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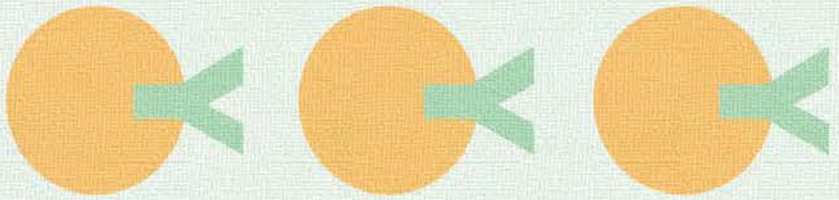


DISSEMINATION BOOKLET

MULTIFUN Consortium

Published by Trinity College Dublin.

Editor Dr. Adriele Prina-Mello

School of Medicine, Trinity College Dublin

ISBN: 9781871409338

Table of Contents

Dissemination preface

Nanomedicine promises to bring better health care, better health economics and truly personalized medicine. Drug delivery has and will greatly benefit from nanotechnology-driven innovation, thus providing safer and more efficient therapies. Nanotechnology is also driving the development of novel diagnostics tools and treatment for personalised monitoring of the treatment.

Therefore, patients suffering from any cancer will benefit from early diagnosis and more effective and targeted therapeutic modalities. The currently used cancer therapies lack selectivity leading to side effects responsible for prolonged and expensive patient recovery, and, even more importantly, are often followed by tumour relapse. In this context, reduced cancer mortality and morbidity should be achieved by 1) the identification of cancer lesions at earlier stages and 2) the selective targeting of cancer cells. The development and validation of nanotechnologies based on multifunctionalised (MF) magnetic nanoparticles (MNP) to selectively target and eliminate cancer (stem) cells. Such nanostructures will offer improved cancer detection at early stages by targeting cancer stem cells (CSC) with tailored contrast agents for magnetic resonance imaging (MRI). Moreover, MF-MNP will enable a minimal-invasive, highly selective and multimodal therapeutic approach to eliminate cancer (stem) cells.

Targeting CSC allows early detection of cancer development that will require identification of specific markers and the development of corresponding selective ligands (e.g. antibodies or peptides) thus offering an early stage tumour detection. Acgieving this will provide better chances to successfully remove the cancer, to avoid long and harmful patient recovery and tumour relapse at later points.

Thereforem multimodal therapeutic approach applied at cellular level will compound the effects provided by different cell apoptotic mechanisms as described below. Hence, the delivery of minimally-invasive cancer theragnostic nanotechnology for developing and validating new tumour detection and elimination approaches, expected to significantly reduce side effects and later relapse.

The funding of detection and therapeutic approaches based on nanotechnologies has been consolidated around 30 M€ in the last 6 years in research areas within the Seventh Framework Programme (FP7). Different FP7 calls have been launched and funded several projects concerning the use of functionalised nanosystems for disease and cancer detection, (NAMDIATREAM, NANOTHER, SONODRUGS, VIBRANT) and therapeutic approaches based on drug delivery (SONODRUGS, MAGNIFYCO, NANOTHER), siRNA delivery (NANOTHER) and/or magnetic heating (MAGNIFYCO, NANOTHER, NANO3T). Different nanocarriers have been designed to target cell markers responsible of diseases (VIBRANT, SONODRUGS) including cancer cells (NANOTHER, SONODRUGS, MAGNYFICO, NANO3T) at different tumours stages.

Thus, Europe has established itself as a major driver for the global market, especially in the medical products. The European NanoMed Map, recently developed by the European Technology Platform on Nanomedicine through the funded Coordinated Support Action project, NanoMed2020, shows that the nanomedicine community counts at least 1500 European main actors; these include more than 500 industrial players and SMEs active in the field, and 1000 actors conducting high-quality research in universities and research centres across Europe.

Integration of academic research and industrial expertise, as well as integration of expertise among different scientific fields, are now key issues for effective transfer of scientific discoveries into marketable products. Although scientific and technological innovations are the basis of nanomedicine, the greatest barriers between potential patient benefits and their realization are neither scientific nor technical. Translating scientific and technological excellence into effective therapeutic and diagnostic modalities requires experts from different fields to cross-disciplinary boundaries in order to create truly multidisciplinary environments, and to

