCF7 cellular line as a model. Two different strategies were designed: a magneto-actuated immunosensor for the quantification of the breast cancer cells and a magnetic genosensor for the detection of the PCR-amplified genetic material from the cells. For that purpose, different commercial antibodies against specific epitopes of the cellular membrane were firstly studied by flow cytometry and confocal microscopy. Such antibodies were then covalently immobilized on magnetic particles to capture the tumor cells by immunomagnetic separation for the preconcentration of the cells from complex samples and immunosensing with an specific antibody (Figure 1). The magneto genosensing approach is based on a double-tagging RT-PCR amplification of the transcripts from the cells and the quantification of the amplicon by amperometry technique or visual readout based on lateral flow. Finally, the results of these strategies are compared in terms of the analytical performance, showing promising features for being used as RDTs.

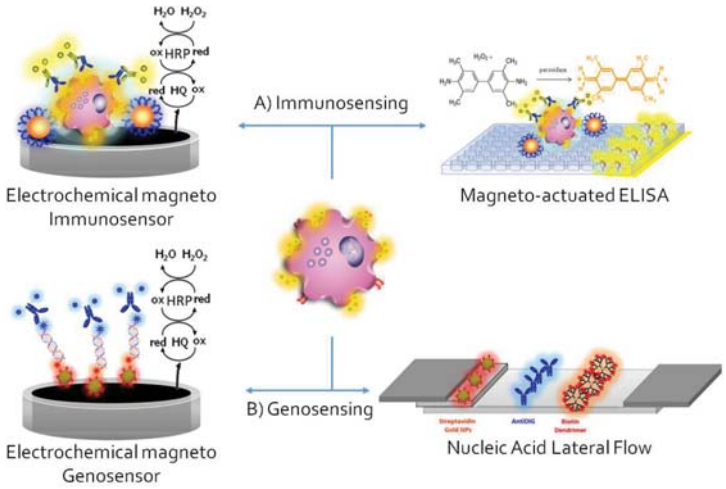

Figure 1. Schematic procedure of the different rapid test compared in this work for the detection of circulating tumor cells based on magnetic separation.

Synthetically-driven Assessment of Magnetic Properties of Nanocrystals and Nanostructures for Magnetic Separation Applications

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Nanoparticles of magnetic materials are very useful in different bio-related applications, on which the combination of chemistry and magnetic performance will determine their final purpose. Examples of magnetic nanoparticles and nanostructures synthesized and manipulated by wet-chemistry methods will be detailed to demonstrate how to exert control over the final magnetic behavior and over their ultimate functionalities, considering particularly magnetic separation and guidance.

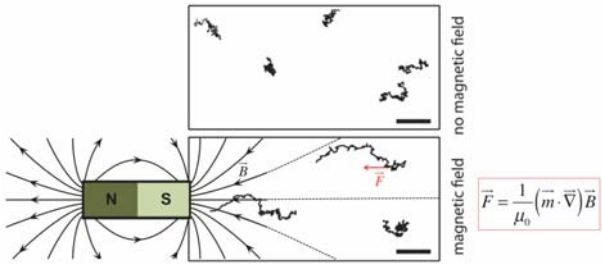

Figure 1. Representative trajectories of magnetic swimmers in the absence and presence of an applied magnetic field.

References

R. Otero-Lorenzo, M. A. Ramos-Docampo, B. Rodríguez-González, M. Comesaña-Hermo, V. Salgueiriño. *Solvothermal Clustering of Magnetic Spinel Ferrite Nanocrystals: A Raman Perspective*. *Chemistry of Materials* **2017**, *29*, 8729-8736.

P. Schattling, M. A. Ramos-Docampo, V. Salgueiriño, B. Stadler. *Double-Fueled Janus Swimmers with Magnetotactic Behavior*. *ACS Nano*, **2017**, *11*, 3973-3983.

Hydrodynamic Effect on Transport and Capture of Bio-entities in a Magnetic Aqueous Two Phase System (ATPS)

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Aqueous Two Phase System (ATPS) comprise of two immiscible aqueous solutions (aqueous polymer or salt solutions), is used for transport and extraction of different bio-entities like proteins and cells. ATPSphenomenons have been traditionally used as separation platforms for cells and biological entities based on their affinity and biocompatibility. This study reports on an analytical model for prescribing the transport and capture of magnetic microsphere from one phase to another phase, mimicking a typical immunomagnetic micro extraction of bio-entities conjugated with magnetic microspheres, in an ATPS with the help of externally applied magnetic field. The microchannel considered for this study consists of two inlets (I_1, I_2) and two outlets (O_1, O_2), where two co-flowing streams are flowing parallelly asshown in Fig. 1. Pressure driven flow is assumed for two immiscible streams, with a planar interface in between the two immiscible solutions. Also, it is assumed that there is no perturbation effect on the interface during cross stream migration of the microsphere at the interface. No slip boundary conditions are assumed at all the walls with equal velocity and stress boundary condition at the interface is also assumed.

Influence of different physical properties of the fluids is carried out for understanding the effect of fluidic force on transport and capture of magnetic microsphere in the ATPS configuration. The parametric variation involving different flow rates, flow rate ratios, viscosities of the fluids and viscosity ratios is observed. Finally influence of the hydrodynamic effects in the microchannel of magnetic microsphere is characterized by capture efficiency. It is found from the analytical solution that both flow rates and viscosities of both the fluids haveexclusive impact on capturing the microsphere in the ATPS.

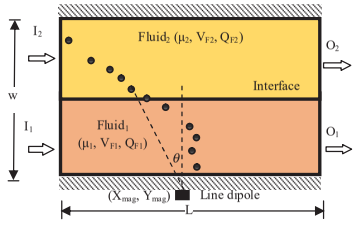

Figure 1: Schematic diagram of the microchannel with two inlets (I_1, I_2) and two outlets (O_1, O_2) with a line dipole at a position (X_{mag}, Y_{mag}) intermediate between inlets and outlets.

A standardised description of magnetic beads for DNA extraction

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Magnetic beads based on magnetic nanoparticles are used for in-vitro diagnostics to extract bacteria, viruses, DNA and other components from water, blood and other body liquids by means of magnetic fields. Currently, the world-wide market of in-vitro analysis kits involving magnetic beads amounts to more than 2 billion €. Several implementations of "liquid biopsy" procedures for detecting circulating tumour DNA based on magnetic separation techniques have been described. Speed and efficiency of the magnetic separation process are decisive for accuracy, safety and cost-effectiveness of the diagnostic method. However, no harmonised definitions and measurement methods exist for the characterisation of the magnetic performance of the beads. In 2017, the International Organization for Standardization (ISO) started to develop in ISO/TC229 "Nanotechnologies" a first international standard (ISO 19807-2) defining the main characteristics of superparamagnetic beads that are used for nucleic acid extraction. Here, we compile the main requirements on such a standardised description of the magnetic beads. The starting point for all interaction between different market participants involving magnetic beads is a clear definition of the terminology for characteristics and procedures. The properties of the beads can be divided into four topical groups: chemical composition, size characteristics, magnetic characteristics (superparamagnetism, mean magnetic moment per bead, saturation magnetisation) and surface characteristics. Different market participants require different sets of characteristics to interact with each other as shown in Fig. 1. As an example, the manufacturers of beads and separation units will focus on the size and magnetic characteristics of the beads in their interaction, whereas the end users are more interested in surface functionality for DNA capture. For each standardised quantity, a measurement procedure must be declared, that is widely available and well established. This offers each party the opportunity to understand the declared value of a characteristic and to verify it.
We propose to decouple the description of the magnetic properties of the beads from the specification of the magnetic separation unit, although the complete information is needed to assess the final performance during a separation process. Within ISO, an ongoing discussion is underway to determine which characteristics should be classified as mandatory for the proper labelling of magnetic beads for DNA extraction.

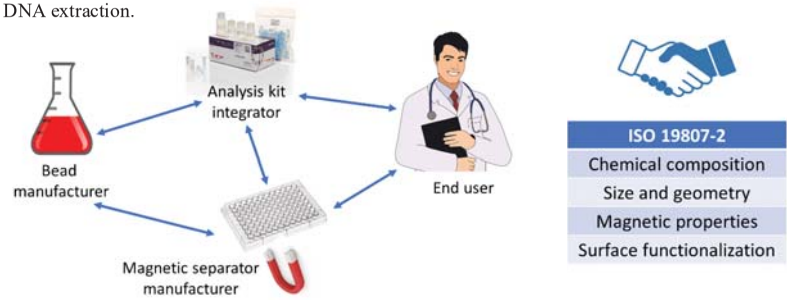

Fig. 1: Market participants benefiting from an international standard on magnetic beads.

Acknowledgement: This project was supported by the EMPIR program co-financed by the Participating States and from the European Union's Horizon 2020 research and innovation program, grant no. 16NRM04 "MagNaStand".

Single cell magnetometry by magnetophoresis vs. bulk suspension magnetometry by SQUID-MPMS

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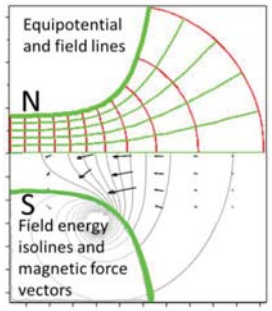
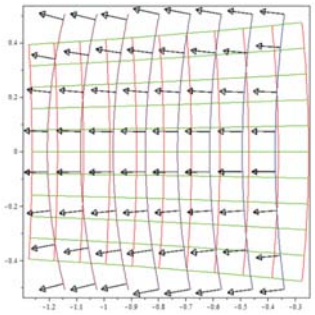
Paramagnetic constituents of a cell have strong effect on cell's volume magnetic susceptibility even at low volume fraction because of their high susceptibility relative to that of the diamagnetic cell constituents. The effect can be measured at a single cell level by measuring cell terminal velocity in viscous media using a microscope equipped with a well-defined field and gradient magnet configuration (a method dubbed cell tracking velocimetry, CTV). The sensitivity of such a microscopic-scale magnetometry was compared to that of a reference method of superconducting quantum interference-magnetic properties measurement system (SQUID-MPMS) using a red blood cell (RBC) suspension model. The RBC hemoglobin oxygen saturation determines the hemoglobin molecular magnetic susceptibility (diamagnetic when fully oxygenated, paramagnetic when fully deoxygenated or converted to methemoglobin). The SQUID-MPMS measurements were performed on an average of 5×10^3 RBCs in 20 μL physiological phosphate buffer at room temperature, those by CTV on an average of 1,000 individual cell tracks per sample in a mean magnetic field of 1 T and gradient of 300 T/m. The mean RBC magnetic susceptibilities were statistically the same between the two methods, for all three forms of the hemoglobin, but only the magnetophoretic analysis provided information about the RBC susceptibility distribution in the sample. In particular, mean volume magnetic susceptibility of deoxygenated RBCs was $(-5.58\pm1.48)\times10^{-6}$, in agreement with previous study [1], and slightly higher than that of water, -9.05×10^{-6}. A minimum of 5,000 RBCs were necessary to perform a single SQUID-MPMS measurement where only 1 RBC was sufficient to perform the same by CTV. We conclude that the magnetophoretic method is 5,000× more sensitive than SQUID-MPMS and may provide means to study emergence of paramagnetic reaction products in the cell.

[1] Zborowski M et al. Red blood cell magnetophoresis. Biophysical Journal 2003; 84: 2638-45



Non-uniform magnetic field globally



Quasi-uniform local magnetic field for magnetophoresis

High Efficient Magnetic Solid Extraction for Heavey Metal Ions Removal

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Water pollution from heavy metal ions such as Cr(VI) and Cd(II) has become one of the most important issues of the world because of its great threat to the environment and human beings[1]. The solid extraction technology which based on adsorption principle is the most promising method for the low concentration heavy metal ions removal. However, the traditional solid extraction technology faces many challenges such as the confliction between the adsorption capacity and adsoption rate, low selectivity, and batch/batch continuous operation mode which limit its industrial applications for the low efficiency. Focus on these problems, we investigated the effect of composition and structure on the separation selectivity and adsorption capacity[2,3]. On this bases, we explored a serial of magnetic adsorbents with high capacity and selectivity such as amine, -C=S, or extractant modified magnetic synthetic polymer/nature polymer/inorganic particles for Cr(VI) and Cd(II) ions removal[4,5]. In addition, novel gas-assisted superparamagnetic extraction technology was proposed for continuous separation of ions uploaded magnetic particles [6].

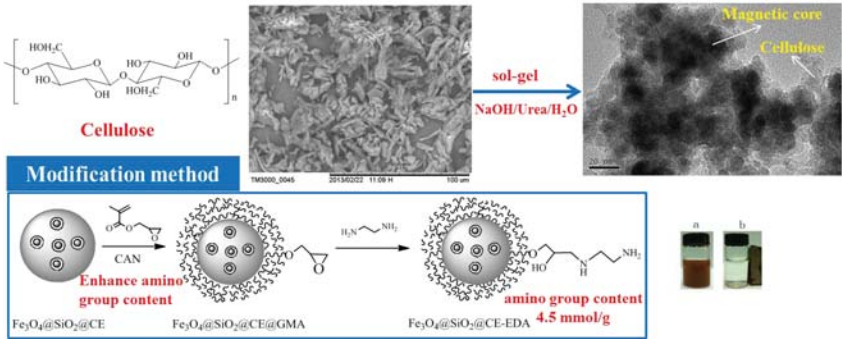

Figure: $Fe_3O_4@SiO_2@CE$-EDA magnetic nanocomposite for Cr(VI) removal

References

[1] Fowler, B.A., Monitoring of human populations for early markers of cadmium toxicity: A review, Toxicology and Applied Pharmacology, 238 (2009) 294-300
[2] Sun, X.T., Yang, L.R.*, Li, Q., Zhao, J.M., Li, X.P., Wang, X.Q., Liu, H.Z.*, Amino-functionalized magnetic cellulose nanocomposite as adsorbent for removal of Cr(VI): Synthesis and adsorption studies. Chemical Engineering Journal, 2014. 241: p. 175-183.
[3] Sun, X.T., Yang, L.R.*, Xing, H.F., Zhao, J.M., Li, X.P., Huang, Y.B., Liu, H.Z.*, Synthesis of polyethylenimine-functionalized poly(glycidyl methacrylate) magnetic microspheres and their excellent Cr(VI) ion removal properties. Chemical Engineering Journal, 2013. 234: p. 338-345.
[4] Dong, T.T., Yang, L.R.*, Pan, F., Xing, H.F., Wang, L., Yu, J.M., Qu, H.N., Rong, M., Liu, H.Z.*, Effect of immobilized amine density on cadmium(II) adsorption capacities for ethanediamine-modified magnetic poly-(glycidyl methacrylate) microspheres. Journal of Magnetism and Magnetic Materials, 2017. 427: p. 289-295.
[5] Sun, X.T., Yang, L.R.*, Xing, H.F., Zhao, J.M., Li, X.P., Huang, Y.B., Liu, H.Z.*, High capacity adsorption of Cr(VI) from aqueous solution using polyethylenimine-functionalized poly(glycidyl methacrylate) microspheres. Colloids and Surfaces a-Physicochemical and Engineering Aspects, 2014. 457: p. 160-168.
[6] Dong, T.T., Yang, L.R.*, Zhu, M.H., Liu, Z.N., Sun, X.T., Yu, J.M., Liu, H.Z.*, Removal of cadmium(II) from wastewater with gas-assisted magnetic separation. Chemical Engineering Journal, 2015. 280: p. 426-432.

Tryptophan Functionalized Magnetic Nanoparticles inhibit Lysozyme Amyloid Fibrillization

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Inhibition of amyloid fibrillization and clearance of amyloid fibrils are essential for the prevention and treatment of amyloid-related diseases. Magnetic nanoparticles have drawn a lot of attention in biomedical applications due to their unique properties as small size, large surface area, biodegradability and relative non-toxicity (1, 2). We have studied effect of uncoated (MNPs) and tryptophan coated superpara-magnetic nanoparticles (Trp-MNPs) on lysozyme amyloid fibrillization *in vitro*. The nanoparticle properties as hydrodynamic diameter and zeta potential were characterized using several techniques. Thioflavin T fluorescence assay was used for monitoring of the interference of both types of nanoparticles with amyloid fibrillization. The high fluorescence signal is observed after binding of ThT to lysozyme amyloid fibrils (LF) (Figure 1, red line). In presence of MNPs and Trp-MNPs the fluorescence intensities are lower (Figure 1, blue and black lines) suggesting their ability to inhibit formation of lysozyme amyloid fibrils. The higher inhibitory activity was observed for Trp-MNPs in comparison with MNPs. To better understand the mechanism of nanoparticle inhibitory activity the kinetic of lysozyme fibrillization alone and in presence of MNPs and Trp-MNPs was investigated. The fibrillization kinetic of lysozyme displayed a sigmoidal profile typical for the formation of amyloid fibrils. Presence of MNPs and Trp-MNPs leads to shortening of the lag phase and significant decrease of steady-state fluorescence intensities. The inhibitory effect of both nanoparticles was confirmed by atomic force microscopy.

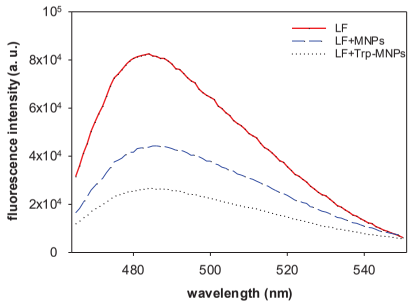

Fig. 1. ThT fluorescence spectra of lysozyme fibrils LF (red solid line) and lysozyme fibrils in the presence of MNPs (blue dash line) and Trp-MNPs (black dotted line). Ratio of LF:MNPs/Trp-MNPs) = 1 : 2.5.

We found that studied magnetic nanoparticles are able to inhibit formation of amyloid aggregates, the higher inhibitory activity was observed for tryptophan functionalized MNPs pointing to the importance of presence of tryptophan aromatic rings on nanoparticle surface. These results indicate their possible application for treatment of amyloid diseases associated with lysozyme or other amyloidogenic proteins.

Acknowledgement. This work was supported by VEGA grants 2/0145/17 and 2/0030/18, by the Slovak Research and Development Agency under the contracts No. APVV-14-0120 and APVV-14-0932, SAS-MOST JRP 2015/5 and MVTS COST 083/14 action BM1405.

References : 1. Bellova *et. al.*, Nanotechnology 21, 2010, 065103
2. Sen S., Konar S., *et al.*, The Journal of Physical Chemistry B 118, 2014, 11667-11676

Dextran-coated nanoparticles as an inhibitor of lysozyme amyloid aggregation

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Hen egg-white lysozyme (HEWL) as one of the proteins with very well characterized molecular structure and physico-chemical properties serves as a model protein for study of protein amyloid aggregation *in vitro*. HEWL is homologous to human lysozyme whose mutant variants are associated with systemic lysozyme amyloidosis. The patients suffering from this incurable disease have massive amyloid deposits in the liver and kidneys leading to their damage. In recent years increasing attention has been devoted to the application of nanoparticles in inhibiting amyloid fibril formation by disturbing the protein self-assembly processes.

Therefore, we investigated the effect of superparamagnetic iron oxide (Fe_2O_3) nanoparticles coated with carboxymethyldextran (CMD), dextran (DEX) or diethylaminoethyldextran (DEAE) on amyloid aggregation of HEWL. We have found that the interference of nanoparticles with HEWL caused dose-dependent inhibition of fibril formation and the IC_{50} values were determined to be in the μg/ml range. The most effective inhibitors were negatively charged CMD and neutral dextran (DEX) nanoparticles. The kinetic profiles for HEWL fibrillization in presence of the different types of magnetic nanoparticles (MNP) have shown that CMD and DEX nanoparticles prolonged the lag phase and decreased the values for plateau phase. None of the studied nanoparticles caused significant changes in SH-SY5Y cell viability relative to the control after 48 h exposure at concentrations close to IC_{50} values.

Our results indicated that all three types of MNP were able to prevent the amyloid fibrillization of lysozyme. The extent of the inhibition was observed to be dependent upon their physico-chemical properties. The studied dextran-coated nanoparticles possess no cytotoxicity, which is promising for further investigation of these nanoparticles as the candidates for therapeutics of amyloid-related diseases.

Acknowledgement
This work was supported by the research grant from the Slovak Grant Agency Vega 2/145/17, SAS-MOST JRP 2015/5, MOST 105-2923-E-002-010-MY3 and MVTS COST 083/14 action BM1405.

Discovery of New Antibacterial Mechanisms of Influence of Magnetite Nanoparticles (MCS-B)

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The influence of basic physical factors caused by magnetite nanoparticles (constant magnetic field and sorption) on microorganisms by examining the reactions of the intensity of free radical lipid peroxidation (FRLP) and bacteriostatic action was studied. It was well established that the magnetite nanoparticles caused unequal reaction in intensity of FRLP on different groups of microorganisms. It was determined that the most significant factor that influenced on the ultimate indicator of the intensity of luminescence on candida albicans, escherichia coli and pseudomonas aeruginosa was constant magnetic field which induced by nanoparticles. On the contrary, sorption was the most significant factor on staphylococcus aureus. It was found that the rate of consumption of free radicals lipid reduced reliably on all microorganisms after their processing by magnetite nanoparticles. The results of microbiological studies of escherichia coli, klebsiella pneumoniae and staphylococcus aureus showed that bacteriostatic effect was detected after exposure by magnetite nanoparticles. Visually, it was detected by decreasing the number of colonies on the nutritious medium in comparison with the control (Fig.1). It was revealed an interesting fact that saline NaCl, which had previously been processed by magnetite nanoparticles also significantly had a marked bacteriostatic effect on the studied microorganisms. This effect could be explained by mechanism of change the polarization structure water of microorganisms by magnetite nanoparticles. It was discovered that degree of expression of bacteriostatic action which induced by magnetite nanoparticles had correlation with marks of reactions intensity of FRLP. Maximum bacteriostatic effect on staphylococcus aureus was expressed in second variant application of magnetite nanoparticles where mechanism of sorption was more significant than action of the magnetic field. On the contrary, maximum bacteriostatic effect on escherichia coli and klebsiella pneumoniae was revealed in third variant, where time exposition of contact with microorganisms nanoparticles and, consequently, action of a constant magnetic field was determinative.