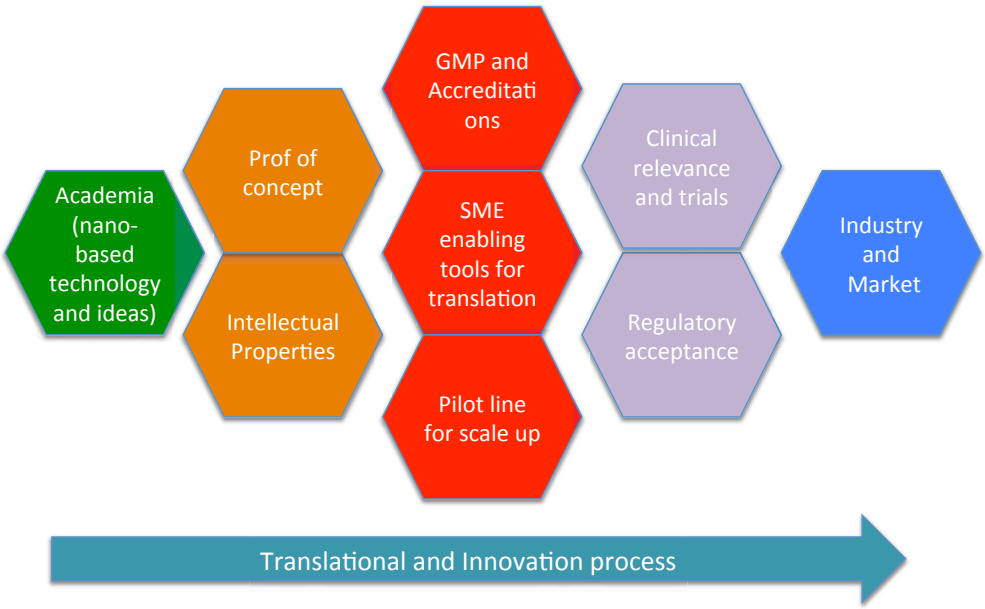
make outcomes available to the whole stakeholder community.

In this context, several EU projects and professional networks are integrating to add value to European nanomedicine: These include the European Technology Platform on Nanomedicine; the Cluster, "Targeted Nano-Pharmaceuticals and Early Diagnostics"; the European Foundation for Clinical Nanomedicine (CLINAM) and its European Summit for Clinical Nanomedicine; as well as large-scale FP7 projects such as MULTIFUN, NAMDIATREAM, SAVEME, NANOFOLS and 3MICRON, among many others.

The European Technology Platform on Nanomedicine (ETPN) has identified several challenges for the realization of an effective open innovation model across Europe. Improving the dialog between different academic disciplines, industries, clinical organizations and regulatory agencies is considered a top priority by ETPN, and this was discussed extensively at the yearly CLINAM summit, which aims at presenting and reflecting on the latest nanoscience-related advances in medicine by supporting clinically focused research and interaction across the whole community of nanomedicine stakeholders.

To facilitate such interaction, it is essential to organize multi-disciplinary events where nanomedicine stakeholders can gather with the aim of sharing each other's potentials and needs. Together with ETPN, CLINAM, and the existing professional clusters and networks in nanomedicine, large-scale European projects can act as catalysts for the creation of multidisciplinary exchange grounds. The sharing of knowledge and ideas that have developed in the course of one project, with other researchers working in the field and with the extended stakeholder community, can bring us closer to achieving real health and societal benefits.

This was the goal of the dissemination events organized by the large-scale collaborative EU-funded FP7 project, MULTIFUN (Multifunctional nanotechnology for selective detection and treatment of cancer. The aim of the project was development is to develop and validate a novel and minimally-invasive nanotechnology system to improve cancer diagnosis and treatment. MULTIFUN nanotechnology is based on multifunctionalised magnetic nanoparticles to selectively target and eliminate breast and pancreatic cancer (stem) cells. The improved magnetic features of the MultiFun magnetic nanoparticles will lead to potential medical applications such as contrast agents and magnetic heating inductors.