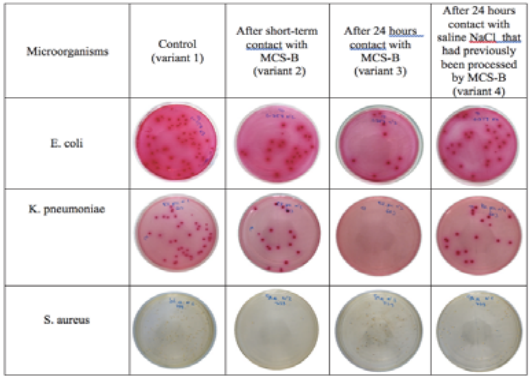

Fig.1 Study bacteriostatic action of magnetite nanoparticles (MCS-B) and saline NaCl that was processed by nanoparticles on different groups of microorganisms.

On the structure-driven functional properties of single-core and multicore magnetic nanoparticles

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The applications of magnetic nanoparticles (MNPs) in biology and medicine depend on their physical properties which are intrinsically linked to their shape and size, as well as their physical and chemical composition. Logically, there *must* be a correlation between the physical structure of magnetic nanoparticles and their response to physical stimuli, i.e. their function. Yet, despite the vast reports of MNPs in the scientific literature, it is not immediately obvious what this relationship is. This is especially puzzling given that overwhelming choice of magnetic material is either magnetite or maghemite, or mixtures thereof, which are phases of iron oxide that are magnetically almost indistinguishable. Unravelling the complex structure-function relation is thus a vital prerequisite for the optimisation of MNPs for biomedical applications including magnetic hyperthermia therapy. In the recent FP7 funded NanoMag consortium [1], we have quantitatively studied the physical structure of more than 25 different nanoparticulate samples. Using the standard nomenclature described in our previous work [2], we have systematically measured the mean core diameter ($\overline{D}_c$) and the core-cluster diameter ($\overline{D}_{cc}$) through a combination of transmission electron microscopy, asymmetric flow field flow fractionation with multi-angle light scattering and small angle x-ray scattering. It is clear that to understand the structure-function relationship we need to treat any given sample as an ensemble of sub-samples, divided with respect to their number- or volume-weighted frequency against some characteristic property. By performing a 'deep dive' analysis into the exemplar nanoparticle systems, highlighted in Figure 1, we have identified two new parameters that are critical to the MNP function; the number of cores ($\overline{N}_c$) and the core cluster packing density (ϕ_{cc}). We have used this to build a functional map that can be used to characterise the entire phase space (Figure 1), and have combined this with kinetic Monte Carlo simulations to validate the way that the coercive field, the blocking temperature and the intrinsic loss parameter should vary, as a function of both the mean core size and the number of cores per particle, $\overline{D}_c$ and $\overline{N}_c$.

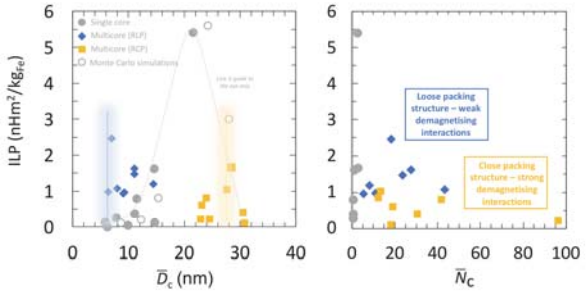

Figure 1 (left) Comparison of variation of the ILP with the mean core size at a drive for experimental data (solid symbols) and obtained from Monte Carlo modelling (open symbols). All values were obtained at a frequency of 989 kHz, with Monte Carlo simulations performed at a field strength of 6 kA/m samples studied in the deep dive analysis are indicated by the coloured lines. (right) Variation of ILP with mean number of cores per sample, showing how the packing density of the core cluster can cause order of magntiude changes in measurements.

[1] http://www.nanomag-project.eu [2] J. Wells et al J. Phys. D Appl. Phys. 50 (2017) 383003

Magnetic targeting of SPIONs under arterial flow conditions: *Ex vivo* and *in vivo* feasibility.

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Background: Superparamagnetic iron oxide nanoparticles (SPIONs) can be utilized to accumulate particle-bound drugs or particle-loaded cells in specified vasculature regions under an external magnetic field. Using this approach, SPIONs can be easily directed to specified microvasculature regions, but little is known about the possibility of magnetic targeting in medium and large arteries.
Methods: We developed an *ex vivo* flow-through model based on human umbilical artery. Artery fragments (13 cm long) were embedded in agarose and perfused with medium containing one of three different types of SPIONs with differing physicochemical characteristics. To evaluate the magnetic capture efficacy, arteries were subsequently cut into 11 segments and iron content was investigated by atomic emission spectroscopy and histology. *In vivo*, the efficacy of magnetic targeting was investigated in a rabbit model of balloon injury. Following the injury of lower abdominal aorta, an immediate intra-arterial infusion of SPIONs was performed under external magnetic field. Afterwards, the animals were sacrificed and the aortas dissected for histochemical analyses.
Results: SPION-1 with lauric acid shell had the largest capacity to accumulate at the specific artery segment. SPION-2 (lauric acid/albumin-coated, with improved biocompatibility) were also successfully targeted, although the peak in the iron content under the tip of the magnet was smaller than for SPION-1. In contrast, we did not achieve magnetic accumulation of dextran-coated SPION-3. Effects of magnetic field parameters, as well as flow time and rate on capture efficacy were analyzed using SPION-1. Reduction of the magnetic field gradient from 40T/m to 30 T/m resulted in the iron peak decrease, in parallel with slightly increased accumulation in the other segments. Increasing the distance of the magnet tip to the artery led to reduction of the iron content by about 75%. The experiments using SPIONs suspended in human plasma and whole blood samples demonstrated the confounding role of clotting in the *ex vivo* model, due to the lack of histocompatibility between umbilical artery and blood samples. Further experiments are currently ongoing with serum and washed red blood cells.
In vivo, the feasibility of MDT to abdominal aorta was tested by targeting of SPION-2 to the injured aortic region directly after the ballooning. Prussian blue staining confirmed that it is possible to accumulate intra-arterially administered SPIONs at the arterial wall.
Conclusions: The umbilical artery model allows standardized investigation of magnetic targeting under flow and can provide critical information in order to predict the capture efficacy of SPIONs *in vivo*. Our *ex vivo* data implied the possibility of an efficient *in vivo* targeting of certain types of SPIONs to medium and large arteries. The successful accumulation of SPION-2 at the aortic injury region in a rabbit model indicates that magnetic drug targeting is a feasible approach to target the vascular injury regions and atherosclerotic lesions.
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Nanoparticle-cell interaction – Surface chemistry triggers inflammation response

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Superparamagnetic iron-oxide nanoparticles (SPIONs) are suitable for biomedical approaches. Their long-term effects are of particular interest since they may remain in the human body. Thereby an inflammatory response may be induced, which has to be considered evaluating the biocompatibility of newly designed nanoparticles. We intend to analyse the effect of different nanoparticles on the expression levels of inflammation associated genes in more detail.
FaDu (hypopharyngeal squamous cell carcinoma) cells were incubated for 3h, 24h and 48h with different SPIONs (starch-coated fluidMAG-D, polyethylenimine-coated PEI-M, PEG-5kDa coated BNF-Dextran, silica-iron oxide composite SiliFe) after wide physico-chemical characterization. Their influence on the viability of the cells was investigated via the PrestoBlue Viability Assay. For gene expression analysis quantitative real-time PCR was performed to determine the expression level of selected inflammation-associated genes (e.g. *c-fos, icam1, cyp1a1, pdgfb*).
At first we confirmed biocompatibility of the selected SPIONs (up to 100 µg/cm^2) with the exception of PEI-coated nanoparticles, as expected. Depending on the coating of the SPIONs a unique interaction pattern with the cells was observed by laser scanning microscopy. Positively charged PEI-M particles formed large aggregates and exhibited a strong interaction with the cells already after a 3h incubation. Neutrally charged fluidMAG-D showed a slight agglomeration and cell interaction behavior after 3h, which was more pronounced after 24h. Neutrally charged PEG-5kDa particles interacted only weakly with FaDu cells even after a 24h incubation. The expression of *c-fos, icam1, cyp1a1* and *pdgfb* was not significantly altered after a 3h incubation with 25 µg/cm^2 fluidMAG-D, PEI-M or SiliFe each as compared to controls. Prolonged incubation up to 48h led to a SPION and gene-dependent expression profile.
In conclusion, short-term application of neutrally and negatively charged SPIONs neither interferes with cell viability and cellular uptake, nor alters expression of inflammation-associated genes. Future work will focus on the application of SPIONs on 3D-multicellular spheroids and its consequences on cellular metabolism.

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MICRO-PATTERNING OF HARD MAGNETIC FILMS FOR MEMS

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Micro-magnets have much potential for use in the fields of bio-technology, energy transformation and management, information technology... However, since these magnets are not commercialized, solutions based on the magnetic patterning of hard magnetic films has been developed, such as *topographic patterning,* in which µ-magnets are produced through the use of clean-room procedures (e.g. etching), *thermomagnetic patterning (TMP),* in which the direction of magnetization of the film is locally modified through localized heating with a ns-pulsed laser, and *Micro flux concentration (µFC),* in which patterned soft magnetic micro-structures serve to concentrate the flux of an externally applied field in the vicinity of a hard magnetic layer.

In this study we explore the use of µFC for the patterning of high performance NdFeB films. Firstly we carry out a comparison of the stray fields produced by identical films patterned using both µFC and TMP, results are compared with simulations, and the µFC technique is modelled. Secondly, we propose the use of a recently developed compact table-top pulsed magnetic field generator for the facile application of µFC to the patterning of hard magnetic films.

In order to evaluate the performance of µFC compared to TMP, equivalent pieces of out-of-plane textured NdFeB films were patterned using these techniques. In the case of µFC, a positive field of 7 T and then a negative field of -2.4 T, which corresponds to the coercivity of the NdFeB film, were applied using the superconducting coil of a VSM. In the case of TMP, the NdFeB sample was magnetised in a field of 7 T, then irradiated with a ns-pulsed laser through a photo-resist mask with a 50 µm wide stripe pattern in the presence of a reverse field of -0.5 T. Scanning Hall probe measurements of the stray fields produced by the samples were performed at different scan heights above the surface of the samples (selected data shown in Figure 1). Compared to the TMP sample, the µFC sample shows roughly twice the average peak-to-peak magnetic field intensities at all distances measured, indicating that magnetization reversal is achieved over a greater depth with µFC. This result is particularly interesting for cell trapping applications.

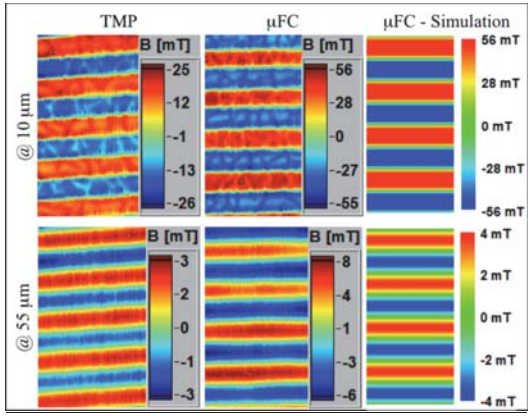

Fig.1: SHPM measurement of the z-component of stray field produced by TMP and µFC patterning of a NdFeB film, simulation assuming reversal through the depth of the film.

A new approach to isolate bacteria based on Magnetic In-Situ Hybridization and Hybridization Chain Reaction

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Traditional microbiological approaches for studying bacteria are culture-dependent methods largely underestimating microbial diversity. Indeed, it is estimated that less than 1% of the microorganisms present in an environment such as soil can be cultivated. There is therefore a great interest in exploring new approaches to physically isolate specific subpopulations including unculturable bacteria. In this work, a new labeling approach based on a combination between Magnetic In-Situ Hybridization (MISH) and HCR (Hybridization Chain Reaction) was developed to selectively trap bacteria onto micromagnet arrays.

In-situ hybridization consists in using a labeled complementary DNA or RNA probe sequence that will hybridize specifically to a target sequence within a permeabilized, yet intact, bacteria. Hybridization Chain Reaction (HCR), relies on the formation of an overlapping construct between two complementary DNA probes (H1 and H2) upon exposure to a DNA fragment containing both the sequence specific to the target (probe sequence) and a triggering (initiator) sequence (Figure A.). The long DNA fragment produced after self-assembly of the amplifier probes H1 and H2 can be used to establish a link between the target sequence in bacteria and magnetic nanoparticles (MNP) remaining outside the cells. To this end, H1 and H2 fragments are biotinylated to allow anchorage of streptavidin coated MNP on the part of the probe extending beyond the cell envelope. Following this approach, specific targeting of *Escherichia coli* DH5a, *Pseudomonas putida*, and *Acinetobacter sp*. ADP1 using heterologous probes was demonstrated.

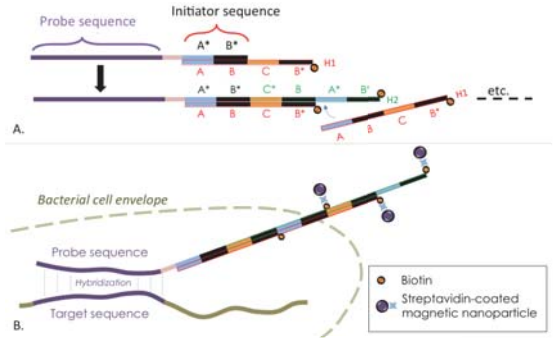

Figure. A. Principle of HCR probe amplification. **B.** Following *In-Situ* Hybridization, Hybridization Chain Reaction allows the formation of a biotinylated DNA tail long enough to allow anchorage of streptavidin-coated superparamagnetic nanoparticles beyond the cell envelope.

Influence of magnetic nanoparticle coating on cell viability and uptake

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Introduction. Magnetic nanoparticles (NPs) represent traditional contrast agents for cell tracking by magnetic resonance imaging (MRI). The nanoparticle core is important for the detection, but the coating, influencing the colloidal stability of NP suspension, cell viability, and cellular uptake, plays a crucial role as well. We tested various coatings of NPs based on Mn-Zn ferrite as potential cell labels.

Materials and Methods. Ferrite NPs of the composition $Mn_{0.61}Zn_{0.42}Fe_{1.97}O_4$ (MZF) were synthesized according to [1]. Physical properties of the particles were studied by X-ray diffraction (XRD), SQUID magnetometry, and MR relaxometry. The MZF cores were coated by silica, titania, mesoporous silica, citrate, mesoporous silica with mannose, and citrate with mannose. PANC-1 cells were labeled by 48-hour incubation with different coated MZF NPs at the concentration of 0.2 mmol($Mn_{0.61}Zn_{0.42}Fe_{1.97}O_4$)/L. Then the adhered cells were harvested and counted, and the viability was analyzed. In addition, the cell gain was evaluated as a ratio of the number of viable harvested labeled cells to the number of viable unlabeled control cells, which reflects the cell viability, proliferation, and adherence. After fixation, the cell suspensions were subjected to relaxometry (0.5 T, 37°C) to estimate the metal content inside the cells (see the Table).

Results and Discussion. XRD, magnetometry, and relaxometry demonstrated suitable properties of the particles as contrast agents for MRI. The coatings ensured suspension stability with the exception of the mesoporous silica combined with mannose, which tended to precipitate. Mannose coating was reported to form a stabilizing layer (2) but the combination with silica led to the precipitation. In contrast, the suspension of citrate-stabilized NPs combined with mannose was stable. Although the viability of all harvested labelled cells was high, the exposition to silica-coated or citrate-stabilized NPs led to lower adherence and thus lower gain of cells. Differences in the NP uptake reflected both their coating and size. Specifically, particles stabilized with citrate and mannose or titania-coated particles were easily internalized, whereas the uptake of silica-coated particles was lower (see Table). However, mannose might also induce receptor-mediated internalization, leading to higher uptake.

Table. Cell viability, gain, relaxation rate, and cellular metal content after labelling by various nanoparticles.

Coating	Cell viability/gain (%)	Cell relaxation rate* R_2 (s^{-1} mL/(10^6 cells))	Mn (pg/cell)	Zn (pg/cell)	Fe (pg/cell)
Silica	86/39	0.68	0.09	0.07	0.30
Titania	87/79	12.07	1.59	1.26	5.38
Mesoporous silica	90/71	4.80	0.63	0.50	2.14
Citrate	90/58	9.69	1.28	1.01	4.32
Mesopor. silica+mannose	94/78	6.31	0.83	0.66	2.81
Citrate+mannose	81/77	23.37	3.07	2.44	10.42
Unlabeled control	93/100				

*The contribution of unlabeled cells to relaxation rates was deducted.

Conclusion. MZF nanoparticles proved to be suitable as cellular labels for MRI tracking. Nanoparticle coating significantly influenced the NP uptake by cells. Specific coatings like titania or agents enabling receptor-mediated internalization such as mannose may increase the nanoparticle uptake and improve the cell detection by MRI.

Grant support

Czech Science Foundation - project No. 16-04340S, Ministry of Health of the Czech Republic (Institute for Clinical and Experimental Medicine – IKEM, Project IN 00023001), Ministry of Education, Youth and Sports of the Czech Republic LM2015062-CzechBioImaging.

References

[1] Kaman O et al. *J Magn Magn Mater* 2017; 427: 251–257.
[2] Horák D et al. *Bioconjugate Chem* 2007; 18: 635-644

Combined treatments of magnetic intra-lysosomal hyperthermia with Doxorubicin promotes synergistic anti-tumoral activity

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Cancer is a leading cause of death with millions of new people diagnosed with cancer every year. The conventional cancer therapies include surgery, radiotherapy and/or chemotherapy. Doxorubicin belongs to the anthracylin chemotherapeutic drugs and is one of the commonly used anticancer drugs. For decades, Doxorubicin is a cytotoxic drug used for the treatment of many cancer types including breast, lung, stomach, bone cancers and endocrine tumors. However, one major difficulty in anti-cancer therapy is the multidrug resistance that appears during treatments. Moreover, Doxorubicin may be hampered by its significant dose-related adverse effects, including cardiotoxicity, myelosuppression, gastrointestinal distress, alopecia, stomatitis… which lead to dose-limited of Doxorubicin use. Hyperthermia has been recently introduced as an adjuvant therapy for cancer and presents promising opportunities to treat cancers, especially in combination with chemotherapy or radiotherapy. Indeed, many clinical experiments have demonstrated that the addition of hyperthermia to radiotherapy or chemotherapy significantly improves tumor control and patient survival rates. Among hyperthermia methods, magnetic hyperthermia is a promising way for site-specific heating that reach deeper tissue, in which magnetic nanoparticles (MNPs) play an important role to relay the externally delivered high frequency alternating magnetic field (AMF). Indeed, direct injection of MNPs into solid tumors, followed by AMF exposure, has been shown to induce tumor regression. However, conventional hyperthermia methods including standard magnetic hyperthermia do not thermally discriminate between the target and the surrounding normal tissues, and this non-selective tissue heating can lead to side effects.

In this context, nanotherapy based on Magnetic Intra-Lysosomal Hyperthermia (MILH) generated by MNPs that are grafted with ligands of receptors overexpressed in tumors appears to be a very promising therapeutic option. Strikingly, in such approach, no perceptible temperature rise in the cell medium occurred during AMF exposure. Thus, MILH differs from standard magnetic hyperthermia whereby tumor eradication is achieved with large doses of MNPs which cause a temperature elevation of the whole tumor. As a proof-of-concept, we previously showed that minute amounts of iron oxide MNPs (Gastrin-MNPs) targeting the gastrin receptor (CCK2R) are internalized by tumoral cells through a CCK2R-dependent physiological process, accumulated into their lysosomes and killed tumoral cells upon AMF application through lysosomal cell death [1,2,3]. The aim of this study was to analyze whether combination of MILH with chemotherapy could increase the efficiency of eradication of cancer cells. Endocrine tumoral cells were incubated with Gastrin-MNPs, treated with Doxorubicin, the commonly used drug to treat endocrine tumors, and exposed to AMF. The impact of combined treatments was analyzed on cell viability and cell death, comparatively to individual treatments. Mechanisms of cell death were also studied following the different treatments. Here, we report that combination of MILH with Doxorubicin increased the efficiency of eradication of endocrine tumor cells with synergism or additivity, according to Doxorubicin concentrations used. We demonstrated that these two treatments activated two different cell death pathways that were respectively dependent on Caspase-1 and Caspase-3 activation. Finally, these findings suggest that MILH can decrease required dose of chemotherapy drugs such as Doxorubicin and thereby reduce their side effect.

References

[1] Sanchez C et al. (2014) Targeting a G-Protein-Coupled Receptor Overexpressed in Endocrine Tumors by Magnetic Nanoparticles To Induce Cell Death. ACS Nano 8(2):1350-63.
[2] Connord V et al. (2015) Real-time Analysis of Magnetic Hyperthermia Experiments on Living Cells under Confocal Microscope. Small 11(20):2437-45.
[3] Clerc P et al (2017) Targeted Magnetic Intra-lysosomal Hyperthermia produces lysosomal reactive oxygen species and causes Caspase-1 dependent cell death. J. Controlled Release (in press).

Magnetic vortex nanodiscs for intracellular cancer cell disruption

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Disc-shaped particles are a new generation of magnetic nanoparticles that can face important biomedical challenges as the search for new cancer therapies. Indeed, their ability to vibrate under external magnetic fields can be exploited to damage cancer cells integrity and/or deliver antitumor drugs. Moreover, thanks to their magnetic vortex configuration [1], they possess zero magnetization at remanence eliminating the problem of agglomeration. When an AC field as small as 10 Hz and 100 Oe is applied, the discs vibrate exerting forces capable of triggering cancer cells apoptosis. This capability has been demonstrated using micrometric discs (1-2 μm in diameter) [2, 3]. The scaling down of the size could favor the internalization by the cells, opening up new possibilities of actuation from the inside, and reduce the magnetic material injected in the body. Nevertheless, no work has been reported on nanosized discs.