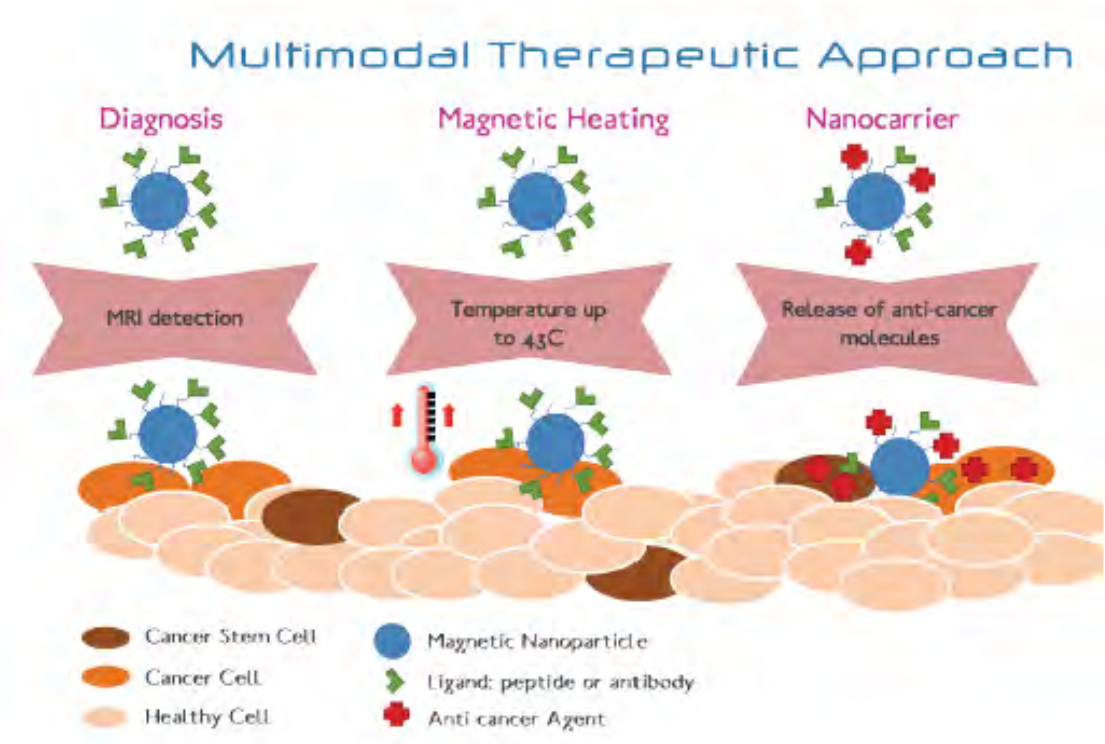


Contribution to Nanomedicine Translation

The international and multidisciplinary dimensions of the project went beyond the composition of its partners, as it gathered other projects and expertise by ar-

ranging outreach events in the fields of nanotechnology-enabled molecular imaging, therapeutics, and market strategies for nanomedicine. Some of the most influential experts and projects in the field were brought together during the Nanotechnology workshop at the World Molecular Imaging Conference, 2012, in Dublin, and also at the Final MULTIFUN workshop. MULTIFUN also brought results to the floor of the EuroNanoForum 2013 conference (its own as well as those of other European projects); this was a unique opportunity to communicate the huge potential of nanotechnology, as an enabling technology for medical applications, to a large panel of research, industry and public authority representatives. Through the success of these events, MULTIFUN has catalyzed the creation of a common ground for discussion of scientific and industrial innovation strategies amongst those with different experiences and interests. A major result of such outreach activities was the highly productive engagement of many MULTIFUN partners internationally.

Fruitful interactions have also been established with other large-scale FP7 projects, major European organizations and industrial partners, as described in the present document. This booklet aims to provide an overview of the multidisciplinary activities initiated by the MULTIFUN dissemination leading partners such as Trinity College Dublin, IMDEA and ATOS through their participation in MULTIFUN projects (FP7 NMP LSP projects funded over the period 2009–2015) and also as part of its broader pan-European engagement.



Workshop at the World Molecular Imaging Congress 2012 (WMIC 2012)

Nanotechnology in Diagnostics, Monitoring and Treatment of Cancer:
Advances in Molecular Imaging

WMIC 2012 Workshop

MULTIFUN partner, Trinity College Dublin, lead the project consortium into the organisation and scientific discussion of the Workshop, titled, **Nanotechnology in Diagnostics, Monitoring and Treatment of Cancer Advanced in Molecular Imaging**, which was held within the fifth annual meeting of the World Molecular Imaging Congress (WMIC), the premier event in molecular imaging worldwide. WMIC 2012 attracted more than 1750 attendees to the Dublin Convention Centre from September 5th–8th with over 60 companies exhibiting the latest instruments and techniques for clinical and pre-clinical imaging.

The goal of the this workshop was to bring together and show some of the most important scientific outcomes of EU projects aimed at developing innovative nanotechnology-based imaging probes and drug delivery carriers for diagnostic and theranostic purposes.

Overall, WMIC 2012 was attended by scientists and stakeholders, covering an impressive worldwide distribution with 41% of people coming from Europe, 40% from North America, 16% from Asia and the remaining 3% from other regions. At the event, presentations were delivered by scientists from academic, clinical and research institutions, with industrial leadership in the field being represented by over 60 exhibiting companies, presenting the latest instruments for clinical and pre-clinical imaging.

More than 110 students and researchers attended the workshop, where 46 International and 4 Irish scientists from diverse areas of the Molecular Imaging field had a chance to present their latest results and share their research experience.

Presentation Highlights

Over 110 scientists convened at the 1-day workshop to present their approaches to diagnosis, monitoring and treatment of some of the major diseases of the 21st century, in particular cancer. Over 20 oral presentations were organised into four sessions, followed by a poster session.

The first session was titled, **Advances in imaging and diagnosis by nanotechnological tools**, and it showcased results and technologies from the NAMDIATREAM project.

Prof. Yuri Volkov from Trinity College Dublin (Ireland) led the workshop and discussion around Diagnostic, Monitoring, Treatment and personalized medicine as opportunities for routine diagnostics and dynamic treatment monitoring. Prof. Luigi Bonacina from the University of Geneva (Switzerland) presented the second-harmonic generating nanoparticles, which aim to provide an inherently nonlinear, photostable, infrared-excitable microscopy probe with multimodal detection capabilities. Prof. Joerg Schotter from the Austrian Institute of Technology (Austria) presented the Plasmag system as a homogeneous immunological detection tool; the system is based on optical detection of the magnetic relaxation times of hybrid magnetic-core, gold-shell nanorods. Prof. Igor Nabiev from Trinity College Dublin (Ireland) presented results relating to applications of fluorescent semiconductor quantum dots for ultrasensitive marker detection and cancer diagnostics. All four presentetations also provided an overview of such applications in the “real world” of clinical diagnostics.

NAMDIATREAM industrial partners also presented their advances and their role in the project. Dr. Daniel Ciepielewski outlined Nikon’s (France) approach to nanotechnology for advanced imaging. Dr. Frans Nauwelaers highlighted BD Bioscience’s (Belgium) efforts to evolve flow cytometry from cellular to sub-cellular analysis through nanotechnology-based imaging. Finally, Dr. Patrick Hole discussed the use of NanoSight NTA (UK) for the measurement of exosomes and other nanosized biological particles.

The second session concerned **Advances in imaging and theranostics using nanotechnological tools** and presentations were from the MULTIFUN project. MULTIFUN (Multifunctional Nanotechnology for selective detection and treatment of cancer) is a large-scale EU-funded collaborative project whose aim is to develop multifunctional magnetic nanoparticles (MNPs) for diagnosis and treatment for breast and pancreatic cancer. The project objectives related to diagnosis were introduced by Prof. Rodolfo Miranda from IMDEA Nanoscience (Spain) as: (i) synthesis of MNPs as contrast agents

for MRI, (ii) development of specific biomarkers for targeting breast and pancreatic cancer stem cells (early diagnosis), and (iii) development of new technologies for the detection and quantification of nanoparticles in tissue, blood and urine.

MULTIFUN strategies for the synthesis and dispersion of magnetic nanocrystals for advanced MRI imaging and hyperthermia were presented by Prof. Maria del Puerto Morales from CSIC (Spain). She also pointed out the main technical challenges to the development and characterization of these nanoparticles.

MULTIFUN strategies for multifunctionalization of magnetic nanoparticles for theranostic applications, specifically for breast and pancreatic cancers, were described by Dr. Aitziber L. Cortajarena from IMDEA. Dr. René Botnar from King's College London (UK) reported results concerning the *in vitro* behaviour of hydrophilic magnetic nanoparticles that are to be used as MRI contrast agents. Then, Dr. Ingrid Hilger from the University of Jena (Germany) offered an overview on the clinical use of magnetic nanoparticles and the challenges that face the development of such strategies. The last presentation of this session was by Dr. Stephanie Teughels from Pepric (Belgium) and it described new devices for quantitative monitoring of the biodistribution and kinetics of magnetic nanoparticles and labeled cells and tissues.

The third-session presentations were delivered by members of the **Targeted Nano-Pharmaceuticals and Early Diagnostics** cluster, which brings together about 25 research projects funded by the EU Sixth and Seventh Framework Programmes for Research (FP6 and FP7), under the priorities, NMP and HEALTH. Dr. Adriele Prina-Mello from Trinity College Dublin (Ireland) introduced the cluster, whose aim is to develop state-of the-art nanotechnological methods for diagnosing, monitoring and treating a wide variety of diseases, including cancer. In particular, this session hosted presentations from the SAVEME, NANOFOL and 3MICRON projects. Dr. Louis Shenkman (Israel) showed results from the SAVEME project, which is developing a novel modular nanosystem platform, integrating advanced functionalized nano-core particles and active agents. NANOFOL was presented by Dr. Artur Cavaco-Paulo (Portugal) who outlined the development of folate-based nanobiodevices for integrated diagnosis/therapy targeting chronic inflammatory diseases. Finally, 3MICRON aims at developing a three-modality contrast-imaging system using multi-functionalized magnetic polymeric microballoons, as described in the presentation of Prof. Hans Herbert (Sweden).

The last session of the Workshop centred on **Imaging cell and tissue interaction with nanomaterials**. Advanced experimental model systems for imaging cancer cells were the subject of the presentation given by Prof. Frauke Alves, Her team at the Max Plank Institute (Germany) has optimized *in vivo* models (mouse) for lung, pancreas and breast cancer to evaluate the sensitivity and specificity of the photoluminescent quantum dots developed in the NAMDIATREAM project; which were compared to standard controls and existing detection labels. New developments in imaging of *in vitro* models were also on show here, in the presentations of Dr. Dania Movia and Dr. Adriele Prina-Mello from Trinity College Dublin. Dr. Movia proposed 3D cell systems as novel *in vitro* models for studying nanomaterial interactions with tissues. The results of imaging and cytotoxicity testing of single wall carbon nanotubes (SWNTs) in such 3D, tissue-like models were presented. Dr. Prina-Mello then described the use of a high-content screening system for the analysis of cellular responses to nanomaterials, emphasizing its effectiveness as an imaging and decision-making tool.

The application of Raman techniques to clinical imaging was outlined by Dr. Furio Gramatica from the Don Gnocchi Foundation (Italy). In particular, Dr. Gramatica proposed a self-assembled SERS platform for biomarker detection, which can be implemented into a "lab-on-a-chip" system, ideal for clinical applications such as hospital lab tests and point-of-care tests.

The implications of nanotoxicology in nanomedicine were addressed by Dr. Marcello Cacace from CNR (Italy). Dr. Cacace pointed out that both nanomedicine and nanotoxicology examine new and sometimes unforeseen effects emerging from bionano interactions; one seeks to exploit these effects for the benefit of our health, whereas the other aims to find, understand and minimize those that are undesirable.