We previously fabricated vortex state $Ni_{80}Fe_{20}$ discs down to 30 nm in radius using a cost-effective lithography technique [4]. We now have performed the first *in vitro* experiments using nanodiscs with cancer cells. Although not biofunctionalized, almost 20% of the cells uptake nanodiscs and accumulate them in lysosomes. The nanodiscs show no cytotoxicity effects by themselves but cause dramatic damages to 30% of the cells after magnetic stimulus, probably due to the rupture of lysosomal membrane and release of hydrolytic enzymes into the cytoplasm. In light of these preliminary results, the intracellular actuation of nanodiscs could become a new pathway to selectively kill cancer cells.

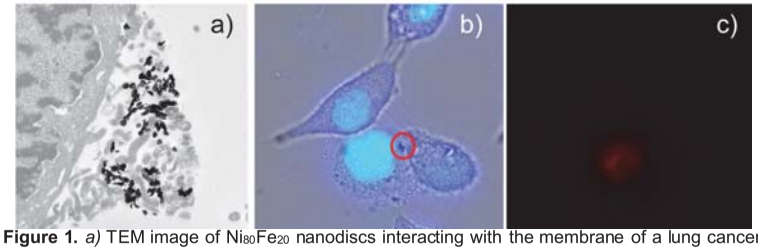

Figure 1. *a)* TEM image of $Ni_{80}Fe_{20}$ nanodiscs interacting with the membrane of a lung cancer cell. Fluorescent micrographs of the *in vitro* experiments: the cell that has internalized nanodiscs (red circle (*b*)) dies after the application of the magnetic field (*c*).

[1] R. P. Cowburn *et al*., *Physical Review Letters* **83** (1999) 1042.
[2] D-H. Kim *et al*., *Nature Materials* **9** (2010) 165.
[3] S. Leulmi *et al*., *Nanoscale* **7** (2015) 14904.
[4] M. Goiriena-Goikoetxea *et al*., *Nanotechnology* **27** (2016) 17530.

Magnetic particles as tools for the development of remotely actuated tissue engineered constructs for tendon tissue regeneration

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That can be achieved modulating the scaffold architecture, properties and composition.

The incorporation of magnetic nanoparticles (MNPs) within 3D polymeric constructs constitutes a novel and attractive strategy towards the development of magnetically-responsive system that may eventually combine therapeutic and diagnostic functionalities in Tissue Engineering/Regenerative medicine approaches. The development of tissue engineering (TE) approaches for tendon regeneration requires biomechanically-stimulating culture environments as tendon tissue functionality is known to be highly dependent on mechanical loading. Therefore, magnetic systems present additional advantages for such applications as cells naturally respond to magnetic forces, and consequently, the application of a magnetic field may enhance stem cells biological performance, and ultimately stimulate cell proliferation and/or differentiation. This work reports on recent studies concerning the development of specific scaffolds architectures based on various polymers, doped with MNPs and fabricated using different technologies enabling responsive systems for culturing stem cells, stimulating their tenogenic differentiation. Moreover, we've found that ex-vivo application of pulsed electromagnetic field therapy (PEMF) applied in combination with magnetic responsive materials, may also enable to modulate the inflammatory response and consequently promote a better tissue regeneration.

Acknowledgments:
Project "Accelerating tissue engineering and personalized medicine discoveries by the integration of key enabling nanotechonologies, marine-derived biomaterials and stem cells", supported by Norte Portugal Regional Operational Programme (NORTE 2020), under the PORTUGAL 2020 Partnership Agreement, through the European Regional Development Fund (ERDF).

Antioxidant Polymer-Modified γ-Fe_2O_3 Nanoparticles for ROS Scavenging

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Many fatal diseases, including cancer, neurodegenerative and cardiovascular disorders, infections or aging, are associated with oxidative stress characterized by enhanced formation of reactive oxygen species (ROS). Though the mechanism of oxidative stress-associated disorders is not known, restraining of prooxidative processes should be beneficial.

Maghemite (γ-Fe_2O_3) nanoparticles were obtained by coprecipitation of starting Fe(II) and Fe(III) salts and the particle surface was treated with poly(L-lysine) (PLL) containing phenolic compounds (PPLL), optionally with heparin (Hep) and gallic acid- or hydroquinone-modified chitosan (CS-G or CS-H). Amount of U87MG cell-associated magnetic nanoparticles was determined by colorimetric Fe assay and antioxidant activity of γ-Fe_2O_3@PLL(PPLL) particles was analyzed by flow cytometry using 5-(and 6)-chloromethyl-2',7'-dichlorodihydrofluorescein diacetate, acetyl ester (CM-H_2DCFDA) dye. Inhibition of oxidative stress was determined by multiplication of *S. aureus* bacteria in the presence of the polymorphonuclear (PMN) cells and/or formation of intercellular ROS was examined using oxidant-sensing 6-carbohy-2',7'-dichlorohydrofluorescein diacetate (DCFH-DA) dye.

According to flow cytometry, application of γ-Fe_2O_3@PLL(PPLL) particles defined above decreased the endogenous ROS level by 10-18 % (Figure). Moreover, γ-Fe_2O_3@Hep-CS-H nanoparticles significantly increased the *S. aureus* bacteria viability, which was 2.7 times higher than that of the control. Using DCFH-DA, the highest inhibition of oxidative burst (90 % of the control) was obtained for γ-Fe_2O_3@Hep-CS-G particles when PMN cells were stimulated with phorbol 12-myristate 13-acetate.

PLL chains of the γ-Fe_2O_3@PLL(PPLL) particles seem to be a key component in the nanoparticle-cell interaction; moreover, PLL itself acted as an antioxidant in glioma cells. γ-Fe_2O_3@PLL(PPLL) particles were well internalized by the cells exerting antioxidant effect and reducing endogenous ROS. Also γ-Fe_2O_3@Hep-CS-G(H) particles reduced oxidative stress, which is significant in terms of combating above mentioned serious diseases.

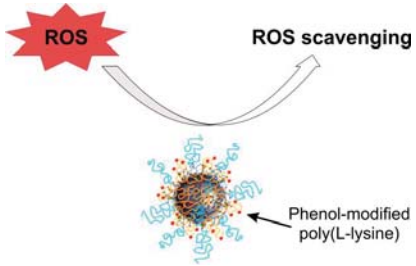

Figure. ROS scavenging by PPLL-modified γ-Fe_2O_3 nanoparticles.

Acknowledgement: The research was funded by the Czech Science Foundation (No. 17-04918S).

Labeling of phosphates-coated MNPs with yttrium-90: a potential tumour treatment radiopharmaceuticals

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Radiation therapy is an effective cancer treatment option in conjunction with chemotherapy and surgery. Radiolabeled nanocarriers can be designed and used for cancer diagnostic and therapeutic purposes when tagged with appropriate radionuclides. Magnetic nanoparticles (MNPs) have been intensively used for a wide variety of biomedical applications. In the present study, MNPs coated with imidodiphosphate (IDP) or inositol hexaphosphate (IHP) were synthesized and labeled with ^{90}Y. ^{90}Y is a high energy β-emitter with optimal nuclear-physical characteristics ($E_{max\beta}$ of 2.27 MeV and $T_{1/2}$ of 64.1 h). The aim was to investigate the possibility of use of radiolabeled coated MNPs in the therapy as multifunctional agents capable to localize both radioactivity and magnetic energy at a tumor site.
MNPs were synthesized by co-precipitation of ferric and ferrous salts in a basic solution. Characterization of the MNPs was performed using X-ray powder diffraction, transmission electron microscopy, Fourier transform infrared spectroscopy, dynamic light scattering and laser Doppler electrophoresis. The heating ability of MNPs was quantified through the specific power absorption (SPA) measurements. The MNPs were labeled with 37 MBq $^{90}YCl_3$, at room temperature for 1 h and were used in *in vitro* stability studies in saline and human serum and *in vivo* studies in healthy Wistar rats.
The coating of MNPs with phosphates made them biocompatible, increased their colloidal stability and allowed the binding of the radionuclide ^{90}Y to the available functional groups on the surface of the MNPs. IDP and IHP have not hitherto been used for coating of MNPs and the results of this study showed that the phosphate groups influenced the modification of the surface of MNPs. The obtained values for the specific power absorption of MNPs (46.95–80.76 W/g) in different physiological media indicated their possible application for hyperthermia treatment. Both types of coated MNPs were ^{90}Y-labeled in a reproducible high yield (>98 %). *In vitro* studies of ^{90}Y-MNP in saline and human serum showed their high stability after 72 h. The biodistribution analyses of ^{90}Y–Fe_3O_4–IDP and ^{90}Y–Fe_3O_4–IHP MNPs after intravenous administration to healthy Wistar rats revealed that the maximum of the injected dose was observed in the liver (91.78% and 85.23%), followed by the spleen (3.53% and 7.89%) and the lungs (2.16% and 2.60%) at 72 h post injection, respectively (Fig. 1). An insignificant amount of radioactivity after 72 h was detected in femur, a target organ for free ^{90}Y, indicating that ^{90}Y was tightly attached to the surface of the MNPs. The results of this comprehensive study showed the great promise of radiolabeled biocompatible phosphate magnetic complexes for therapeutic uses combining magnetic hyperthermia and radiotherapy

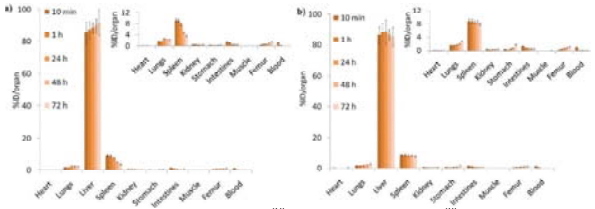

Fig. 1: Biodistribution results of a) ^{90}Y-Fe_3O_4-IDP and b) ^{90}Y-Fe_3O_4-IHP MNPs

Probing Magnetogenetic Activation of hRas with MagIcS Nanoparticles Inside Living Cells

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Dynamic control of cellular functions still represents an important challenge in academical and biomedical research. Activated small GTPases settles in many signaling pathways responsible for growth and cellular differentiation making it a prime candidate for controlling cellular functions. Earlier studies successfully demonstrated magnetogenetic control of the small Rho-GTPase Rac1 by exploiting magnetic microparticles inside living cells[1]. However, this approach was limited by the size of the used particles. Here we present a novel approach for magnetogenetic control of GTPase activation by utilizing engineered Magnetic Intracellular Stealth (MagIcS) nanoparticles based on the natural protein cage ferritin[2]. Switching to MagIcS nanoparticles reduced the effective size to only 25nm in diameter enabling free diffusion in the cytoplasm and, important for their application inside living cells, this semisynthetic nanobiomaterial is intrinsically non-toxic. For probing hRas activation at the plasma membrane using MagIcS nanoparticles, a FRET biosensor based on Raichu-hRas was reengineered by substitution of the FRET pair CFP/YFP for mTFP1/mNeonGreen, which is orthogonal with a nonfluorescent GFP mutant fused on the surface of ferritin. Characterization of the modified biosensor yielded FRET ratios which were comparable with FRET ratios obtained by Raichu-hRas. With these approaches at hand, after magnetic translocation of MagIcS nanoparticles functionalized with the catalytic segment of the guanine nucleotide exchange factor (GEF) mSOS to the plasma membrane (Fig. 1), site-specific activation of hRas on a subcellular scale was observed. In conclusion, by exploiting MagIcS nanoparticles, magnetogenetics represents a non-toxic and robust tool with great prospects for remote control of GTPase activation inside living cells.

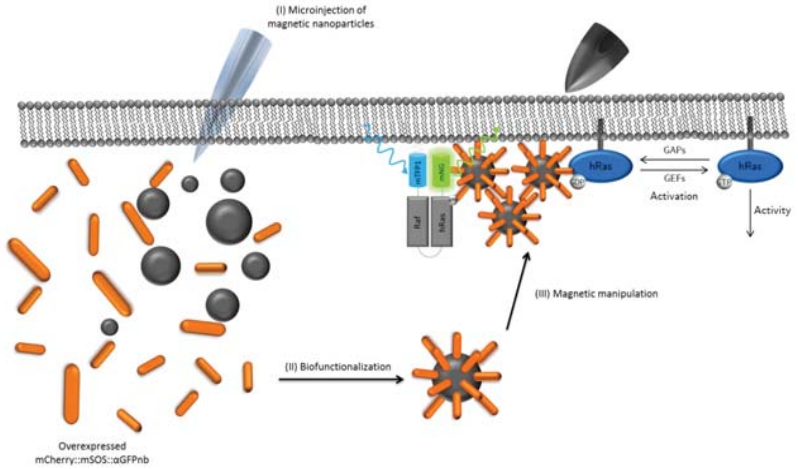

Figure 1: Strategy of magnetogenetic activation of hRas with MagIcS nanoparticles inside living cells. (I) Microinjection of magnetic nanoparticles and overexpression of mSOS-fusion protein. (II) αGFP-nanobody based biofunctionalization. (III) Translocation of biofunctionalized magnetic nanoparticles to the plasma membrane by magnetic forces and activation of small GTPase. Probing of activation using FRET biosensor.

[1] Etoc, F. et al. (2013): Subcellular control of Rac-GTPase Signalling by Magnetogenetic Manipulation Inside Living Cells. Nature Nanotechnology 8. 193-198.
[2] Liße, D. et al. (2017): Engineered Ferritin for Magnetogenetic Manipulation of Proteins and Organelles Inside Living Cells. Adv. Mater. 29 (42).

$NOBF_4$ coated Au-Fe_3O_4-Nanoparticles in radiation therapy: The benefit of combined generation of ROS and RNS

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Heterostructured nanoparticles like Au-Fe_3O_4-nanoparticles are attractive candidates for advanced nanomaterials. These nanoparticles do not only benefit from the unique properties of each pristine material but also can exhibit novel physical and chemical properties. Both nanoparticles species alone are possible candidates as sensitizers for radiation therapy and so the combination of both materials can be very advantegous.
The Au-Fe_3O_4-nanoparticles have been synthesized by thermal decomposition of an iron precursor on the surface of pre-synthesized gold nanoparticles. Afterwards these nanoparticles were surface-modified through a ligand exchange reaction using nitrosyl tetrafluoroborate ($NOBF_4$) to attain water solubility and thereby the desired performance for nano-oncological applications. The combination of gold nanoparticles, which can emit photo-/Auger electrons after irradiation with X-rays and the superparamagnetic iron oxide nanoparticles, which effectively catalyze the Fenton reaction and producing hydroxyl radicals after irradiation, makes them ideal candidates as radio sensitizers. Additionally the iron oxide nanoparticles can stabilize the nitrosonium ions and prevent its hydralyzation. The simultanous gneration of superoxide and nitrogen oxide in near distance at the particle surface leads to an effectiv increase of the peroxynitrite formation after irradiation of cells loaded with these nanoparticles. The peroxynitrite generation is high enough to overpower the cellular antioxidant system and leads to oxidative damage such as lipid peroxidation and apoptotic cell death. Compared to iron oxide and gold nanoparticles the Au-Fe_3O_4-nanoparticles prove to be the best candidate as sensitizers in radiation therapy.

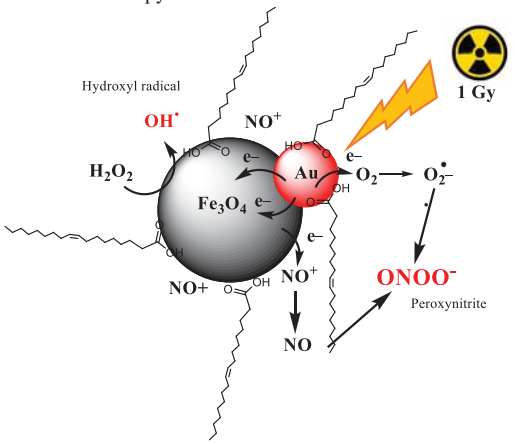

Figure 1: Possible reaction mechanisms after irradiation of the Au-Fe_3O_4-nanoparticles:
a) Generation of the **hydroxyl radical** via the Fenton reaction
b) Generation of the **superoxide** radical by electron transfer to adsorb oxygen
c) Generation of the **nitric oxide** radical by electron transfer to adsorbed nitrosonium cation
d) Generation of **peroxynitrite** by reaction of superoxide with nitric oxide

The effect of the rotating magnetic field on bioethanol production by yeast strain immobilized on ferromagnetic nanoparticles

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The efficiency is an important parameter in bioprocessing and many studies are being conducted in order to find new methods to improve it. In general, the immobilization techniques are effectively used to enhance the efficiency of production process. The cell productivity may be affected by applying the electromagnetic fields (EMFs). For example, these fields have been proven to influence growth dynamics or cellular metabolic activity.

The special case of the EMF is a rotating magnetic field (RMF), which is created due to the superposition of EMFs generated by windings situated around the same axis and properly powered. The electromagnetic force action (as the result of interaction between induce currents and magnetic field) causes moves of fluid or ferromagnetic particles around its axis (Rakoczy et al., 2016, Biochem Eng J, 109, 43–50) creates microscopic dynamos. These dynamos can increase the interfacial mass transfer between cell and fluid through influence on the cell surface where stagnation zone commonly occurs. Moreover, the RMF force causes the particles movement around the generator axis, which can be treated as the non-invasive stirring device at the microscopic level. (Moffat, 1991, Phys Fluids A, 3, 1336–1343). The RMF has been proven to oxygen transfer intensification (Rakoczy et al., 2017, Chem Eng J, 327, 608-617) and mixing improvement (Rakoczy et al., 2017, Chem Eng Process, 112, 1-8).

The main objective of this work was to analyze the effect of the rotating magnetic field on the bioethanol production. For this purpose, the yeast strain ATCC 4098 was employed and in further steps, it was modified by immobilization on Fe_3O_4 nanoparticles synthesized by a solution method. The RMF exposition was conducted for the various frequency of field (f = 10-50 Hz, $B_{max} \approx 18$ mT) continuously up to 72h. The process was performed for sugar-rich medium (33 w/w%) to maximize the amount of produced bioethanol. The growth rate, metabolic activities, and bioethanol productivity were analyzed. The experimental system containing the RMF generator is presented in fig. 1.

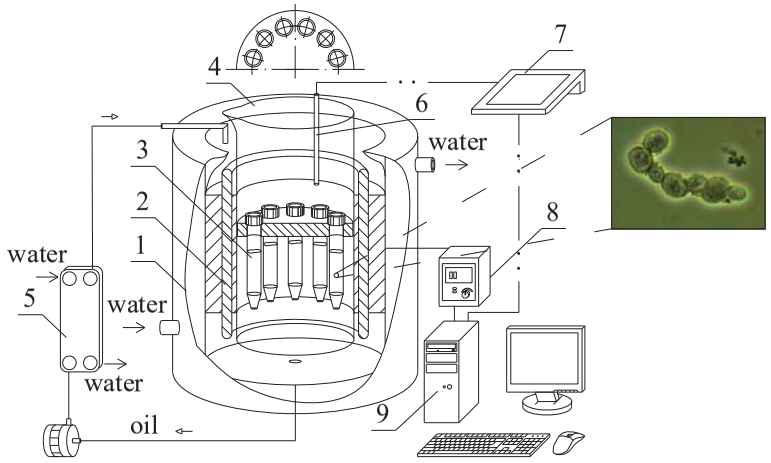

Fig 1. The experimental system: 1 – tank, 2 – RMF generator, 3 – cultures, 4 – glass container, 5 – plate heat exchanger, 6 – temperature probe, 7 – multifunctional meter, 8 – current inverter, 9 - PC

Antitumor drug-functionalized magnetic nanoparticles internalized by human adipose derived stem cells for *in vitro* tumour tissue-like structures targeting

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Magnetic nanoparticles (MNPs) functionalized with different therapeutics delivered by mesenchymal stem cells represent a promising approach to improve the typical drug delivery methods. This innovative method, based on the "Trojan horse" principle, faces however important challenges related to the viability of the MNPs-loaded cells and drug stability.
In the present study we report about an in vitro model of adipose-derived stem cells (ADSCs) loaded with palmitate-coated MNPs (MNPsPA) as antitumor drug carriers targeting a 3D tissue-like osteosarcoma cells (Fig. 1). Cell viability, MNPsPA-drug loading capacity, cell speed, drug release rate, magnetization and zeta potential were determined and analysed. The results revealed that ADSCs loaded with MNPsPA-drug complexes retained their viability at relatively high drug concentrations (up to 1.22 pg antitumor drug/cell for 100% cell viability) and displayed higher speed compared to the targeted tumour cells in vitro. The magnetization of the sterilized MNPsPA complexes was 67 emu/g within a magnetic field corresponding to induction values of clinical MRI devices. ADSCs payload was around 9 pg magnetic material per cell, with an uptake rate of 6.25 fg magnetic material/min/cell. The presented model is a proof-of-concept platform for stem cells-mediated MNPs-drug delivery to solid tumors that could be further correlated with MRI tracking and magnetic hyperthermia for theranostic applications.

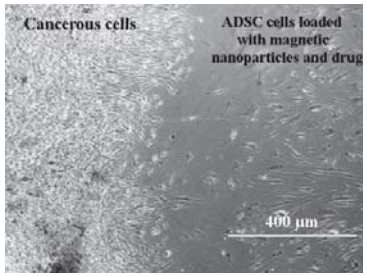

Fig. 1. Osteosarcoma cells (left) and stem cells (right) after 48 h of "interaction". The stem cells loaded with MNPs and antitumor drug maintained their viability and moved towards osteosarcoma (cancerous) cells.

Acknowledgment – Work supported by the Nucleu Programme (Project PN 16 37 01 02 and Project PN 18 06 01 01).