Outcomes

The World Molecular Imaging Congress attracted 1200 abstracts; hosted 250 oral presentations and four poster sessions, and provided 14 educational sessions, 7 spotlight sessions and 5 industry workshops. Within this world-class, international framework, the workshop effectively engaged leading molecular imaging experts and industrial players to share their views and results on current and future applications of nanotechnology for disease diagnosis and treatment, and to highlight the pathways to implementation of nanotechnologies in clinical molecular-imaging practice. The cutting-edge presentations were delivered by scientists, clinicians and industrial leaders from across Europe.

Trinity College Dublin under an SFI Conference and Workshop grant (awarded to Prof. Volkov and collaborator, Dr Prina-Mello) provided nearly 150 travel grant awards to attendees receiving the best scores. In addition, this grant provided for 3 young investigator awards and 10 poster awards to recognize innovative research by young investigators, including junior faculty. Indeed, throughout the workshop, young scientists were provided the opportunity to highlight their achievements and share their experiences with established researchers in an informal networking atmosphere.

Workshop Program

(I) Advances in imaging and diagnostics using nanotechnological tools

Nanotechnology in diagnostic monitoring and treatment of cancer: Advances in molecular imaging
Harmonic nanoparticles for biolabelling
Plasmonic magnetic detection for advanced clinical diagnostics
Quantum dots as fluorescent probes for advanced imaging
Nanotechnology in advanced imaging
Evolving flow cytometry from cellular to sub-cellular analysis
The use of NanoSight NTA to measure exosomes and other nanosized biological particles

(II) Advances in imaging and theranostics using nanotechnological tools

MULTIFUN: Project highlights
Magnetic nanoparticles for advanced MRI imaging and hyperthermia
Functionalization of magnetic nanoparticles for theranostics
Nanoparticles and advanced MRI detection and imaging
Clinical use of magnetic nanoparticles
New devices based on advanced imaging

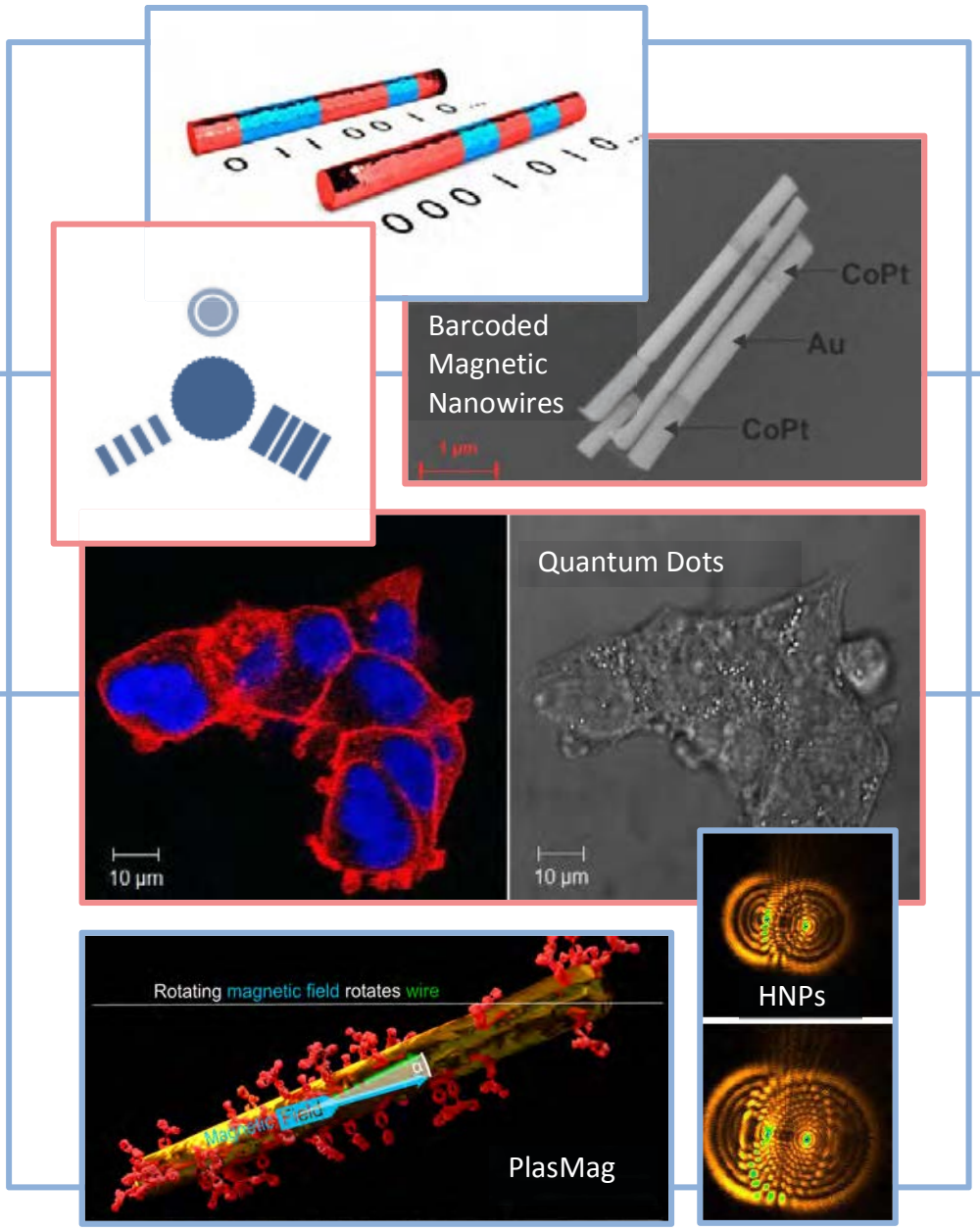
(III) Cluster: Targeted nanopharmaceuticals and diagnostics

Overview of targeted nanopharmaceuticals and diagnostics cluster activity
Folate-based nanobiodevices for integrated diagnosis/therapy targeting chronic inflammatory diseases
SaveMe: A modular active nano-platform for advanced cancer management — Core nanosystems, tumour targeting and penetration, molecular imaging and degradome based therapy
3 Micron: Multimodality microballoons

(IV) Imaging cell and tissue interaction with nanomaterials

Advanced models for imaging cancer
Nanotoxicology implications in Nanomedicine: unanswered questions and future directions
Imaging cell and tissue interaction with nanomaterials: From 2D to 3D models
Raman spectroscopy in clinical diagnostics
High content screening of cellular response to nanomaterials interaction as imaging and decision-making tool

(I) Advances in imaging and diagnostics using nanotechnological tools



Nanotechnology in diagnostic monitoring and treatment of cancer: Advances in molecular imaging

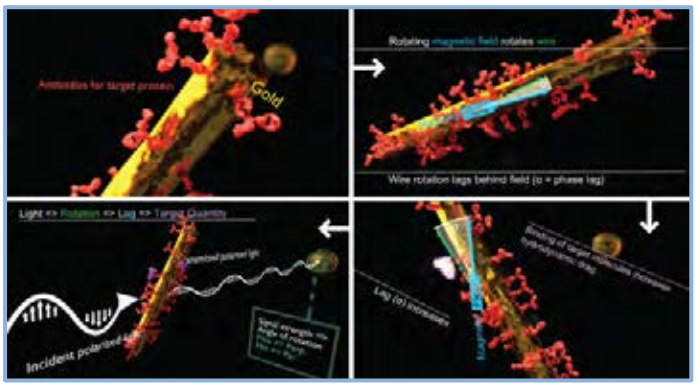
Yuri Volkov; Department of Clinical Medicine, School of Medicine, Trinity College Dublin, Ireland.

The recent advances in nanotechnology have opened up new opportunities in the diagnosis, monitoring and treatment of cancer. In particular, the opportunity to develop cutting-edge nanotechnology-based toolkits for multi-modal detection of biomarkers of the most common cancer types and cancer metastases is permitting the identification of cells indicative of early disease onset. This is critical for cancer, considering over 3.2 M new cases and 1.7 M cancer-related deaths are registered in Europe every year, largely because diagnostic methods have an insufficient level of sensitivity, limiting the potential for early disease identification.

In fact, it is foreseen that advances in molecular imaging for *in vivo* and in *vitro* research will be focused on increasing the specificity, sensitivity and reliability of clinical, laboratory and point-of-care devices. The NAMDIATREAM project is built on the innovative concepts of super-sensitive and highly specific "lab-on-a-bead", "lab-on-a-chip" and "lab-on-a-wire" nano-devices, which utilize photoluminescent, plasmonic, magnetic and non-linear optical properties of certain nanomaterials. This offers ground-breaking advantages over present technologies in terms of stability, sensitivity, time of analysis, probe multiplexing, assay miniaturization, reproducibility, cost and safety.

Through the implementation of these innovations, the project will deliver the following: (1) Photoluminescent nanoparticle-based reagents and diagnostic chips for high throughput, early diagnosis of cancer and treatment efficiency assessment; (2) Nanoparticles enabling plasmon-optical and nonlinear optical monitoring of molecular receptors within body fluids or on the surface of cancer cells; (3) Multiparametric screening of cancer biomarkers in diagnostic material using segmented magnetic nanowires; (4) Validation of nano-tools for early diagnosis and highly improved specificity in cancer research and (5) OECD-compliant nanomaterials with improved stability, signal strength and biocompatibility.

Direct lead users of the results will be the diagnostic and medical imaging device companies involved in the consortium, along with clinical and academic partners.