Bio-Nano-Magnetic Materials for Localized Mechanochemical Stimulation of Cell Growth and Death

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Abstract: In the last decade we have seen a rapid increase in our understanding of the mechanisms by which the local mechanical environment of a cell influences its phenotype. We have used magnetic nanoparticles to apply force to specific regions of cells, which permit the modulation of cellular behavior through the use of spatially and time controlled magnetic fields. In one case force has been used to direct the outgrowth on neuronal growth cones and in another it has been used to kill model cancer cells that preferentially express specific receptors. We conclude by identifying exiting future applications of these new materials.

Introduction: Recent work from our and others' laboratories suggested that apart from carrying drugs and inducing hyperthermia, magnetic nanoparticles can also be used for the mechanical stimulation of cells to induce a therapeutic effect.[1] Mechanics plays important roles in numerous physiological and pathological processes, and is particularly important for cancer, where forces acting on cells modulate the metastatic process, accounting for over 90% of cancer-related deaths. Throughout metastasis, cells not only undergo morphological change but also actively interact with their physical environment through motility and adhesion-related pathways. The most straightforward approach to translate this into the clinic would appear to be to interfere with these critical mechanical processes to block metastasis. An alternative approach would be to use mechanical stimulation to selectively kill tumors and circulating tumor cells. This could be achieved either through damaging the target cell's membrane or its intracellular structures, or through activating select mechanotransduction pathways to induce cell death. Another area where mechanical force may induce a therapeutic effect is chronic spinal cord injury, where the glial scar tissue that forms after the injury poses not only a physical barrier for regenerating neurons but also secretes repulsive factors that inhibit axon elongation. Importantly, external mechanical stretch has been shown to enhance axon elongation under control conditions [2] and against concentration gradients of known axon repellents.[1a] Thus, stretch growth of regenerating axons through regions of low permissivity may complement existing therapeutic approaches after spinal cord injury, such as engineered biomaterial scaffolds, cell transplantation, and local delivery of neurotrophic factors. To this end, delivering sufficient force to axonal growth cones *in vivo* appears to be a challenge to overcome. Ideally, this would be achieved non-invasively, since a very long therapy period may be required depending on injury severity.

Results: In this paper we examine the advances that have been made in bio-polymer-magnetic nanoparticle synthesis that have made it possible to target specific receptors on cells and transduce significant levels of force to them.

Figure 1. (below) Example of mechanically-induced cancer cell death in vitro. Schematic of MCF7 breast cancer cells targeted with FeAu composite nanorods that are tip-functionalized with Heregulin (HRG). Periodic pulling of ErbB3 receptor caused 2.4-fold increase in Erk phosphorylation, which, when combined with low dose B-Raf inhibition results in 15-fold increase in cell death. (Top,left) Phase contrast images of MCF7 cells targeted with HRG-conjugated nanorods. (Bottom,left) ERK and pERK immunofluorescence images of the same regions. Color images are overlays of ERK (red) and pERK (green). (Right) MCF7 cell death in response to specific targeting and mechanical stimulation combined with B-Raf inhibition. From [1b].

External-Gradient Ferrofluid-Enabled Clinical-Scale Cell Separator

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Biologically active, highly magnetic colloidal nanoparticles known as ferrofluids (FF)[1]played a key role in developing the field of circulating tumor cells through their use in CELLSEARCH® (developed at Immunicon Corp., marketed by Veridex Division of J&J, acquired by Menarini/Silicon Biosystems in 2017), the first and only actionable FDA-approved test for detecting and enumerating such cells in cancer patients. Key to the success of CELLSEARCH® is that the FF's Brownian energy promotes rapid rare-cell labeling without mixing and can be separated with external magnetic gradients. To exploit these properties in clinical-scale separation, we have developed an external-gradient FF-enabled [**X-GRAFFE**] system. We first determined that for FF, indirect magnetic labeling (i.e., mAb incubation, unbound mAb removal, and common-capture FF incubation) of target cells results in superior purities and yields as compared to using a direct mAb–FF conjugate. We chose to marry our device to a cell processing system (e.g., Fresenius Kabi's Lovo), which can be used for upstream processing of leukapheresis products to remove plasma, platelets, and some erythrocytes. Moreover, such a system can be used to adjust volumes (to optimize cell concentrations for labeling steps), incubate cells with mAb, remove unbound mAb, and incubate cells with FF.

In preliminary small-scale separations in test tubes with quadrupoles, we observed that cells separated evenly on the inner walls of test tubes – effectively monolayered. Furthermore, we found that it was possible to effectively remove non-target cells that are entrapped during these separations simply by passing buffer over the collection surface while the test tubes remained in the magnetic field gradient. Hence, we designed a large (i.e., 200 x 270 mm) planar magnet array to spread collected cells over as large an area as is practicable to determine whether non-target cells could be removed in the presence of a magnetic field gradient; this would not only shorten and simplify the process, but could reduce damaging stresses on the cells. We affixed the planar magnet array onto a shaft such that a separation chamber placed on the array could be rocked back and forth, allowing buffer to be gently passed over cells on the magnetic collection surface within the chamber. Since it is desirable to separate and wash cells within an inexpensive, disposable, and sterile chamber that would be compatible with existing single-use cell processing sets, we created a chamber from a blood bag with inlet and outlet ports on either side of the bag. Initially, separation bags were placed over the planar magnet array, on top of which was placed a cover to fix the depth of the chamber to 10mm, thus forming a quasi-rectilinear chamber with the surface area of the bag and a depth of 10mm. A peristaltic pump was connected to the inlet port of the bag to deliver sample, wash buffer, and recovery buffer, and a waste bag was connected through a valve to the outlet port of the bag to prevent backflow.

While initial experiments were promising, filling, emptying, and buffer wash steps were problematic as the flexible bag tended to collapse, resulting in lower yield, purity, consistency. To resolve these issues,the flexible bag was pressurized within a rigid frame to create a rigid chamber. This strategy allows separation and washing to take place on a flat collection surface without the walls of the chamber falling away from the magnetic surface or collapsing during processing. To remove non-target cells from the magnetic collection surface, a protocol was developed wherein wash buffer along with a controlled bolus of air to form a meniscus is repeatedly passed over the magnetic collection surface during rocking operations. With this system, CD3+cell separations with purities > 97% and yields ranging from 85–92% were achieved with no appreciable reduction in cell viability. These yields are significantly superior to current commercial systems, and the entire process can be completed in about 1 h. The economics of manufacturing and utilizing a common-capture FF with an indirect approach, paired with the inexpensive blood-bag-based chamber, the simplified purification protocol, and the extraordinary yields make this system important with the advent of cellular therapy.

1. Liberti P.A., et al. (1996) Bioreceptor Ferrofluids: Novel Characteristics and their Utility in Medical Applications. In: Pelizzetti E. (eds) Fine Particles Science and Technology. NATO ASI Series (Series 3: High Technology), vol 12. Springer, Dordrecht.

Gallate-Induced Nanoparticle Uptake by Tumor Cells: Structure-Activity Relationship

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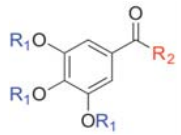
Surface modification of nanoparticles may impact on nanoparticle-cell interaction and thus the consequent biological effects; phenolic–nanoconjugates have been shown to exert potential anti-tumor effects. Our previous study demonstrated that tea catechins significantly enhanced cellular uptake of magnetic nanoparticles (MNPs). In the current study, we asked whether the trihydroxy benzene structure, such as gallate, may act as the pharmacophore in the enhancement effects of MNP-cell interaction.

Using confocal microscopy, co-administration of methyl gallate and dextran-coated MNPs in glioma cells induced co-localization of internalized MNPs and lysosomes. Using a colorimetric iron assay, cell-associated MNPs (MNP_{cell}) were quantitated, which was increased by 1.2-5.4 fold in response to gallic acid or methyl gallate in a concentration-dependent manner. The application of magnetic field exerted a synergistic effect on MNP_{cell}, which was increased by 1.5-3.8 fold in response to gallic acid or methyl gallate. Figure 1 shows that the ester (2&3) and amide (4) derivatives of gallate exerted significantly higher internalization effects than gallic acid (1), which is further potentiated by application of magnetic force. In addition, blockade or reduced number of hydroxyl groups rendered these compounds less effective, which was not due to induction of cytotoxicity. Similar structure-activity relationship on the antioxidant activities was also observed. Our results may provide pivotal information for theranostic application of gallates by facilitating nanoparticle-cell interaction and internalization by tumor cells.



No. of compound	R_1=	R_2 =
1	H	OH
2	H	OCH_3
3	H	$OCH_2CH_2CH_3$
4	H	NH_2
8	CH_3	OH

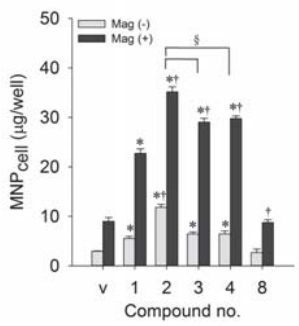


Figure 1. Structure-activity relationship of gallate derivatives on MNP_{cell}. LN-229 cells were incubated with MNPs (100 μg/mL) and various gallate derivatives at 10 μM for 24 h in the presence (+) or absence (-) of the magnet (Mag). $^{*, \dagger, \S}$, $P<0.05$ compared with the corresponding vehicle (**v**) groups, gallate derivatives (**1**) or (**2**), respectively.

Action of low-frequency magnetic field modes on magnetic nanoparticle environments

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The interaction of alternating, static or rotating magnetic field modes with magnetic nanoparticles (MNPs) allows transformation of electromagnetic to mechanical energy. Thus, this option renders magnetic nanoparticles useful in biomedical research, provided they are properly functionalized to bind to malignant cell membranes or even internalize within biological entities and incur damages by exerting magneto-mechanical stresses.[1] Such multimodal fields, may be routinely applied with a novel magneto-mechanical device, which was designed, manufactured and thoroughly characterized in terms of field parameters and MNPs properties.[2] Such a device may be further implemented, as a versatile magnetic force performer, on cellular environments, where the magnetic nanoparticles, following external magnetic field variations, may ignite cellular processes.[3] Different growth modes were observed, with respect to specific cancer cell lines and field parameters, for cells incubated with MNPs and exposed to either static, rotating or alternating low frequency magnetic fields (40-200 mT, 0-16 Hz). Further research focusing on the accurate role of each specific field parameter (frequency, amplitude, field gradient) may optimize mechanical force's magnitude resulting to even more successful non-invasive therapeutic anticancer approaches.

Acknowledgements: This research has been co-financed by the European Social Fund (ESF) and by Greece (Operational Program "Exploitation of field effects on appropriate nanoparticulate carriers for modern biomedical applications" of the Research Funding Program "Human Resource Development, Education & Lifelong Learning 2014-2020").

References

[1] Hetero-nanocomposites of magnetic and antifungal nanoparticles as a platform for magnetomechanical stress induction in Saccharomyces cerevisiae, K. Giannousi, M. Menelaou, J. Arvanitidis, M. Angelakeris, A. Pantazaki and C. Dendrinou-Samara, J. Mater. Chem. B 3, 5341 (2015)

[2] Magneto-mechanical action of multimodal field configurations on magnetic nanoparticle environments, N. Maniotis, A. Makridis, E. Myrovali, A. Theopoulos, T. Samaras, M.Angelakeris, J. Magn. Magn. Mater. 2018, *in press*

[3] Effect of low frequency magnetic fields on the growth of MNP-treated HT29 colon cancer cells, K. Spyridopoulou, A. Makridis, N. Maniotis, N. Karypidou, E. Myrovali, T. Samaras, M. Angelakeris, K. Chlichlia, O. Kalogirou, Nanotechnology 2018, *in press*.

DEVELOPMENT OF MAGNETIC NANOIMMUNOCONJUGATES FOR THE FAST AND EFFICIENT ISOLATION OF TUMORAL EXOSOMES

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Exosomes are nano-sized vesicles limited by a lipid bilayer released to the extracellular media by most cells. Inside, they harbour specific cargo materials (proteins, nucleic acids, amino acids, etc) that can be uptake by other cells. They play therefore an important role in the inter-cellular communication, particularly in metastatic processes. Since they can be found in several biofluids like blood, saliva and urine, they become very interesting biomarkers to study cancer progression. However, isolation of high yields of pure and intact exosomes from complex biological fluids is still a critical step for the implementation of their use in clinical diagnosis.

Nowadays, the most used purification strategies are based on differential centrifugation and precipitation, which are far from being efficient and time consuming. The feasibility of extracting exosomes targeting specific protein markers on their surface by means of immune-magnetic microbeads has already been demonstrated. However, further analysis of the captured exosomes requires their elution from the microbeads and this usually damage their structure. In this sense, magnetic nanoparticles (MNPs), with similar sizes to exosomes, may be more adequate as this would allow collecting a greater amount of exosomes due to their larger surface area. Besides, due to their smaller size they should make feasible the integration of the purification process with the detection of exosomal surface biomarkers without the need of tedious downstream processing.

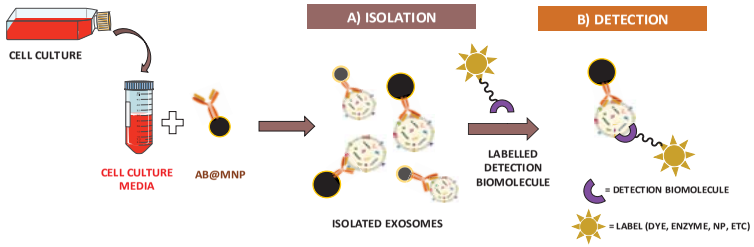

Figure. *Exosome purification using MNPs for a faster and efficient process that should be also compatible with a "one pot" further characterization of selected biomarkers present on their surface.*

In this work MNPs were functionalized with antibodies (Abs) that recognizes specific exosome surface biomarkers. In order to obtain the best capture capability of exosomes, the oriented attachment of the selected Abs was optimized. Once functionalized, these MNPs were characterized to confirm the correct union of the antibodies, and then used to capture exosomes by magnetic immunoseparation. Model samples containing exosomes consisted of cell culture conditioned media from epithelial (MDCK) and mesenchymal (Balbc 3T3) cell lines. Both cell lines are widely used as models of epithelial mesenchymal transition (EMT), a process that occurs in all cancers of epithelial origin (90% of total tumors) and plays a key role in cancer metastasis. During the optimization of the purification process, it was found that to avoid aggregation of the MNPs it is essential not only to control the density of antibodies attached, but also to optimize the blocking of the MNPs' surface once functionalized. Other parameters that were also optimized include the ratio of MNPs:exosomes and the incubation times needed. The optimized process was compared, in terms of efficiency, yield and purity, with currently used methodologies (ultracentrifugation and a commercial kit based on precipitation). The results obtained are very promising thinking in a future integration of exosome purification/detection.

Design of Magnetic Nanoparticles for Magneto-mechanical Cancer Cells Destruction

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Magnetic nanoparticles are increasingly used for a variety of biomedical applications, given their ability to exert heat, force or torque on biological species. One such application is the triggering of cancer cells destruction through the low frequency mechanical vibration of magnetic nanoparticles, attached to the cells membrane. The induction of cancer cells death has been demonstrated in glioblastoma cells [1] and renal human cancer cells [2].

The magnetic particles required for this type of application differ drastically from the SPION particles that are most often used for drug delivery or hyperthermia application. In the latter case the particles need to be superparamagnetic, which limit their size to roughly 20 nm. Such particles, usually made of iron oxides, are easily produced using chemical synthesis. On the other hand the particles required for magneto-mechanical applications need to be much bigger, in the micron-size range for at least one of their dimension, in order to achieve a sizeable mechanical effect (which, in addition, scales with the particle's magnetization). These particles are thus ferromagnetic. A second requirement, which may seems at odd with the preceding, is that they must have a vanishingly small magnetization with no applied magnetic field and a low initial susceptibility in order to prevent clumping.

In this presentation we will illustrate how magnetic particles made with top-down, lithography techniques allows to fabricate micron-wide disks particles with magnetic properties matching these requirement (Fig. 1). Among the various tested design, permalloy disks with vortex magnetization is one of the best suited system [3]. Other types of systems, as for instance synthetic antiferromagnetic particles, will be described and compared as well [4].

This work has been funded by the H2020 European project ABIOMATER 665440 and by the EuroNanoMed II project Nanoviber (ANR-16-ENM2-0008-02).

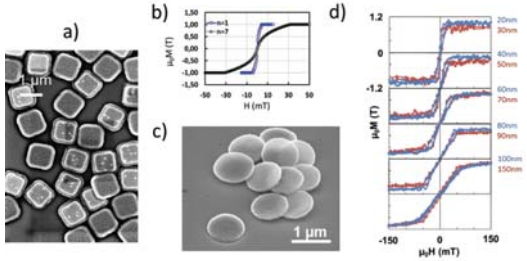

Fig. 1 (a) Synthetic antiferromagnetic $(NiFe/Ru)_n/Ru$ particles and (b) magnetization for two values of n; (c) NiFe vortex particles and (d) magnetization for different thicknesses.

[1] D.H. Kim, *et al.*, Nat. Mater. 9, 165 (2010) ; [2] S. Leulmi, *et al.,* Nanoscale 7, 15905 (2015) ; [3] S. Leulmi, *et al.*, APL 103, 132412 (2013); [4] H. Joisten, *et al.*, APL 97, 253112 (2010).

Oriented functionalization of magnetic nanoparticles with cadherins

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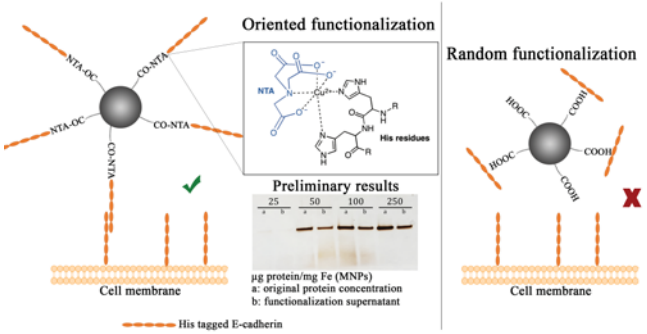
Magnetic nanoparticles (MNPs) have shown a great potential for biomedical applications due to their stability, biocompatibility and superparamagnetic behaviour below 20 nm.[1,2] Thus, they are widely used in biosensing, magnetic hyperthermia, or controlled drug delivery, to name but a few applications. Specifically, in order to achieve active targeting systems for therapy, MNPs are frequently vectorized by means of their biofunctionalization with biomolecules. However, there is no universal MNPs biofunctionalization protocol; large differences among MNP types and their coatings and the vast diversity of biomolecules to be attached are factors that difficult this critical step. Each particular case requires optimization to find the most adequate method to preserve the biological activity of the targeting biomolecule; for instance, its proper orientation once attached to the MNPs is crucial for maintaining its activity and must be achieved through innovative functionalization methodologies.

In this sense, we decided to take advantage of the affinity of chelated metals for histidine residues present in His-tagged biomolecules to develop a protocol for oriented functionalization of MNPs with cadherin recombinant fragments. Our final aim is to selectively attach the cadherin-functionalized MNPs to cell membranes for localized magnetic hyperthermia studies. Cadherins are a family of Ca^{2+}-dependent cell-cell adhesion proteins with a precise spatio-temporal pattern during embryonic development. Among them, E-cadherin is the most studied, having a relevant role in maintaining the cohesion of epithelial tissue. Moreover, during the epithelial-mesenchymal transition, E-cadherin disappearance is associated with metastasis.[3,4]

MNPs were synthesized by thermal-decomposition and covered with an amphiphilic polymer (poly(maleic anhydride-alt-1-octadecene)).[2] Their functionalization with Cu^{2+} chelated by nitrilotriacetic acid (NTA) via carbodiimide chemistry was optimized. Afterwards, those MNPs were incubated with E/EC1-2-cadherin fragments, allowing the Cu^{2+}-His interaction to take place. The attached protein was quantified from the supernatant. We are currently testing the use of different passivating agents (TRIS, polyethylene glycol) to stabilize the MNPs in cell culture media. The cytotoxicity of the MNPs, as well as preliminary studies of immobilization on the membrane of MDCK cells are also underway.



References: 1) Revia, R. A. & Zhang, M. *Mater. Today (Kidlington)* **19,** 157–168 (2016). (2) Moros, M. *et al. Nanoscale* **2,** 1746 (2010). (3) Stemmler, M. P. *Mol. Biosyst.* **4,** 835 (2008). (4) Moros, M. *et al. J. Phys. D. Appl. Phys.* **49,** (2015).

Effect of Surface Chemistry and Associated Protein Corona on the Long-Term Biodegradation of Iron Oxide Nanoparticles In Vivo

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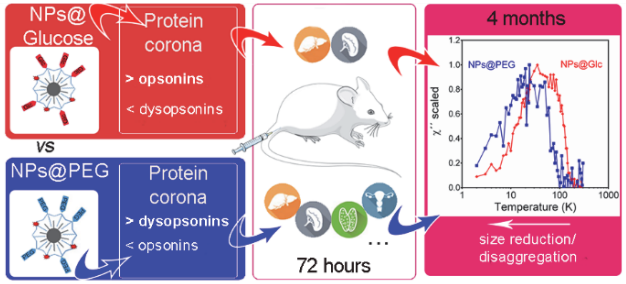
Once a nanoparticle (NP) is administered *in vivo*, it interacts with the components of the physiological environment, especially with proteins, resulting in the formation of the so-called protein corona (PC). PC can dramatically change the nanomaterial size, aggregation state, and interfacial properties, dominating in an uncontrolled way the biological behaviour of NPs. (1) Although it is widely accepted that the presence of this PC would ultimately determine the fate of the nanomaterial, its role in the biotransformation and degradation of NPs *in vivo* has been scarcely investigated.

Here we report how the surface modification of identical superparamagnetic iron oxide NPs with either glucose (Glc) or poly(ethylene glycol) (PEG) affects the PC identity, the NPs biodistribution, and more importantly, the degradation over time. Although NPs@Glc and NPs@PEG bound similar amount of proteins *in vitro*, the differences found in the composition of both PCs corresponded to the NPs biodistribution *in vivo*. Whereas NPs@Glc were mostly accumulated in the liver and spleen, NPs@PEG were detected in various organs, including thymus or reproductive system organs. Moreover, by employing magnetic measurements we have found that the biodegradation kinetic and therefore clearance of both NPs types was unequal, as NPs@PEG core suffered a faster degradation over time than NPs@Glc, in both liver and spleen. Differences in the degradation rate observed *in vitro* and *in vivo* could be related not only with the attached molecules, but also with the associated PC, which composition may directly affect the degradation rate by lysosomal enzymes or indirectly by driving NPs accumulation in different cells. (2)



References

(1) Monopoli, M. P.; Aberg, C.; Salvati, A.; Dawson, K. a. Biomolecular Coronas Provide the Biological Identity of Nanosized Materials. *Nat. Nanotechnol.* **2012**, *7*, 779–786.

(2) Stepien, G.; Moros, M.; Pérez-Hernández, M.; Monge, M.; Gutiérrez, L.; et al. Effect of Surface Chemistry and Associated Protein Corona on the Long-Term Biodegradation of Iron Oxide Nanoparticles in Vivo, ACS Applied Materials & Interfaces **2018,** 10, 4548-4560

Functionalisation of T lymphocytes for magnetically controlled immune therapy

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Breast cancer is the most common cancer related cause of death in women. With many factors such as genetics, hormones or lifestyle having an effect on the development of breast cancer, there is a classification of various subgroups: triple negative, luminal A or luminal B, HER2 positive or HER2 negative, basal-like or normal-like. In addition, gene mutations e.g. in BRCA1 or BRCA2 are to be found in every subgroup. The complexity of all those factors results in an individual treatment protocol consisting of chemotherapy, hormone therapy, antibody therapy, irradiation or surgery for every single patient.

On the contrary, the immune status of the tumour, especially the amount of tumour infiltrating lymphocytes (TIL) is important for all patients. The presence of $CD8^+$ killer T cells in the tumour area directly correlates with the patient`s clinical outcome, independent of molecular subgroup and treatment protocol. For this reason it seems desirable to increase the number of TIL.

One way to accumulate T cells in the tumour area is to make them magnetisable and attract them with an external magnetic field. Magnetisation can be achieved by superparamagnetic iron oxide nanoparticles (SPIONs) which can be bound to the cells' surfaces or – to avoid further immune reactions – can be internalised into the cells.

SPIONs with different coatings that were used for this study were synthesised according to an alkaline coprecipitation process. At first, we started with iron oxide cores coated with lauric acid ($SEON^{LA}$). The coating can be done *in situ* for reproducible particles or afterwards. Due to high toxicity, we improved the biocompatibility of those particles by adding an albumin layer ($SEON^{LABSA}$). Unfortunately, the protein coating decreased the amount of particle uptake into the cells. To increase the uptake, we aminated the $SEON^{LABSA}$-particles with various amounts of aminating reagents. The higher the amination degree the higher the uptake but also the higher the toxicity. The coming challenge will be to find a balance between sufficient uptake and acceptable toxicity.

With our promising preliminary data we already can demonstrate that it is possible to attract SPIONs bearing T cells by an external magnet (fig. 1).

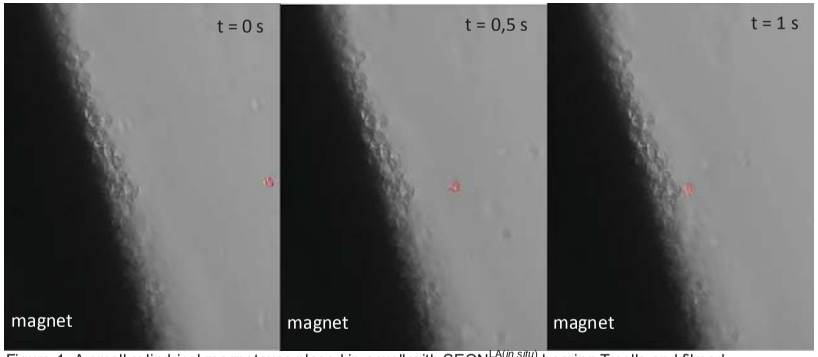

Figure 1: A small cylindrical magnet was placed in a well with $SEON^{LA(in\ situ)}$ bearing T cells and filmed. Cells accumulate on the magnet`s surface. (Colours and contrast have been adjusted for enhanced visualisation.)

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Differential modes of interaction and passage of magnetic nanoparticles through an *in vitro* blood-placenta model

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A prerequisite for any application of superparamagnetic iron-oxide nanoparticles (SPION) is the knowledge about their impact onto the human body, whereby the interaction with biological tissues plays a decisive role. SPIONs might offer the opportunity to selectively target the pregnant woman, the foetus or the placenta during pregnancy. In this study, an *in vitro* model of the blood-placenta barrier (BPB) was established and optimized to study the interaction and passage of SPIONs through this interface.

For the co-culture *in vitro* BPB model, the human choriocarcinoma trophoblastic cell line BeWo as well as primary placental pericytes were used. The *in vitro* BPB was incubated with differently charged SPIONs: neutral starch-coated, cationic polyethylenimine-coated and anionic carboxymethyldextran-coated particles. The interaction and the cytotoxicity of the SPIONs with the two cell types was investigated independently for both cell types with the PrestoBlue cell viability assay, real time cell analysis, flow cytometry and diverse microscopic analyses. The passage of the SPIONs through the BPB model was quantified by magnetic particle spectroscopy and atomic absorption spectroscopy.

For the establishment of an *in vitro* BPB model, the co-culture of BeWo cells with pericytes was compared to a BeWo mono-culture regarding the formation of a cellular barrier. For different BeWo seeding densities, histologic cross sections showed the formation of a tighter and thinner cell layer for the co-culture. TEER measurements and molecular permeability assays confirmed this observation by showing a 2- and 3-fold increase compared to the mono-culture. Additionally, the visualization of the cell-cell-contact proteins ZO-1 and β-catenin by immunological staining showed an enhanced expression again supporting the barrier-enhancing effect of co-cultured BPB towards mono-culture systems.

Investigations of the cellular interaction and cytotoxicity of SPIONs with the two cell types revealed the influence of the particle charge and concentration, the incubation time as well as the cell type on these parameters. Especially cationic PEI-coated particles showed pronounced cytotoxicity and interaction capability especially in the pericytic cells.

The incubation of the transwell-based BPB model with the three different SPION types did not reveal major disruption of the barrier integrity, while a particle charge- and incubation time-dependent incorporation into the barrier's cell layers could be shown. The quantification of the SPION distribution within the distinct compartments of the transwell BPB model showed that the BPB prevents the free passage of particles from the upper donor compartment into the lower acceptor compartment again confirming the high barrier integrity. However, minor amounts of particles in the range of 1 ng were detected in the lower compartment. Detection of SPIONs in the basolateral pericyte cell layer in the range of 4 ng indicates a transcellular passage of these particles through the BPB.

In conclusion, the established *in vitro* BPB model based on the co-culture of BeWo cells and pericytes on transwell inserts was shown to be a suitable model for the investigation of behavior and passage of SPIONs in this biological interface. Together with the possibility to sensitively quantify amounts of SPIONs in this model, a first prediction of the *in vivo* behavior of these particles is possible.

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Precision MRX®: A Versatile Iron Oxide Nanoparticle Platform for the Clinical Diagnosis and Treatment of Cancer

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Background: Iron oxide nanoparticles (NPs) have been used for a variety of *in-vivo* and *ex-vivo* applications within the biomedical sciences. Moreover, when intended for clinical *in-vivo* applications, NPs need to meet rigorous requirements to ensure safety as well as bio-functionality including blood circulation time and specificity for cellular targets. PrecisonMRX® NPs are extensively characterized superparamagnetic NPs composed of a 25nm magnetite cores that are currently employed in a variety of *in-vivo* applications including non-invasive/in vivo diagnosis of cancer, Magnetic Particle Imaging, MRI, and magnetic hyperthermia.

Objective: Here we report on the extensive pre-clinical development and functionality of PEGylated and antibody-conjugated NPs for *in-vivo* and *ex-vivo* detection of HER2+ tumor cells by Magnetic Relaxometry (MRX).

Results: We observed: 1) specific binding and detection of HER2 positive tumor cells in-vitro (5000 cells); 2) specific detection of HER2+ tumors in mice; 3) binding and amplitude of magnetic signal to be proportional to the level of HER2 expression in-vitro and in-vivo; 4) the nanoconstruct remains stable in circulation; 5) the particles do not induce a pro-inflammatory response nor activate complement; 6) the particles are biodegradable; and do not induce acute or delayed signs of morbidity in vivo.

Conclusion: Precision MRX® nanoparticles offer great clinical promise including the *in-vivo* detection of tumor cells by magnetic relaxometry. Given the stability and safety of these NPs, further development for use in MRX, MPI, MRI and hyperthermia applications as well as expansion into therapeutic drug delivery and disease monitoring in response to treatment will be discussed.

Development of a protocol to assess cell internalization and tissue uptake of nanoparticles

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Several applications of nanoparticles rely on the internalization and accumulation of nanocarriers in specific cell compartments and tissues. The internalization process has been extensively explored for multiple particles and conjugations. However, the methods currently employed for characterizing such processes, although well described, are time consuming and do not provide *in vivo* information, which is a crucial barrier towards translational applications. Here, we hypothesize that the ACB technique can be employed to assess cell internalization of magnetic nanoparticles, with possible applications in screening assays to track specific biomarkers and cell types. We tested a simpler and easier alternative to quantify cell internalization and to track specific cell-types in mixed cell cultures with considerable advantages, such as extrapolation to tissue accumulation after perfusion. We utilized citrate coated, manganese ferrite nanoparticles and evaluated the internalization process in mouse macrophages cells (J774.A1), for protocol validation, and in an embryonic neural stem cell culture (E14.5) after differentiation in astrocytes and neurons, to assess internalization specificity. Respecting the particles toxicity limits, we tested different concentration of particles, in different incubation times. Sequentially, we imaged the cell cultures to confirm internalization and nanoparticles localization, labeling nucleus and cell body to assure that the particles were inside the cells and not simply attached to the well or to the cell membrane. We tested doses of 500-5000. Our results showed a linear behavior on internalization for different doses and an optimum incubation time of 2 hours. Figure 1 summarizes our findings.

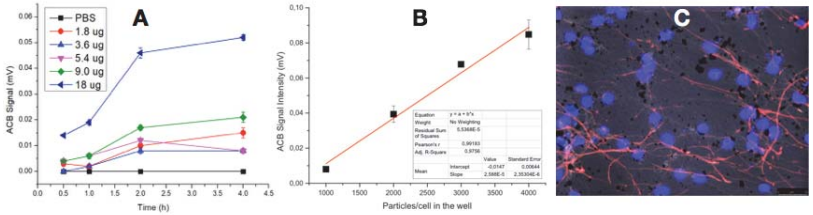

Figure 1. (A) ACB Signal Intensity obtained after internalization of nanoparticles by macrophages; (B) ACB Signal Intensity obtained after internalization of nanoparticles by astrocytes and neurons and; (C) Confocal Microscope Image indicating nanoparticles internalization.

Development of a uniform phantom for quantification of magnetic nanocomposites in biological tissue for a cross-calibration of X-ray and Magnetic Resonance Imaging.

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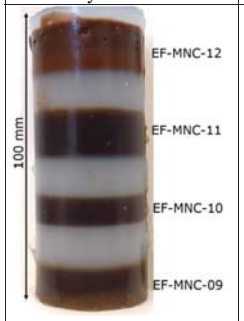
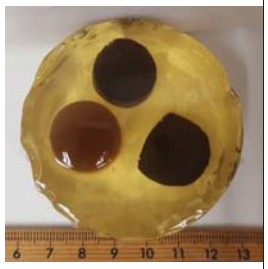
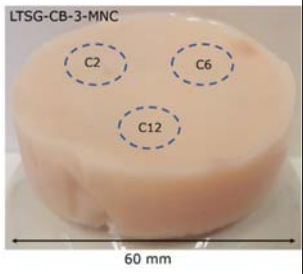
The use of a uniform body tissue and magnetic nanocomposites (MNC) phantom for X-ray computed tomography (XCT) and magnetic resonance imaging (MRI) provides the possibility of co-registration, and thus a more precise visualisation of MNC in, e.g., tumour sites as well as their quantification. In this paper we present a promising approach for a uniform long-term stable phantom which mimics MNC enriched biological tissue, e.g., tumours after magnetically assisted cancer treatments. For this study two different immobilisation matrices – silicon rubber and gelatin - have been used to create three different phantom types:

a. a silicon rubber Ecoflex® (EF) loaded with MNC → EF-MNC
b. a long-term-stable-gelatin (LTSG) loaded with MNC → LTSG-MNC
c. a long-term-stable-gelatin (LTSG) and raw homogenized chicken breast mixture loaded with MNC → LTSG-CB-MNC.

The phantoms were produced as stacks of cylinder discs with base diameter of 30 mm as shown in figure a), and as cylinder discs with base diameter of 60 mm where three smaller cylinder discs with different MNC concentrations are embedded within the matrix component as presented in figures b) and c). The MNC concentrations in these phantoms range from 0 mg/ml to 4.5 mg/ml. The phantoms have been analysed with NMR and MRI regarding the T2 relaxation times, with Vibrating Sample Magnetometry (VSM) for detection of the real MNC concentration and homogeneity and visualised in 2-D and 3-D with MRI and XµCT. The cross-calibration of an XµCT and a MRI scanner has been performed using these various phantoms. A sensitivity range for both imaging modalities has been determined.

a) EF-MNC cylindric phantom with 4 MNC-loaded cylinder discs.	b) LTSG matrix with 3 MNC concentrations.	c) Raw biological tissue - LTSG-CB - matrix with 3 MNC concentrations.
		

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Facile Synthesis of Magnetic-Silica-Mannan Nanocomposites for Enhancement in Internalization and Immune Response by Dendritic Cells

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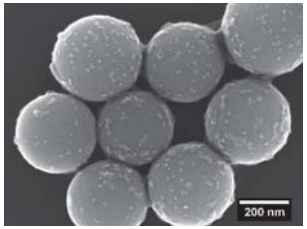
Magnetic nanoparticles (MNPs) have drawn much attention in various applications, for instance, catalysis, sensors, medical diagnosis, and so on. Especially in biomedicine, their attractive properties such as biocompatibility, low toxicity, stability in physiological environment are of great interest. Moreover, superparamagnetic property is also very attractive and unique, which offers a potential possibility in targeted drug delivery area. Vaccination is one of the applications that can utilize a magnetic delivery system to direct antigens and adjuvants to the targeted immune cells. As mannan, a polysaccharide from cell wall of fungi species, has been reported as a good adjuvant, we were interested in producing magnetic-silica-mannan nanocomposites (MS-mannan) as a delivery system to target dendritic cells and induce their immune response. In this work, we synthesized magnetic-silica nanocomposites (MS) via a simple thermal decomposition method and characterized them with FESEM equipped with energy dispersive X-ray spectroscope (Figure a). The nanocomposites clearly showed spherical shape with magnetic nanoparticles deposited on the surface. Subsequently, the obtained nanocomposites were modified with mannan obtained from cell wall of *Saccharomyces cerevisiae,* which is a non-pathogenic fungus. The obtained MS-mannan demonstrated negative charge as confirmed by a zeta potential technique. Under an external magnetic field, MS-mannan particles could facilitate intracellular uptake (Figure b). Due to dual-functionality of mannan and magnetic nanoparticles and the abundance of mannose-receptors on dendritic cells, the MS-mannan nanocomposites greatly enhanced cellular uptake compared to the MS particles. Moreover, the dendritic cells exposed to MS-mannan nanocomposites showed high percentage of cell viability and performed a good immune response by releasing more IFN-γ cytokine compared to the particles without mannan coated. The nanocomposites from this work can be beneficial for the development of effective delivery of antigen to targeted immune cells with adjuvant properties for enhanced immunization.

a) 

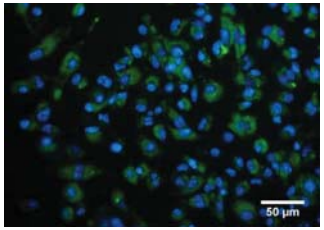
b) 

Figure. (a) FESEM image of magnetic-silica nanocomposites (MS), (b) Fluorescent microscopy image of magnetic-silica-mannan nanocomposites (MS-mannan) internalized by dendritic cells.

Synthesis of PDMAEMA-Iron Oxide Nanocubes for Plasmid Gene Delivery into Dendritic Cells

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Dendritic cell (DC) is an important antigen presenting cell, and they have been focused on vaccines, gene therapy, and cancer immunotherapy applications. However, DCs are difficult to be transfected *in vitro* using traditional non-viral molecules.

Herein we are interested to deliver plasmid genes to dendritic cells using magnetic iron oxide nanocubes (MNCs) coated with cationic polymer under the induction of an external magnetic field. The MNCs were coated with positively charged polymer, poly(2-dimethylamino)ethyl methacrylate (MNC-PDMAEMA), before adsorbed with plasmid gene (pMAX-GFP) on their surfaces. The MNCs were synthesized by thermal decomposition method before PDMAEMA was assembled onto the MNC surfaces. The MNCs were synthesized in three different sizes of 15, 40 and 90 nm in diameter as confirmed by transmission electron microscopy (TEM). After coating, the MNC-PDMAEMA exhibited positive charge with the zeta potential of +23 to +26 mV, and the obtained particles showed superparamagnetic character. The plasmid gene loading particles were tested for cytotoxicity and cellular uptake with dendritic cells using MTT assay and a fluorescence microscope. The MNC-PDMAEMA showed low cytotoxicity and well taken up in bone marrow-derived dendritic cells (BM-DCs) under an external magnetic field.

Plasmid gene coated on MNC-PDMAEMA could be a potential non-viral approach for *in vitro* transfection of dendritic cells under an induction of a magnetic field.

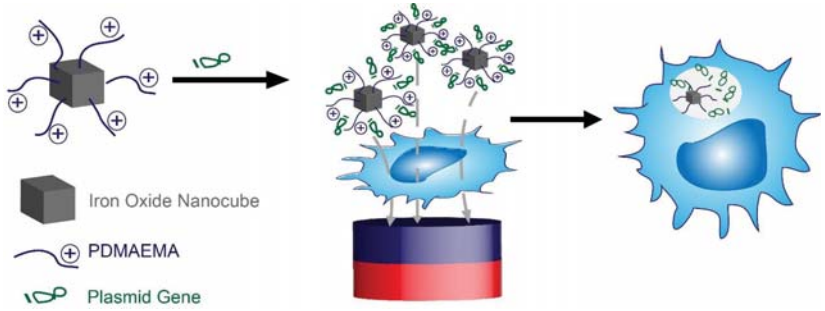

Figure. Schematic model depicting the MNC-PDMAEMA particle containing plasmid gene internalize into dendritic cells under the induction of a magnetic field.

Glioma-specific targeting of superparamagnetic iron oxide nanoparticles conjugated with cmHsp70.1 monoclonal antibodies (SPION–cmHsp70.1) is improved by ionizing radiation

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The stress-inducible 72 kDa heat shock protein Hsp70 is known to be expressed on the membrane of highly aggressive tumor cells including high-grade gliomas, but not on the corresponding normal cells.[1] Membrane Hsp70 (mHsp70) is rapidly internalized into tumor cells and thus targeting of mHsp70 might provide a promising strategy for theranostics. Superparamagnetic iron oxide nanoparticles (SPIONs) are contrast negative agents that are used for the detection of tumors with MRI. Herein, we conjugated the Hsp70-specific antibody (cmHsp70.1) which is known to recognize mHsp70 to SPIONs to assess tumor-specific targeting before and after ionizing irradiation.[2]

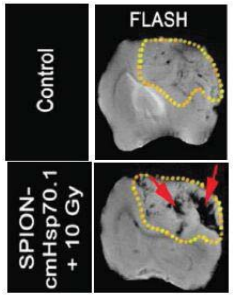

Fig.1. FLASH (gradient echo) MR scans for control and irradiated (10 Gy) rats treated with SPION–cmHsp70.1 conjugates. Retention of the nanoparticles in the tumor presented as hypotensive zones (red arrows).

In vitro experiments demonstrated the selectivity of SPION–cmHsp70.1 conjugates to free and mHsp70 in different tumor cell types (C6 glioblastoma, K562 leukemia, HeLa cervix carcinoma) in a dose-dependent manner. High resolution MRI (11T) on FLASH images showed the retention of the conjugates in tumor (Fig.1). Biodistribution analysis using measurements of second harmonic of nonlinear response to a weak *ac* magnetic field in parallel to it *dc* field *H* showed a 7-fold increase in tumor-to-normal brain uptake ratio of SPION–cmHsp70.1 conjugates in glioma-bearing rats relative to SPIONs (Fig.2). This retention within Hsp70-positive glioma was further enhanced after a single dose (10 Gy) of ionizing radiation. Elevated uptake of the magnetic conjugates in the tumor (4-fold) due to radiosensitization proves the combination of radiotherapy and application of Hsp70-targeted agents in brain tumors. Our approach has the potential to be clinically applied for Hsp70-targeted anti-tumor diagnostic and/or therapeutic approaches.

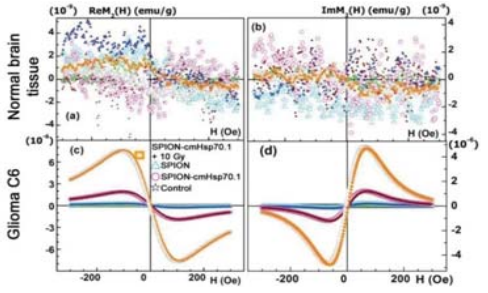

Fig.2. Biodistribution of nanoparticles in normal brain and tumor of the non-irradiated rats treated by SPIONs, SPION–cmHsp70.1 and irradiated rats (10Gy) treated with SPION–cmHsp70.1

[1] G. Multhoff, C. Botzler, M. Wiesnet, et al, *Int. J. Cancer* **61** (1995) 272–279.