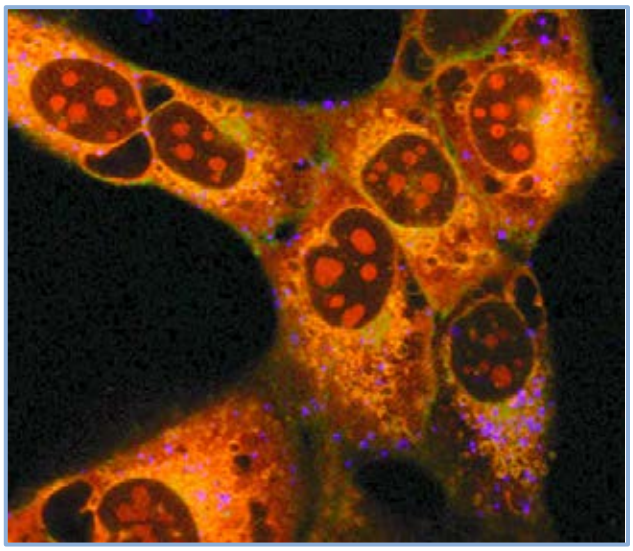
Yuri Volkov, (PhD, MD, MA, FTCD)

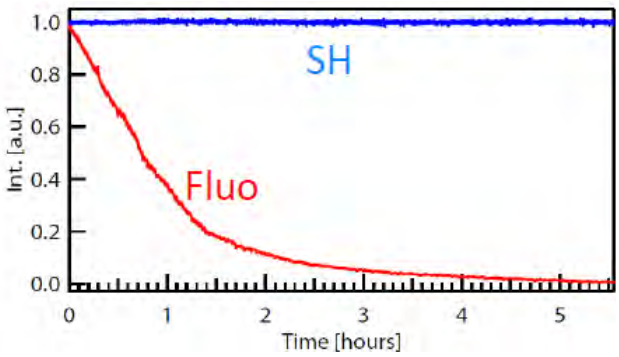
Yuri Volkov received his MD from the Moscow Medical University and subsequently a PhD in biomedical sciences from the Institute of Immunology, Moscow. He is a Professor at the Department of Clinical Medicine and former Director of Research at the School of Medicine, Trinity College Dublin (TCD). Prof. Volkov coordinates a large-scale EU FP-7 project, NAMDIATREAM, for early diagnostics and monitoring of malignant diseases, with 22 European academic, research, clinical and industrial partners. He is the lead TCD partner for the EU FP-7 project, MULTIFUN, and Principal Investigator on a number of other grants. Prof. Volkov is the author of over 100 scientific publications, including articles and book chapters, and he is an inventor on several patents.

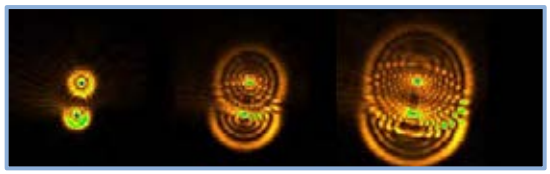
Harmonic nanoparticles for biolabelling

Luigi Bonacina, GAP-Biophotonics, University of Geneva, Geneva, Switzerland.

In the quest for the next generation of imaging bio-markers, successful probes have to prove to be non-toxic, bright, stable against long term excitation, and able to generate a sharp contrast against background fluorescence. In all these respects, Harmonic Nanoparticles (HNPs) are receiving an increasing attention as they also open a series of alternative detection possibilities simply not accessible with the present generation of fluorescent dyes and quantum dots. HNPs are a family of inorganic nanometric crystals (size < 100 nm) of different materials (KNbO3, BiFeO3, LiNbO3, KTP, etc.) sharing the characteristic of non-centrosymmetric crystal structure. This property determines their large non-linear optical efficiency, and, in fact, in the last years they have been mostly investigated as localized sources for second harmonic generation. After introducing this approach and comparing it with respect to other nanoparticles-based optical labelling strategies (quantum dots, up-conversion NPs), I will highlight some recent applications in the field of (multi-photon) imaging and optical detection of HNPs labelled biological samples.







Luigi Bonacina, PhD

Luigi Bonacina obtained a Master degree in Optics from the University of Milan in 1999. Successively, he moved to the Ecole Polytechnique Fédérale de Lausanne, where he completed a PhD in 2004 in the Chemistry Department with a project on ultrafast spectroscopy. In 2012, he was appointed a permanent research position at the Physics Department of the University of Geneva, in the Biophotonics group. His main research interests include the development of nonlinear and phase-coherent optical techniques for imaging and spectroscopy of biological systems. Recently, he has focused his activities on multi-photon nanotechnology-based approaches for cancer detection and treatment.

Plasmonic magnetic detection for advanced clinical diagnostics

Joerg Schotter[1], Stefan Schrittwieser[1], Frank Ludwig[2], Jan Dieckhoff[2], Katerina Soulantica[3], Guillaume Viau[3], Sergio Mozo Lentijo[3], Colin Self[4], Patrick Hole[5], Joanna Sullivan[5], Annegret Guenther[6], Andreas Tschoepe[6]; (1) Health and Environment Department, Austrian Institute of Technology, Austria; (2), Institute of Electrical Measurement and Fundamental Electrical Engineering, Technical University Braunschweig, Germany; (3) INSA, UPS, LPCNO, and CNRS, Université de Toulouse, LPCNO, France; (4) Selective Antibodies Ltd., United Kingdom; (5) NanoSight Ltd., United Kingdom; (6) Universitaet des Saarlandes, Experimentalphysik, Germany.