[2] M. A. Shevtsov, B. P. Nikolaev, V. A. Ryzhov, et al, *Nanoscale* **7** (2015) 20652-20664.

Magnetic manipulation of implanted cancer cells for the optimization of the standard syngeneic mouse tumor model

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Transplantable syngeneic mouse tumor models consisting of the implantation of histocompatible tumor cells in immunocompetent mice, have played a fundamental role in the development of novel therapeutic interventions and are currently widely used in translational research. However, irregular distribution patterns of inoculated cells can induce inconsistent tumor size ranges among animals in the same treatment groups. Because of this variability in tumor volume values, more animals and multiple biological repetitions might be needed in order to achieve statistically significant results. We propose the optimization of the standard transplantable subcutaneous mouse tumor model by magnetically restricting the distribution of injected cancer cells. We used magnetic nanoparticles (fluidMAG-D, Chemicell GmbH) to label, and an NdFeb magnet to manipulate, magnetically-tagged CT26 cancer cells subcutaneously implanted in BALB/c mice. Our data provide evidence that the proposed approach can facilitate the formation of tumors with smaller variability in tumor volume values, evident by the calculations for certain measures of dispersion and central tendency. For the proof-of-principle, the efficiency of the optimization method was examined by using a low dose administration scheme (35 mg/kg) of the chemotherapy drug fluorouracil (5-FU) as a potential new anticancer drug under investigation. Under these experimental conditions, the 5-FU-induced tumor growth inhibition was statistically significant only after implementation of the optimized method. We have devised a simple, cost-effective procedure that is applied along the standard experimental process and significantly improves the detection limits of the standard protocol. We surmise that optimization and refinement of current murine preclinical models will accelerate the pace and development of novel therapeutic strategies.

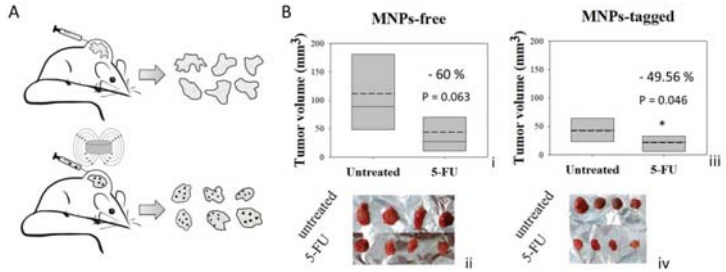

Figure. *Schematic representation of the proposed optimization method* ***(A)*** *and mean tumor volume and photographic observation of tumors excised from mice that were subjected to the standard method for generating subcutaneous syngeneic tumors* ***(Bi-ii)*** *or the proposed optimization protocol* ***(Biii-iv)****. 5-FU, a widely used anticancer drug, was used representing a potential anticancer compound under investigation (horizontal solid lines indicate median and dashed lines indicate mean).*

Reducing non-specific binding on magnetic beads

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Non-specific binding of proteins is an issue that affects all magnetic beads, increasing the noise and therefore reducing the signal to noise ratio of the assay. This causes the sensitivity of the assay to be reduced, resulting in higher amounts of analyte required to generate a positive signal, and an increased risk of false positive results. The non-specific binding can be due to various mechanisms, including hydrophobic, electrostatic and hydrophilic interactions. These interactions can come from a variety of sources on the bead, dependent on the manufacturing process. In general, for protein coated beads, the two major sources of non-specific binding would be the protein and the underlying bead surface chemistry. The source of these interactions were investigated by looking at the different layers of a typical bead to determine the contribution to the levels of non-specific binding. Approaches to reducing or eliminating these negative interactions were also evaluated, with a focus on identifying suitable wash solvents that can be easily introduced into the assay procedure. Optimisation of these washes ensured that the non-specific binding was minimised and that the appropriate levels and type of solvent were utilised.

Magnetically-Actuated Alginate Scaffolds: Effects on Macrophage Cytokine Secretion

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Creating bioengineered tissue to treat or replace damaged tissue is challenged by the need for efficient vascularization to support tissue survival and engraftment after implantation. Angiogenesis is a complex biological process critical for vascularizing bioengineered tissue that relies on a combination of biochemical cues, multiple cell types, and mechanical cues. The incorporation of mechanical cues in novel tissue design is largely limited to the physical characteristics of the biomaterial chosen serve as the tissue scaffolding. Consequentially, crucial dynamic-mechanical cues, such as shear-stresses or cyclic strain generated *in vivo*, are lacking. Previously, our lab demonstrated the ability to induce biological responses from vascular cells, i.e., endothelial cells, and cardiac cells stimulated in magnetically actuated scaffolds[1-3]. This method utilizes a magnetically-responsive scaffold in combination with a remotely applied time-varied uniform magnetic field to impart cyclic strain within the scaffold.

In the current study, we investigate the impact of our system on macrophage behavior. Macrophages are key mechanosensitive-accessory cells known to modulate the behavior of surrounding cells during important regenerative processes, such as angiogenesis, through the secretion of cytokines and growth factors, making them valuable targets for regenerative medicine. Using primary bone-marrow derived macrophages from Balb/c mice seeded onto magnetic scaffolds, we show that magnetically-actuated scaffolds significantly alter macrophage cytokine, chemokine, and growth factor secretion in a manner that is dependent on both the frequency of the applied magnetic field and the biochemical environment. At a low frequency of 0.5Hz, in the presence of inflammatory biochemical cues, lipopolysaccharide and interferon-gamma, magnetic stimulation resulted in increased secretion of the leukocyte chemokines CCL2 and CXCL2, and the cytokine interleukin-10. Additionally, we observed a significant increase in the potent angiogenic growth factor, vascular endothelial growth factor-A (VEGF-A) at low frequency and in both the presence and absence of inflammatory-biochemical cues. Interestingly, we did not observe significant differences in these cytokines and growth factor at a high frequency of 40Hz implying that cells respond differently to different regimens of magnetic stimulation. These results indicate that magnetically-actuated scaffolds can affect the microenvironment and biological processes, like angiogenesis, by altering macrophage cytokine and growth factor secretion. Future studies will investigate the impact of magnetic actuation on the *in vivo* scaffold environment.

References:

1. Sapir, Y.; Cohen, S.; Friedman, G.; Polyak, B., The promotion of in vitro vessel-like organization of endothelial cells in magnetically responsive alginate scaffolds. *Biomaterials* 2012, *33* (16), 4100-9.
2. Sapir, Y.; Polyak, B.; Cohen, S., Cardiac tissue engineering in magnetically actuated scaffolds. *Nanotechnology* 2014, *25* (1), 014009.
3. Sapir-Lekhovitser, Y.; Rotenberg, M. Y.; Jopp, J.; Friedman, G.; Polyak, B.; Cohen, S., Magnetically actuated tissue engineered scaffold: insights into mechanism of physical stimulation. *Nanoscale* 2016, *8* (6), 338699.

Effect of Viscosity on AC Magnetization of Magnetic Nanoparticles for different AC Excitation Field

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Magnetic nanoparticles (MNPs) have been widely studied for biomedical applications such as hyperthermia and magnetic particle imaging (MPI), where magnetization of MNPs under ac excitation field is used. It is well recognized that ac magnetization occurs via Néel and Brownian mechanisms. In biomedical applications of MNPs, the viscosity, which is related to the Brownian relaxation time, is affected by the surrounding medium. Therefore, it is important to clarify how the viscosity affects the ac magnetization mechanism of MNPs. To this end, we prepared three MNPs samples (S1-3) with different viscosities by diluting the water-based MNP suspension with different volume of glycerol as shown in Table 1. The total volume of each sample is 150 μl. The calculated values of viscosity of three samples are listed in Table 1. As MNP sample, we used fractionated Resovist® (FUJIFILM RI Pharma) particles. The typical hydrodynamic diameter of MNPs is 60.5 nm.

We measured the ac magnetization of these three samples for various ac excitation frequency f and amplitude H_{ac} (Table 1). Figure 1 shows ac M-H curves of S1 and S3 for f = 3.5 kHz and $\mu_0 H_{ac}$ = 3.5 mT. As shown, the two curves are different from each other. In Table 1, the strength of fundamental and third harmonic component of ac magnetization, i.e., M_1 and M_3, and the values of coercive field H_c and remanence M_r are summarized. As shown, these values considerably changed with viscosity. This result indicates that Brownian relaxation affects the ac magnetization mechanism under this ac field condition.

In Table I, the results obtained under different excitation conditions are also shown. As can be seen, properties of ac magnetization are not affected by the viscosity when the amplitude and/or frequency of the excitation field become large. This indicates that the Néel magnetization mechanism is dominant in these cases. These results are important to quantitatively evaluate the MNPs for use in biomedical applications.

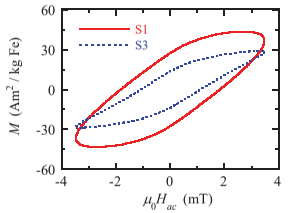

Fig. 1 M-H curves when an ac excitation field with $\mu_0 H_{ac}$ = 3.5 mT and f = 3 kHz was applied.

Table 1 Summary of ac magnetization properties from MNP samples with different viscosities. The values of M_1, M_3, H_c, and M_r under different excitation conditions are shown.

	Volume of glycerol (μl)	Viscosity (mPa · s)	M_1 (Am²/kg (Fe)), M_3 (Am²/kg (Fe)), $\mu_0 H_c$ (mT), M_r (mT)			
			f = 3 kHz		f = 20 kHz	
			$\mu_0 H_{ac}$ = 3.5 mT	$\mu_0 H_{ac}$ = 20 mT	$\mu_0 H_{ac}$ = 3.5 mT	$\mu_0 H_{ac}$ = 20 mT
S1	0	0.957	45.1, 2.24, 1.84, 27.2	90.0, 20.9, 4.32, 49.1	26.9, 1.55, 1.77, 15.9	91.1, 19.0, 6.78, 59.7
S2	80	9.43	33.1, 2.78, 1.33, 16.6	89.0, 21.2, 4.50, 53.0	24.0, 1.54, 1.49, 12.0	92.2, 19.8, 6.69, 60.0
S3	140	411	31.3, 2.79, 1.13, 13.4	88.5, 21.6, 4.51, 53.4	24.4, 1.56, 1.48, 12.1	93.2, 19.9, 6.66, 60.2

Molecular Magnetic Resonance Imaging of Cancer using Double Action Core/Shell Nanoparticles

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Magnetic Resonance Imaging (MRI) has been used for early cancer detection, treatment monitoring and image guided surgery. MRI has excellent spatial resolution and soft tissue contrast but low specificity. Standard contrast enhanced MRI based on tumors vasculature, including Gd-based T_1 contrast agents, do not provide sufficiently high specificity for tumor diagnosis and thus contrast agents providing T_2 contrast have been applied to provide information on tumor specificity.

To improve the tumor contrast we have developed core/shell $NaDyF_4/NaGdF_4$ nanoparticles changing both T_1 and T_2 relaxation times of surrounding water molecules and conjugated them with tumor specific antibodies and proteins. The relaxation times (T_1 and T_2) of the nanoparticles with various score/shell sizes and concentrations were measured at 9.4T and 3T to find the optimum T_1/T_2 ratio for MRI. T_1- and T_2-weighted images using core/shell nanoparticles of the animal models of brain, breast and prostate cancer were collected and combined to provide enhanced contrast and edges (Fig 1). The contrast agents consisting of the core/shell nanoparticles with the optimal core and shell sizes are being developed to provide improved tumor contrast when the T_1 and T_2-weighted MR pulse sequences are applied. The results may improve the efficacy of the new contrast agents, thus potential suitability for the early detection of cancerous tissues.

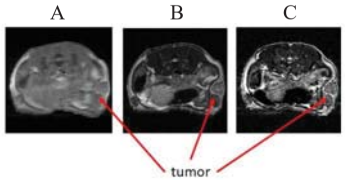

Fig 1. T_1-weighted (A), T_2-weighted (B) and combined T_1/T_2 (C) MR image of the breast cancer in the animal model using core/shell contrast agents. The differences in tumor contrast are visible.

Numerical Investigation of Magnetic Nanoparticles Clearance from Blood Circulation Using Extracorporeal Magnets

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Several biomedical applications of magnetic nanoparticles (MNPs) have already been translated from bench to bedside. In some of these applications MNPs are injected in blood circulation. Although they are destined to be cleared from blood with biological processes, some MNPs may remain in blood, until they are removed by extracorporeal magnetic fields. On the other hand, there are biomedical applications, which are based on the extravasation of MNPs in the interstitial space, e.g., for targeted drug delivery.

The present work aims at studying computationally the clearance of MNPS from blood circulation under the influence of an external magnetic field by the development of a 2D mathematical model that has been suggested for magnetic drug targeting (Figure (a)).

To determine the optimal conditions under which MNPs can be successfully eliminated, the study examines three possible magnetic fields induced by one or two cylindrical magnets, diametrically magnetized and positioned perpendicularly to the blood vessel. The partial differential equations that describe MNPs motion under the magnetophoretic forces are formed for each configuration of magnets. Then, they are numerically solved using a classical fourth-order Runge-Kutta method to predict the trajectories of MNPs for different combinations of the model parameters. Each simulation estimates the percentage of captured particles, i.e., those reaching the walls of the blood vessel, in order to find the combinations which lead to complete (> 95%) clearance. The parameters varied were magnet magnetization and radius, distance between magnet and blood vessel, blood vessel radius and average flow velocity, viscosity of blood and radius of nanoparticles.

The results show that artificial clearance of iron oxide magnetic nanoparticles is possible (Figure (c)) even with the use of a single ceramic (M_s=106A/m) magnet with radius R_m between 1.5 and 2cm, as long as it can be placed close to the blood vessel (for vessel diameters d<3cm). Moreover, clearance rates improve as the blood vessel radius gets smaller, while low blood viscosity ($\eta = 8.9\times10^{-4}$Ns/m^2) is a significant factor for the process. Finally, adding a second magnet allows the clearance from more distal vessels, especially when the magnets are placed with opposite magnetization (Figures (b) and (d)).

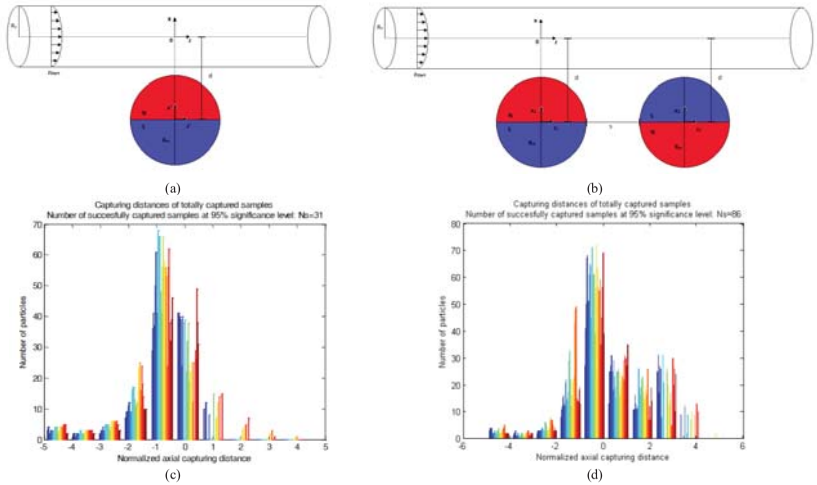

Figure. **(a)** Configuration with (a) single magnet, and **(b)** two magnets oppositely magnetized.**(c)** Normalized capturing distance along the vessel for the single magnet configuration. **(d)**Normalized capturing distance along the vessel for the two magnets configuration. The number of cases of complete MNPs elimination (Ns) increases from 31 to 86.

Differential Magnetometry to detect sentinel lymph nodes in laparoscopic procedures: static results

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We present a novel laparoscopic probe for sentinel node biopsy, to facilitate minimally invasive surgery. Sentinel node biopsy is a procedure to analyze the lymph node status of cancer patients [1]. As a result, it can be determined if the tumor has metastasized, leading to personalized patient care.

Superparamagnetic iron oxide nanoparticles (SPIONs) are used as a tracer to identify sentinel nodes. The main advantages of SPIONs are their long shelf life, safe clinical use, and that they accumulate in the sentinel lymph nodes. The latter makes it possible to perform a pre-operative MRI scan, that can be used as surgical guidance. To locate SPIONs *in vivo* during open surgery, a magnetometer was developed [2]. However, the main drawback of this system is its sensitivity both to diamagnetic tissue and surgical instruments. The principle we use to locate sentinel nodes is Differential Magnetometry (DiffMag) [3]. In DiffMag, the nonlinear magnetic properties of SPIONs are exploited, enabling selective detection. However, our first handheld probe suffers from limited depth sensitivity, which does not meet the clinical need of pathologies where lymph nodes are located deep in tissue.

To meet the clinical demands of increased depth sensitivity, we propose a set-up in which the excitation and detection coils are mechanically separated, as shown in Figure (a). As a result, the size of the excitation coil can be increased and placed outside the body. The detection coil can then be made much smaller, and placed inside laparoscopic equipment. However, the main challenge of this set-up is that the detection coils can move with respect to the excitation coils. As a consequence, the detector signal is obscured by the excitation field, requiring continuous active compensation. We implemented this active compensation and tested it in a static set-up. The results are shown in Figure (b) for three different SPIONs. These first results are promising for sentinel node biopsies, since it is possible to detect small amounts of iron.

[1] Giuliano and Gangi, 2015. [2] Pouw et al., 2016. [3] Waanders et al., 2016.

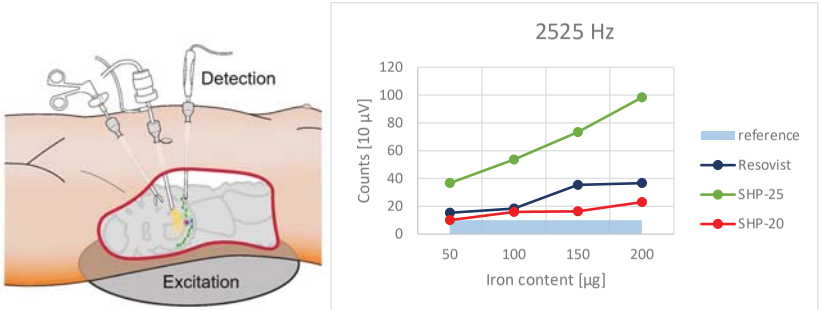

*Figure – **(a)** A novel laparoscopic probe for sentinel node biopsies using our DiffMag technique with separated excitation and detection coils. **(b)** Static results, measured using the DiffMag protocol on samples of Resovist (Bayer Schering Pharma GmbH), SHP-25 and SHP-20 (Ocean Nanotech) containing different amounts of iron in a concentration of 5 mg/mL.*

Characterization of superparamagnetic iron oxide nanoparticles in biological environments

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We present a novel characterization technique for superparamagnetic iron oxide nanoparticles (SPIONs): the Superparamagnetic Quantifier (SPaQ). It was created to measure SPIONs in biological environments, such as blood, lymph nodes and tissue. The SPaQ can be used to improve sentinel node biopsies (SNB). The latter is a procedure to analyze if cancer cells have spread to lymph nodes, leading to personalized patient care. During SNB a tracer material is injected at multiple sites close to the tumor. Via mechanical transport the tracer will accumulate in the first nodes it encounters, namely the sentinel nodes. The sentinel node can then be found using a dedicated probe, and examined for metastases following surgical removal.

When SPIONs are used as a tracer in SNB, it is important to characterize them first. Therefore, we know what we are looking for in the human body. To characterize SPIONs, the magnetization curve is measured, which shows the response of the particles to an externally applied field. In the SPaQ this magnetization curve is measured by application of a constant AC magnetic field that has a low amplitude and a gradually increasing DC offset. The maximum field strength is 15 mT and the AC frequency can be chosen between 1 and 10 kHz. This leads to a scan through different external field strengths, which yields the derivative of the magnetization curve. The resulting curve is shown in Figure (a) for two types of SPIONs. Numerical integration yields the magnetization curve, which is shown in Figure (b).

The sample influences the susceptibility and saturation field strength, which is reflected in the magnetization curve. This allows us to quickly assess newly fabricated particles on their feasibility as a tracer material. In various biomedical applications, a measurement method like the SPaQ's allow for monitoring changes in physiological conditions of the system in a real time fashion. For example, a changing hydrodynamic particle diameter caused by protein corona formation in blood or binding of drugs in controlled drug delivery.

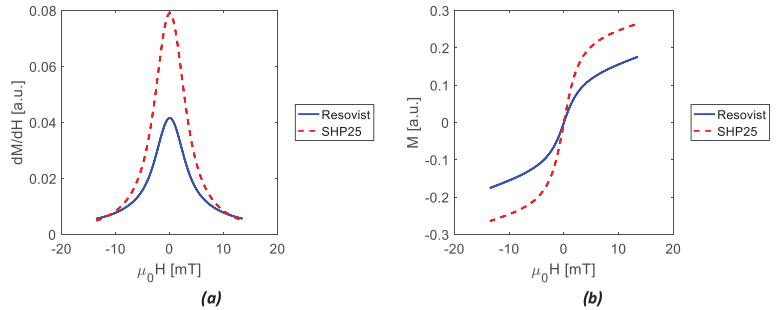

*Figure – SPaQ results, measured on Resovist (Bayer Schering Pharma GmbH) and SHP-25 (Ocean Nanotech) samples containing 750 µg iron in a total volume of 150 µl, at a frequency of 2.5 kHz. **(b)** is a numerical integration of **(a)**.*

In vivo studies of phosphonate-coated magnetic nanoparticles labeled with technetium-99m

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University of Belgrade, Vinča Institute of Nuclear Sciences, Serbia and COST Action RADIOMAG (TD1402)

Notable advances have been witnessed over the past decade on radiolabeling of nanovectors for diagnostic and therapeutic purposes. Multifunctional vectors capable to deliver radionuclides for imaging could be selectively delivered to tumor tissue by passive targeting taking advantage of the enhanced permeability and retention effect of tumor tissues.

The aim of this work was to investigate the potential of ^{99m}Tc- labeled Fe_3O_4 nanoparticles coated with two hydrophilic bisphosphonate ligands, methylene diphosphonate (MDP) and 1-hidroksietan diphosphonate (HEDP) for the potential application as theranostic nanoagents: hyperthermia application and diagnostic imaging.

MDP and HEDP coated Fe_3O_4 nanoparticles (MNPs) were synthesized by co-precipitation method. Bisphosphonate coatings improved biocompatibility of Fe_3O_4 nanoparticles and reduced aggregation. The mean hydrodynamic diameters of MDP- and HEDP-MNPs obtained from dynamic light scattering measurements were 45.64 nm and 21.91 nm, respectively, reflecting the coating with bisphosphonate layer on the 10 nm magnetic cores. Radiolabeling of both bisphosphonate-coated MNPs with ^{99m}Tc were carried out using $SnCl_2$ as a reducing agent. *In vitro* studies of radiolabeled MNPs in saline and human serum presented their high stability after 72 h. *In vivo* behavior of ^{99m}Tc–bisphosphonates and ^{99m}Tc–bisphosphonate–coated MNPs was studied in healthy male Wistar rats by gamma-camera and *ex vivo* biodistribution studies at predetermined time periods.

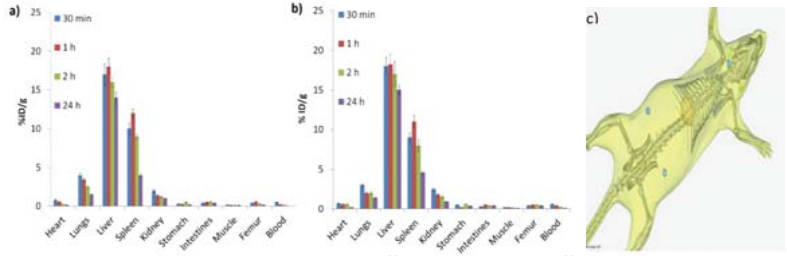

Fig.1. *Ex vivo* biodistribution studies during 24h of a) ^{99m}Tc-MDP-MNPs, b) ^{99m}Tc-HEDP-MNPs and c) cumulative SPECT/CT images of a normal Wistar rat 30 min after i.v. injection of ^{99m}Tc–MDP MNPs

Radiolabeled MNPs cleared fast from the circulation and showed significant liver and spleen uptake after i.v. injection at all studied time points. The highest uptake was observed at 1h post injection in the liver (18.0 ± 1.6 ID/g for ^{99m}Tc–MDP MNPs and 18.2 ± 1.3 ID/g for ^{99m}Tc-HEDP-MNPs), followed by the spleen (12.0 ± 1.0 ID/g for ^{99m}Tc-MDP-MNPs and 11.0 ± 0.9 ID/g for ^{99m}Tc-HEDP-MNPs). The obtained results of specific power absorption, high radiolabeling yield and *in vivo* stability of ^{99m}Tc-bisphosphonate-coated MNPs, demonstrate their high potential for therapeutic and diagnostic imaging applications.

Measuring Saturation Magnetizations of Superparamagnetic Nanoparticles from Liquid Phase

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Superparamagnetic iron oxide nanoparticles (SPIONs) have been extensively used as bio-imaging contrast agents, heating sources for tumor therapy, and carriers for controlled drug delivery and release to target organs and tissues. These applications require elaborate tuning of the physical and magnetic properties of the SPIONs. We present here a search coil based method to characterize the saturation magnetizations (M_s) of different SPIONs. The nonlinear magnetic response of SPIONs to alternating current (AC) magnetic fields induces harmonic signals that contain information of these nanoparticles. By analyzing the phase lag and harmonic ratios in the SPIONs, we can predict the saturation magnetization, the average hydrodynamic size, the dominating relaxation processes of SPIONs, and the distinction between single- and multi-core particles. Our numerical simulations reveal that the harmonic ratios are inversely proportional to saturation magnetizations and core diameters of SPIONs and that the phase lag is dependent on the hydrodynamic volumes of SPIONs, which corroborate our experimental results. Herein, we stress the feasibility of using search coils as a method to characterize physical and magnetic properties of SPIONs, which may be applied as building blocks in nanoparticle characterization devices.

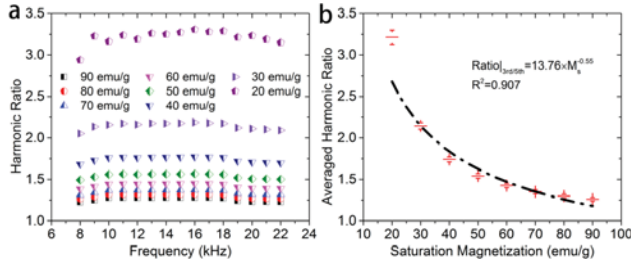

Figure 1. (a) Simulated harmonic ratios as function of frequency with $D = 30\ nm$, effective anisotropy constant $K_{eff} = 1.8 \times 10^5\ erg\ cm^{-3}$ ($1.8 \times 10^4\ J\ m^{-3}$), and M_s of SPIONs varies from 20 $emu\ g^{-1}$ to 90 $emu\ g^{-1}$. (b) Averaged harmonic ratios as function of M_s. Curve fitting gives rise to $R|_{3rd/5th} = 13.76 \times M_s^{-0.55}$, with a coefficient of determination (R^2) of 0.907.

Surface Functionalized Magnetic Nanoparticles Shift Cell Behavior with On/Off Magnetic Fields

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Magnetic nanoparticles(MNPs) are used as contrast agents and targeted drug delivery systems (TDDS) due to their favorable size, surface charge, and magnetic properties. Unfortunately, the toxicity associated with MNPs limits their biological applications. Surface functionalization of MNPs with selective polymers alters the surface chemistry to impart better biocompatibility. We report the preparation of surface functionalized MNPs using iron oxide NPs (MNPs), poly (lactic-co-glycolic acid) (PLGA), and sodium alginate via co-precipitation, emulsification, and electro-spraying, respectively. The NPs are in the nanosize range and negatively charged. Morphological and structural analyses affirm the surface functionalized nanostructure of the NPs. The surface functionalized MNPs are biocompatible, and demonstrate enhanced intracellular delivery under an applied magnetic field (H), which evinces the targeting ability of MNPs. After NP treatment, the physico-mechanical properties of fibroblasts are decided by the selective MNP uptake under "on" or "off" magnetic field conditions. We envision potential use of biocompatible surface functionalized MNP for intracellular-, targeted-DDS, imaging, and for investigating cellular mechanics.

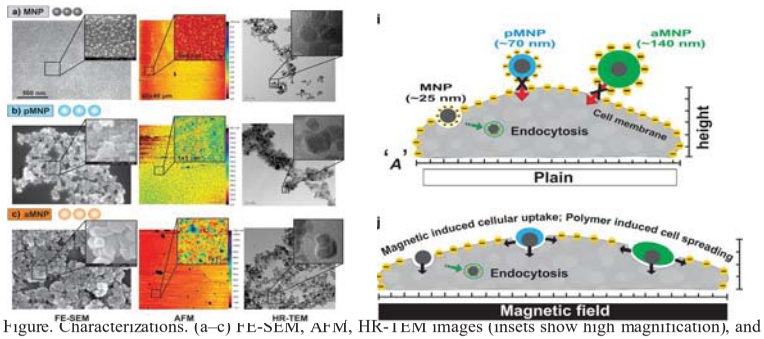

Figure. Characterizations. (a–c) FE-SEM, AFM, HR-TEM images (insets show high magnification), and hysteresis loops (VSM), Experiments were repeated in triplicate, and significant differences between samples are marked with *p < 0.05, and ***p < 0.001. Schematics depict the mechanism of cellular uptake of NPs and cell behavior under on/off magnetic field, respectively.

Reference

Seongbeom Jeon, Ramesh Subbiah, Taufik Bonaedy, Seyoung Van, Kwideok Park, Kyusik Yun, Journal of Cellular Physiology, 2018,233,1168~1178

Synthesis and Mossbauer study of the stability of $^{57}Fe_3O_4$ nanoprobes in living cells for probing the viscoelasticity of cytoplasm with nanosecond time resolution

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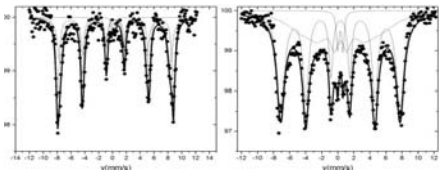
The key to a successful fight against diseases lies in understanding of the fundamental principles of the functioning of living systems. With the development of computer technology, bio-informatics has become one of the most promising branch of biology, capable of exploring the foundations of life at the molecular level. At the same time, the construction of models in bioinformatics is necessarily based on the data of experimental biology. The modeling of many intracellular processes, such as transport processes, diffusion of macromolecules, etc., is constrained by the lack of experimental data on the dynamic properties of intracellular fluids in living cells. The problem of studying intracellular dynamics is related both to the complexity of the internal structure of the cell and to the phenomenon of "macromolecular crowding", a phenomenon in which the interaction of large macromolecules occupying up to 40% of the intracellular volume of a living cell, dramatically changes the hydrodynamic and diffusion properties of the cytoplasm[1]. In recent years, studies on the viscoelasticity of the cytoplasm have been successfully carried out by studying the behavior of nanoscale probes embedded in living cells [2]. However, existing methods rely on optical measurements and therefore have a limited temporal and spatial resolution. We are developing a new method for studying the dynamic properties of intracellular fluid, based on nuclear gamma-resonance spectroscopy. The proposed method is based on the Mossbauer study of Brownian motion of ^{57}Fe-based nanoparticles, injected into cells. The viscoelastic properties of the cytoplasm can be obtained from the analysis of the change in the shape of the spectra of particles and the broadening of the spectral lines. Such study will make it possible to experimentally obtain thermodynamic parameters of the cytoplasm in nanosecond time resolution, which exceeds by several orders of magnitude the resolution of the existing methods.

For the successful implementation of the proposed approach it is necessary to take into account the change in the spectra of probe nanoparticles, which can arise due to their interaction with cells. Previously, we have already demonstrated the intensive transformation of Mössbauer spectra of particles as a result of their stay in living organisms [3]. In this paper, we investigated the stability of magnetite nanoparticles composition when incubated in cell culture. For this purpose, we synthesized sets of isotopically enriched $^{57}Fe_3O_4$ nanoparticles of various sizes and types of coating. Obtained nanoparticles were introduced into the cells and incubated for several days (from 1 to 6 days). After the incubation step, the cell samples were lyophilized and measured with Mossbauer spectroscopy, both at liquid nitrogen temperature and at room temperature. With the help of group analysis of spectra and their comparison with control spectra, we obtained new data on the stability of synthesized $^{57}Fe_3O_4$ nanoparticles to biodegradation in cells. The obtained results allowed us to select the optimal requirements for nanoparticles to be used as nanoprobes for investigation of the viscoelastic properties of the cytoplasm in further studies.



Mossbauer spectra of synthesized 25-nm magnetite nanoprobes after its incubation in living cells for 6 days measured at 77K (left) and 300K (right).

The work was supported by the Russian Foundation for Basic Research under Grant 17-00-00438

1) Ellis, R. J., & Minton, A. P. Cell biology: join the crowd. *Nature*, *425*(6953), 27. (2003)

2) Guigas, G., Kalla, C., & Weiss, *Biophysical journal*, *93*(1), 316-323. (2007)

3) Mischenko, I. N., Chuev et.al.. *Hyperfine Interactions*, *206*, (1-3), 105-108. (2012)

Enhancement of Cytosolic Ca^{2+} Levels and Induction of Actin Polymerization in Skeletal Muscle Cells by Spatiotemporal Magnetic Fields

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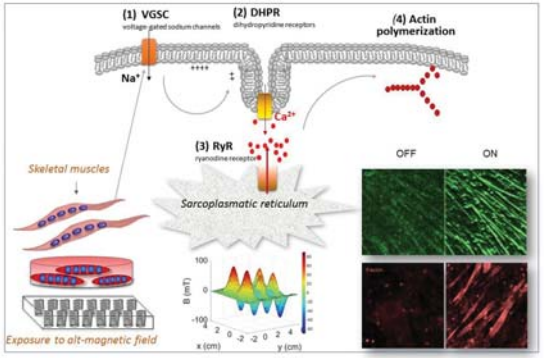
Cell molecular bioelectricity and machinery are substantially defined by the membrane potential controlling intracellular physiology and signal propagation from a motor neuron to a muscle fiber resulting in muscle contraction. Here, we demonstrate that the cell membrane potential can be altered by inhomogeneous static and time-varying magnetic fields [1,2]. We used spatiotemporal magnetic fields (17–70 mT) to control intracellular signaling in skeletal muscle cells. By choosing application of spatial and time-varying gradients of magnetic field, we induced transient depolarization of cellular membranes leading to the following sequence of intracellular events: i) Na^+ influx through voltage-gated sodium channels (VGSC), ii) VGSC- and ryanodine receptor-dependent increase of cytosolic calcium (up to 30%) and actin polymerization (Figure). Importantly, the ion fluxes occurred only, when the field was applied and returned to baseline after the field was turned off. An elaborated mathematical model reveals a key role for the magnetic field-induced eddy current, which mediates a local change in the membrane potential (≈10 mV) triggering the activation of VGSC.



We also review current research into the impacts of high-gradient magnetic fields on living cells and present the results towards a fundamental understanding of the cell response to spatiotemporal magnetic fields. We discuss the use of spatiotemporal magnetic fields for the modulation of cell functions and opening intriguing perspectives for engineering new magnetic devices permitting remote control for clinical applications to treat a number of neuro-myopathies.

[1] V. Zablotskii, T. Polyakova, O. Lunov, A. Dejneka. Scientific Reports 6 (2016) 37407.

[2] M. Rubio Ayala, T. Syrovets, S. Hafner, V. Zablotskii, A. Dejneka, Th. Simmet. Biomaterials (2018) 163, 174.

Influence of various properties of magnetic particles on their pharmacokinetic profile

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Magnetic nanoparticles (MP) are widely investigated in biomedicine as perspective contrast media for MRI, magnetic-guiding drug vehicles, and hyperthermia agents. However often MPs with promising effectivity *in vitro* have poor pharmacokinetic properties *in vivo*. Here we report investigating of the influence of different factors on pharmacokinetic profile of MPs: their rate of elimination from the bloodstream and following biodistribution.

In this study, for quantitative registering of nanoparticles, we used highly sensitive magnetic particle quantification (MPQ) technique [1], which offers convenient and accurate non-invasive detection of magnetic nanoparticles in real time *in vivo*. To detect nanoparticles in tissue, organ of interest was located in the system of magnetic coils. For example, for measuring nanoparticles concentration after its administration in blood, we detect MPs in tail venues and arteries and see monoexponential decreasing of MP quantity with time (Fig. 1).

We identified the chemical and physical properties of the nanoparticles, including size, surface charge, and surface chemistry, as well as the administration parameters that can determine pharmacokinetics of MPs. For example, we have shown the faster elimination from the blood of large and positively charged MPs. In addition, we showed that injection of large doses of almost any nanoagents saturates the macrophages and eventually leads to significant prolongation of the subsequently administered doses of MPs. Biodistribution changes after macrophage blockade with different types of particles were also investigated.

The results of the study can be used for prediction and improvement of pharmacokinetic parameters of nanoagents even of a non-magnetic nature.

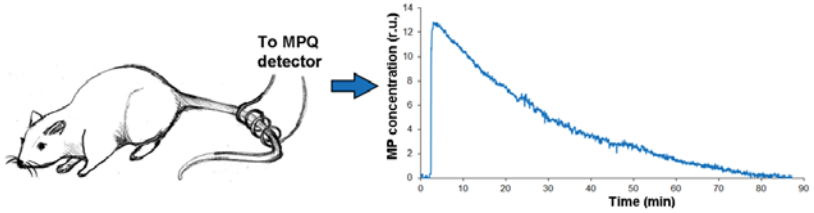

Fig.1. Measurement of the magnetic particle elimination from the bloodstream: scheme of the experiment (right) and example of the obtained result (left).

The work was supported by the Russian Science Foundation (grant № 17-74-20146)

1. M. Nikitin, P. Vetoshko, N. Brusentsov, P. Nikitin, J Magn Magn Mat, (2009)

Solid Phase Multiplex PCR Based on Encoded Magnetic microbeads

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With the development of genomics, multiplex PCR has attracted increasing attention. However, the existing Q-PCR technology is restricted by the fluorescence detection channels, which is difficult to achieve simultaneous detection of more than 6 indexes in a single tube. Solid phase PCR(SP-PCR) allows each analyte of interest to be physically isolated, and it also can overcome the interference of primer dimers that may cause poor specificity in a multiplex PCR reaction. Therefore, SP-PCR is supposed to be one of the most ideal carries for quantitative multiplex detection .

Nowadays, compared with conventional supports like microarrays, magnetic beads as new carries for SP-PCR possess faster reaction kinetics. Our group has already developed a three dimensional encoding library of 100 dual-color magnetic barcodes(DEBs) and successfully realized multiple targets detection of nucleic acids and proteins in a single tube.

As a proof of concept, we focused on the quantitative multiplex PCR for microbiological detection with hilA, sefA, sdf as model targets based on DEBs. Firstly, sequence-specific DNA probes are immobilized on three different DEBs. Then unbounded small amount of forward and Cy5 labelled reverse primers were added to a PCR reaction. After the first 30 cycles for DNA amplification in solution, annealing temperature was raised for nested PCR on microbeads. And finally, the intensities of fluorescence on each bead was recorded by flow cytometry. The results of hilA detection showed the lowest DNA concentration able to be detected by SP-PCR was 0.18fM. This method also has high specificity.

This work indicated the target DNA can be directly amplified on DEBs with high sensitivity and specificity and efficiently quantified according to the intensities of fluorescence, which paves a new way to realize quantitative multiplex detection based on SP-PCR in a single tube.

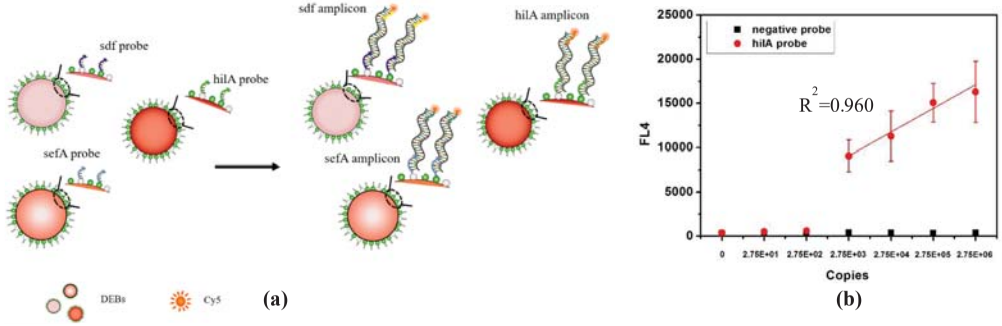

Figure:(a) Schematic illustration showing SP-PCR on DEBs. (b) Sensitivity and specificity of SP-PCR for the detection of hilA on magnetic microbeads

Optomagnetic sensing and biosensing

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Optomagnetic (OM) sensing relies on measurements of the intensity modulation of light of wavelength λ transmitted through a magnetic nanoparticle (MNP) dispersion in response to an oscillating magnetic field, $B(t) = B_0 \sin(2\pi f t)$.[1] Upon application of a magnetic field single-core or multi-core MNPs with linked optical and magnetic anisotropies will change their orientation resulting in a change of the intensity of transmitted light (**Fig. 1a**). The degree of field-induced alignment is determined by the magnitude but not the sign of the applied field and therefore the effect of the particles is observed in the even harmonics of the applied magnetic field. OM measurements can be performed as function of f and/or B_0 and can be realized in a fairly simple setup, which is suited to be used as readout in a low-cost disposable lab-on-a-chip system as the technique requires only a transparent sample container. Although OM measurements may seem restricted to particular nanoparticle systems, we have found that surprisingly many commercially available particle systems show a significant OM signal and hence can be studied and used by this technique.

Measurements typically measure the synchronous 2nd harmonic OM response vs. the frequency f of the magnetic field applied at low amplitude (**Fig. 1b**). Such measurements can be used to infer the distribution of hydrodynamic diameters, D_h, of the MNPs and are thus sensitive to changes of D_h resulting from binding or growth of biomolecules to individual MNPs or to clustering of MNPs.[2] MNP clusters with dimensions comparable to λ interact differently with the light and often show an OM response of opposite sign to that of individual MNPs.[1] This makes OM measurements very sensitive to the formation of MNP clusters. Moreover, measurements vs. f and B_0 can be used to estimate the distributions of D_h and remanent magnetic moments as well as their correlation.[3] Thus, OM measurements are suited for determination of MNP properties and for verification of the colloidal stability of MNP dispersions.

In our group we have moreover developed the OM technique to a powerful tool for real-time studies of nucleic acid detection and amplification. These studies have mainly used BNF 100 nm multicore particles from Micromod and relied on setups with integrated temperature control capable of measuring a single frequency spectrum in 40 s or less. As examples, we have studied in real-time: (1) the target-induced clustering of MNPs,[4] (2) the growth of amplification products,[5] and (3) DNA hybridization and denaturing under changing conditions.[6] In this presentation, we will introduce the technique and give an overview on how we have applied it for sensing and various types of DNA-based biosensing.

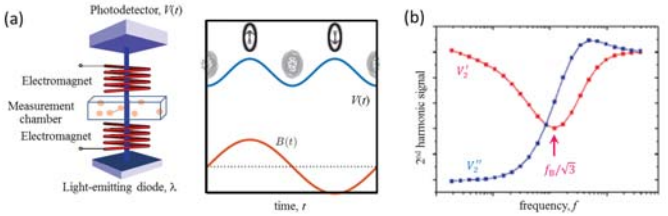

Figure 1 (a) Principle of OM measurements. (b) Example of OM spectrum. The in-phase $\sin(4\pi f t)$ component, V_2', shows a peak at $f_B/\sqrt{3}$, where $f_B = k_B T/(\pi^2 \eta D_h^3)$ is Brownian relaxation frequency (η is the viscosity).

[1] M. Donolato *et al.*, Anal. Chem. **87**, 1622-1629 (2015).
[2] J. Fock, C. Jonasson, C. Johansson, M.F. Hansen, Phys. Chem. Chem. Phys. **19**, 8802-8814 (2017).
[3] J. Fock *et al.*, Nanoscale **10**, 2052–2066 (2018).
[4] Mezger *et al.*, ACS Nano **9**, 7374-7382 (2015).
[5] G.A.S. Minero *et al.*, Analyst **142**, 3441-3450 (2017).
[6] G.A.S. Minero *et al.*, Analyst **142**, 582-585 (2017).

Development of a sensitive induction based magnetic nanoparticle biodetection method

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Magnetic iron oxide-based nanoparticles (MNPs) are utilized in several biomedical applications including diagnostics, therapy, actuation, and imaging. In this project, we focus on functionalized iron-oxide based magnetic multi-core particles with a mean particle size of 100 nm (BNF, micromod Partikeltechnologie GmbH) as magnetic carriers for the detection of influenza virus. Our aim is to develop a novel, portable, rapid, and sensitive detection platform for the influenza virus. AC susceptibility (ACS) measurements vs frequency are used to analyze changes in the Brownian relaxation of suspended functionalized MNPs that are induced by volume amplification of the analyte with padlock-probe-ligation and rolling circle amplification (RCA) [1-4]. We have developed a new and novel ACS measurement system (based on the DynoMag induction technique [1]) where the sample movement into two well-balanced detection coils is based on sample plug-flow driven by a peristaltic pump. A sample volume of 80 µL, consisting of an incubated mixture of MNPs and RCA coils, is injected into a tube by a syringe pump. The sample is moved as a plug to the two detection coils and the magnetic moment in each position is read over a band of excitation frequencies. In the DynoMag system, we use a paramagnetic powder (Dy_2O_3) for ACS calibration. In this new system, we instead use a stable MNP system, initially measured in the DynoMag system, as a liquid calibration sample. The time for making a complete frequency sweep over the relaxation peak is decreased to about 5 minutes (5 Hz – 10000 Hz with 30 data points). The reduction in time is achieved by a) reducing the number of measurement frequencies especially at low frequencies, b) pre-setting the amplifier gain in the electronics, and c) only move the sample twice during a measurement. The obtained standard deviation of the magnetic signal at the relaxation frequency (around 100 Hz) is equal to 10^{-5} (volume susceptibility SI units), which is in the same range obtained with the DynoMag system.

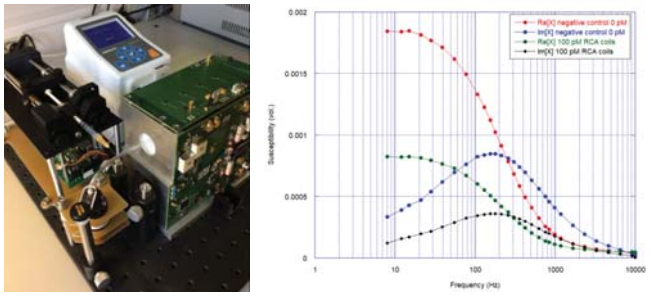

Figure 1 (left) The new induction based magnetic biodetection system and (right) ACS vs frequency at 0 pM and 100 pM of RCA coil concentrations. The concentration of the BNF 100nm MNPs is 0.2 mg/ml.

[1] Astalan, A. P. et al. *Biosensors and Bioelectronics* **19**, 945 (2004).
[2] Nilsson, M. et al., *Science* **265**, 2085 (1994).
[3] Zardán Gómez de la Torre, T. et al, *Biosensors and Bioelectronics* **29**, 195 (2011).
[4] Sobhan Sepehri et al., *APL Bioengineering* 2, 016102 (2018).

Strategies for on-chip DNA processing on magnetic microbeads

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We present the detection of DNA concatemer products of rolling circle amplification (RCA) via optomagnetic (OM) measurements on functionalized magnetic nanoparticles (MNPs). Streptavidin coated magnetic microbeads (MMBs) were employed as a movable substrate to transport the biotinylated target and products between three microfluidic chambers corresponding to the reactions (Fig. 1A): (i) circularization of padlock probes (PLPs) to the target and enzymatic ligation of PLPs to form a circular template (58°C), (ii) RCA of circular templates to form a long single-stranded DNA concatemer[1] (37°C), and (iii) OM detection of depletion of MNPs upon binding to RCA products (anchored to MMBs) (52°C). For the entire assay, it is crucial that all reacted DNA probes are anchored to MMBs in the first step as otherwise fewer RCA products reach the detection chamber. We therefore tested and compared the following strategies for on-chip DNA ligation (Fig. 1A):

(1a) on-chip simultaneous circularization and ligation of PLPs to target and target capture on MMBs.

(1b) on-chip circularization and ligation of PLPs on targets and subsequent target capture on MMBs.

(1c) off-chip PLP circularization on targets followed by on-chip ligation and subsequent capture on MMBs.

(2) as in (1c) but targets are captured on MMBs functionalized with a DNA capture probe.

Fig. 1B shows the measured depletion of MNPs vs. time after the above procedure followed by RCA and OM quantification of the relative amount of free MNPS. In the simplest scenario (**1a**), there was no control over the sequence PLP hybridization → PLP ligation → capture on MMB, which led to an overall poor assay performance with capture of ≈10% of the free MNPs. For the sequential operations in cases **(1b)** and **(1c)**, a capture of ≈34% of the free MNPs with no significant difference between on-chip and off-chip PLP circularization. Upon introduction of the DNA capture probe on the MMBs rather than binding the target directly to the MMBs using the biotin-streptavidin bond in case **(2)**, we found that ≈80% of the free MNPs were captured on the RCA products. This improved performance is attributed to the release of the RCA products from the MMBs in the detection chamber, which reduces diffusion limitations.

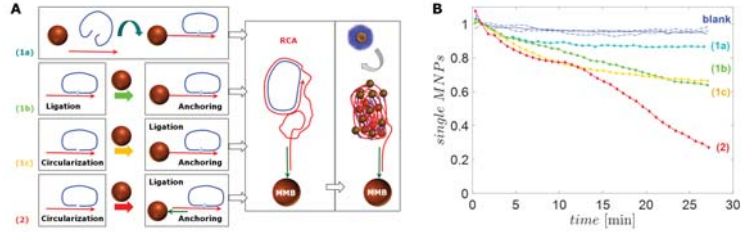

Figure 1: (A) Multi-step DNA processing using MMBs as movable substrate for capture, ligation, RCA and optomagnetic detection. The figure shows schematics of DNA ligation, RCA, and detection, where functionalized MNPs bind to multiple sites of the RCA product and (1a), (1b), (1c), and (2) illustrate the strategies for one-pot vs. sequential PLP circularization, ligation and capture on MMBs. (B) Time-traces of depletion of single MNPs in response to 200 pM target concentration and no-template controls.

1 X. Qi, S. Bakht, K. M. Devos, M. D. Gale and a Osbourn, *Nucleic Acids Res.*, 2001, **29**, E116.

Ultrasensitive *In Vitro* Diagnostics Platform Based on High-Affinity Magnetic Nanoprobes for Simultaneous Detection of Multiple Biomarkers

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Here we demonstrate a rapid ultrasensitive analytical platform based on magnetic nanoprobes for simultaneous detection of multiple biomarkers including small-molecule substances. The platform uniquely combines: (i) the novel format of indirect immunoassay with the extra-high affinity trapping of the magnetic nanoprobes at the readout zone and (ii) extremely high sensitivity of volumetric detection of magnetic nanolabels by non-linear magnetization with portable readers [1-3], which exhibit sensitivity on the level of registration of magneto-radioactive nanoparticles by γ-radiation. The development of the platform involves synergetic combination of original magnetic instruments having extraordinarily wide 7-order linear dynamic range with label-free interferometric devices providing the kinetic data about molecular recognition reactions.

The developed biosensing system features ultrahigh sensitivity (femtamolar limit of detection – Fig.1) in a wide dynamic range of analyte concentrations (more than 3 orders); short assay time (less than 30 min); ease of use due to minimal requirements to resources and operator's skills. The characteristics for detection of several disease biomarkers are considerably better than those offered by much more complicated laboratory methods such electrochemiluminescent assays that employ expensive equipment, or radioimmunoassays based on radioactive isotopes.

Importantly, the developed platform can substantially contribute to reduction of employment of radioisotope labels for *in vitro* clinical diagnostics of low molecular weight molecules. Despite of well-known disadvantages of the radioisotopes (short reagent life-time, licensing, disposal problems, etc.), they still remain in use as the labels for sensitive detection of a number of biomolecules, e.g., for measurements of small-molecular hormones concentration in human serum or plasma. The developed platform can serve as an attractive alternative for ultrasensitive detection of various biomarkers in clinical samples.

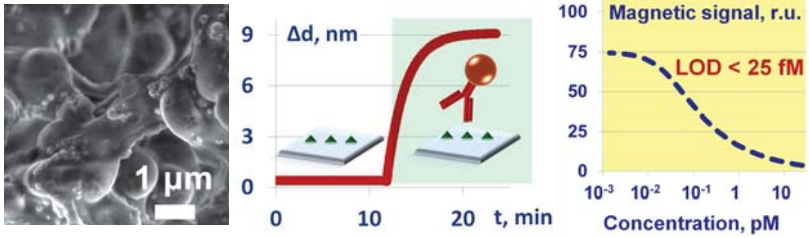

Fig 1. SEM-image of the trapped magnetic nanoprobes (*left*); interferometric characterization of kinetic of magnetic nanoprobes (*center*); dose-response curve for sensing of biomolecules binding (*right*).

[1]. P.I. Nikitin, P.M. Vetoshko. Patents RU2166751, RU2177611 (2000), EP1262766 (2001).
[2]. M.P. Nikitin V.O.Shipunova, S.M. Deyev, P.I. Nikitin. Nature Nanotechnol. 9 (2014) 716–722.
[3]. A.V. Orlov, V.A. Bragina, M.P. Nikitin, P.I. Nikitin. Biosens. Bioelectron. 79 (2016) 423–429.

Magnetic nanoparticles for detection of small molecules: synergetic combination of quantitative volumetric registration with interferometric optimization of immunoassays

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Small molecules (haptens), i.e., toxins, hormones, drugs, antibiotics and vitamins exhibit biological activity even in extremely low concentrations. Development of methods for ultrasensitive quantitative detection of the small molecules is among the high-priority research tasks in many fields of science and industry.

Here we demonstrate several quantitative measuring techniques as candidates for development of an analytical platform for hapten detection with magnetic nanoparticles (MP). As small molecules possess only one antigen determinant, competitive binding of antibody (Ab) with either free hapten in the test sample or that conjugated with protein deposited on the test zone should be recorded. Therefore, the reproducibility of such assay strongly depends on accessibility of the hapten antigen determinant in the conjugate, which varies according to the ways of formation. For quantitative monitoring of reproducibility of these conjugates and kinetics of binding with different Ab, the original optical biosensors have been modified. Antibiotic chloramphenicol and low molecular weight toxins were used as model small molecules for optimization of conjugate design for further application of the results for development of magnetic ultra-sensitive and quantitative assays.

We also propose method of MP distribution mapping provides a tool for rapid, simple and cost-efficient optimization of all stages of the immunoassay without high consumption of reagents. The method has been also used for quantitative monitoring of total MP mass by determination of square under the curve of their distribution along the test strip. According to the experiments, this parameter did not depend on antigen concentration and remained constant for each batch of the test strips. The method for optimization of quantitative immonoassays for detection of small molecules can be used for rapid and cost-effective development of highly sensitive express test systems for food quality control, in vitro diagnostics, criminalistics, environmental monitoring, etc.

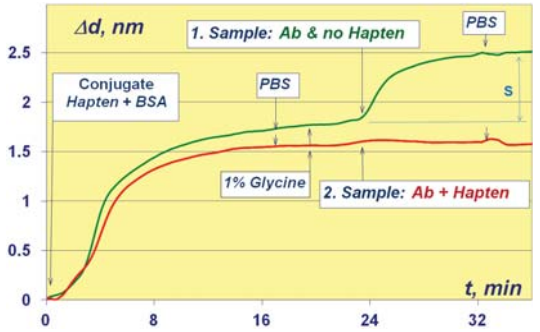

Fig. Sensograms of competitive label-free assay in the presence and absence of haptens..

Rapid Diagnostic Test for Celiac Disease based on Deamidated Magnetic Peptide as a novel biomarker

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Celiac disease (CD) has emerged as an increasingly recognized public health problem over the last half-century, and is now coming to be seen as a global phenomenon contributing to avoidable morbidity and mortality, since it is caused by the intake of gluten proteins present in cereals [1] . The symptoms can vary for an individual over time, and often mimic other diseases, which, combined with low global awareness of the disease, results in many cases remaining undiagnosed or being ineffectively treated [2]. Although the worldwide prevalence of CD is estimated in 1 %, for each diagnosed case of CD, an average of 5-10 cases remain undiagnosed, mostly because of physicians failing to diagnose the disease [3]. From a public health perspective, the at-risk group comprises this huge amount of people living with CD that is not diagnosed as such. The currently available tests for the diagnosis of CD remain within the specialized diagnostic laboratory while the gold standard biopsy is an invasive method requiring anesthesia [4]. Given the high prevalence of the disease, the rapid and accurate identification of CD patients at the general practitioner's site remains thus a major global health issue, since the burden of the CD would be eventually reduced if diagnostic tests were more widely available at community and primary-care level. Moreover, such a test would be useful for the following-up of patients with CD, since relapse may occur at a later time, as well as for the screening of their first-degree relatives. This work addresses the study of novel diagnostic biomarkers for CD based on deamidated gliadin-related peptides improving the sensitivity of the current methods and integrating these biomarkers in a magneto biosensor. Different gliadin peptide sequences based on the generic gliadin, including deamidations modification were studied [5]. Thus, these peptides were immobilized on magnetic particles and used as biomarker to isolated the antibodies present in celiac patient's serum. A reporter antibody was used for the enzymatic labeling and the detection was performed by amperometry. This test, which it is named as CeliFast, has shown a great potential because it is inexpensive, quick and sensitive, require minimal handling, and can be introduced into the general practitioner's armory for ambulatory screening of celiac disease. Moreover, the prototype has been evaluated in anonymized blood samples showing a high capacity to discriminate health from celiac patient. To summarized, CeliFast merges all the competitive advantages of the diagnostic test currently in the market: point of care, rapid, user-friendly, quantitative, and highly predictive.

References

1. Di Sabatino, A et al., *The Lancet*, **2009**, 373, 1480.
2. Byass, P et al., *PLoS ONE*, **2011**, 6(7), e22774.
3. Fasano, A et al., *Gastroenterology*, **2001**, 120, 636.
4. European Society for Paediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN). European Society for Pediatric Gastroenterology, Hepatology, and Nutrition Guidelines for the Diagnosis of Coeliac Disease, Clinical Guideline, 2012.
5. Pividori, MI and Kergaravat, V. Péptido, péptido magnético y método para detectar la enfermedad celíaca. PCT/ES2015/070097.

Stability of biofunctional magnetic nanoparticles in a microfluidic chip for sensitive detection of DNA/RNA viruses

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We present a magnetic bioassay using colloidal functionalized magnetic nanoparticles (fMNP) and a high-Tc superconducting quantum interference device (SQUID) magnetic readout [1]. The target DNA/RNA molecules are specifically detected by padlock probe recognition and ligation forming a padlock-target complex [2]. Using the rolling circle amplification (RCA) method [3], the padlock probes are further converted into single strand concatemer molecules, also called DNA coils (~1 µm). The fMNPs are streptavidin coated iron-oxide based magnetic multi-core particles with a mean particle size of 100 nm (Micromod Partikeltechnologie GmbH). They are tagged with complimentary oligonucleotides for specific binding to the DNA coils. The binding drastically changes the Brownian relaxation dynamics of the particles. A home-built ac magnetic susceptometer based on a high-Tc SQUID gradiometer is used to detect the change in Brownian relaxation dynamics of the fMNPs in a microfluidic chip. The chip enables fast and easy processing and control of the fluid and can be developed further to automate all steps of the assay. The fMNP system needs to preserve its monodispersed colloidal properties inside the microfluidic chip. Therefore, we have studied the stability of the frequency dependent ac magnetic susceptibility of the MNPs in the microfluidic chip. We have observed high stability and reproducibility in the ac magnetic susceptibility of the MNPs without streptavidin coating in the microfluidic chips. However, the streptavidin coated and/or oligo-functionalized MNPs show an instability in the ac magnetic susceptibility [4]: both the real and imaginary ac susceptibility loses 1% of its initial value every 3 minutes. This drop in susceptibility could be attributed to either non-specific binding of the fMNPs to the PDMS chip, or their colloidal instability and sedimentation. Without colloidally stable fMNPs or a method to account for the loss of signal in the microchannels, it is hard to reach beyond the measured 1 pM target analyte sensitivity and reach the projected limit of detection of 66 fM for our bioassay. We are investigating possible ways to address this instability problem such as: passivating the microfluidics with biocompatible materials like polyethylene glycol (PEG) to reduce the non-specific binding of the MNPs to the channel and a method that would allow differentiating the specific binding to the targets DNA coils form the loss of signal due to colloidal instability.

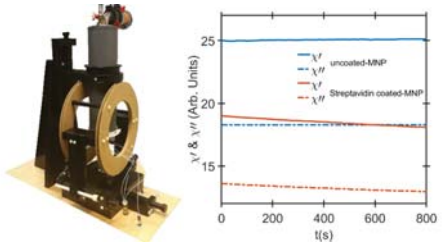

Figure 1 (left) The home-built high-Tc SQUID based ac susceptometer. (right) The real and imaginary ac susceptibility of 80 nm uncoated MNP (blue) and 100nm streptavidin-coated MNP(red) measured as function of time at constant frequencies of 251 and 63 Hz, respectively. The two frequencies are at the peak imaginary ac susceptibility of the uncoated and streptavidin-coated MNPs corresponding to their respective Brownian relaxation times.

Ref:

[1] S. Sepehri et al., APL Bioengineering 2, 016102 (2018).
[2] M. Nilsson et al. Science 265, 2085 (1994).
[3] J. Banér et al., Nucleic Acids Research 26, 5073 (1998).
[4] F. Öisjöen et al., J. Phys.: Conf. Ser. 200 122006 (2010).

Development of self-assembling system based on magnetic and gold nanoparticles for *in situ* monitoring of biomolecules

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Magnetic nanoparticles has become one of the major instruments in addressing key biomedical problems ranging from biosensing to targeted drug delivery. Such nanoagents can combine several functions in a single particle and are of special interest due to capabilities that substantially exceed those of molecular drugs. While many approaches were created to load a nanoparticle with various chemicals for actuation of the target cells, the nanoparticle-based approaches for the localized biosensing with real-time reporting of the marker concentration lag behind. Here we show a smart assembly/disassembly magnetic nanoparticle-based system for the reversible *in situ* biosensing of small molecules within a relatively wide range of concentrations.

The present nanoparticle-based smart system is multifunctional and reacts to a molecular input both as a sensor and an actuator. It is self-assembled via a non-covalent molecular interface from a larger magnetic nanoparticle (a "core") and smaller gold "shielding" nanoparticles. The analyte breaks the non-covalent bond between the core and gold shielding nanoparticles (AuNPs), which leads to system disassembly. That results in a shift of surface plasmon resonance due to spatial separation of AuNPs from the core nanoparticle and to the activation of the "output" receptor on the core particle, which has been previously sterically inaccessible to its target due to shielding nanoparticles. With the help of UV-Vis spectroscopy we observed the reversible surface plasmon resonance shift upon assembly and disassembly of the system both in phosphate buffer and in an opaquer cell culture medium. Next, we evaluated sensitivity of the developed smart agent to its specific molecular input, and demonstrated the reversible character of biosensing in contrast to most of the existing solutions. Indeed, the latter employ irreversible binding to analyte, and the sensing nanoparticles or molecules become insensitive to subsequent changes in concentration of detectible molecules. Thus, the proposed system is an *in situ* biosensor, which can detect changes in concentration of target molecule in its natural environment without specific sample processing [1,2].

Our system combines sensory and actuating functions, therefore, the range of potential applications of the system is significantly wider than for classic biosensors. These properties make our system attractive for use in different areas of biology and medicine with focus on cell biology, physiology and theranostics. Further development of this approach can yield nanoagents capable of non-invasive real-time profiling of biochemical markers in cell culture, tissue slices or in vivo with further reaction with a specific biomedical action.

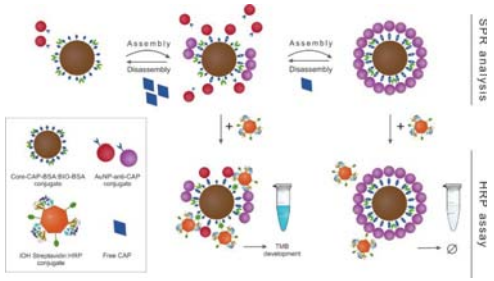

Fig. 1 Design of the multifunctional system and methods for evaluation of its performance.

[1] K. G. Shevchenko, et al., "Smart multifunctional nanoagents for in situ monitoring of small molecules with a switchable affinity towards biomedical targets," Appl. Nanosci., vol. 8, p.195-203, 2018

[2] K. G. Shevchenko, et al., "Surface plasmon resonance as a tool for investigation of non-covalent nanoparticle interactions in heterogeneous self-assembly & disassembly systems," Biosens. Bioelectron., vol. 88, pp. 3-8, 2017.

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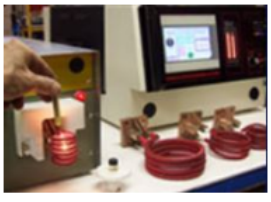

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