We introduce a new biosensor concept (denoted as 'PlasMag'), which is based on highly sensitive plasmon-optical detection of the rotational dynamics of anisotropic magnetic nanoparticles immersed in the sample solution. On the specific binding of analyte molecules to the antibody-functionalized nanoparticle surfaces, their hydrodynamic volumes increase, which translates into a change in their rotational dynamics. A suitable nanoparticle type consists of an elongated core-shell structure with magnetic core and noble metal shell. Thereby, the magnetic core enables control of the nanoparticle alignment by external magnetic fields, while the anisotropic plasmon resonance excitation within the noble metal shell in linearly polarized light allows optical detection of the nanoparticle's rotational dynamics. Compared to existing nanoparticle-based homogeneous immunodiagnostic methods, our approach promises to combine ease of use, minimum sample preparation and a simple setup with high analyte sensitivity.

We present model calculations of the optical, magnetic and hydrodynamic properties of both the required nanoparticles and measurement conditions. According to these, core-shell nanorods with a cobalt core surrounded by a gold shell agitated by a rotating magnetic field are best suited for our biosensing principle. While we currently strive to synthesize such nanorods, we still have to solve stability issues of respective nanorod dispersions. Thus, as an intermediate solution we demonstrate the 'PlasMag' detection principle using aqueous dispersions of nickel nanorods fabricated by electrodeposition into nano-porous alumina templates. Specifically, we show that the phase lag of the long axis of nickel nanorods (magnetic core parameters: medium length 182 nm, medium diameter 26 nm) with respect to externally applied rotating magnetic fields significantly increases on the adhesion of bovine serum albumin (BSA) protein to their surfaces. By fitting our measurement results, we obtain a large increase of the hydrodynamic shell thickness of the nanorods by 22 nm, which is related to both the size of the adsorbed protein molecules as well as changes in the stagnant surface layer of immobile fluid. To validate these results, we independently determine the amount of bound protein molecules by analysis of the electrophoretic mobility of the nanorods, which gives a protein surface density of 5.8 femtomol/mm^2.

Our results successfully confirm the theoretical model calculations on the rotational behaviour of magnetic nanorod dispersions, thus serving as a first proof of the 'PlasMag' concept.

Joerg Schotter, PhD

Joerg Schotter is a staff scientist at the Molecular Diagnostics business unit of the Austrian Institute of Technology (AIT). His primary scientific interest is the application of magnetic techniques to molecular diagnostics, which includes both heterogeneous magnetoresistance-based biosensors and lab-on-a-chip systems as well as homogeneous nanoparticle-based molecular detection methods.

Joerg Schotter was born in Friedrichshafen (Germany) in 1975. He received his Master Degree in physics from the University of Massachusetts (Amherst, USA) in 2000, where he studied the fabrication of ultra-dense magnetic nanowire arrays by electrodeposition into nano-porous diblock copolymer templates. He then moved to the University of Bielefeld (Germany) to prepare his PhD thesis on the development of magnetoresistive biosensors, which he finished in 2004. Since 2005, he is employed by the AIT as staff scientist, where he continues his research on magnetic biosensor systems. He has authored more than 30 original research papers.

Quantum dots as fluorescent probes for advanced imaging

Alena Sukhanova[1], Hilal Hafian[2], Jean-Marc Millot[2], Michel Pluot,[2] Jacques H.M. Cohen,[2] Patrick Chames[3], Daniel Baty[3], and Igor Nabiev[1,4]; (1) School of Medicine, Trinity College Dublin, Ireland; (2) Laboratoire de Recherche en Nanosciences, Université de Reims Champagne-Ardenne, Reims, France; (3) Inserm U1068 and CNRS UMR7258 CRCM, Institut Paoli-Calmettes, Aix-Marseille Université, Marseille, France; (4) Laboratory of Nano-Bioengineering, National Research Nuclear University ("Moscow Engineering Physics Institute"), Moscow, Russian Federation.

High-quality imaging for immunofluorescence diagnosis requires a high sensitivity of labeling and discrimination of the tissue autofluorescence. Multiphoton microscopy with excitation in the near-infrared spectral region has now become the primary fluorescence imaging technique for thick biological specimens. The main problem for fulfilling the important multiphoton imaging task is the inherently low TPACS of organic fluorophores. These fact determine an inherently low spectral sensitivity of two-photon bioimaging with organic dyes whereas the semiconductor quantum dots (QD) two-photon absorption cross-sections (TPACS) are orders of magnitude bigger, making them the best fluorescent label to be used for multiphoton imaging.

With a molecular weight of only 13 kDa, single-domain antibodies (sdAbs) are the smallest Ab fragments capable of binding their antigens with affinities comparable to conventional Abs. A sdAb is only 1/12 as large in volume as a conventional IgG molecule, and their size allows them to bind epitopes inaccessible to conventional IgGs. In addition to their small size, sdAbs are characterized by a low tendency to aggregate, diffuse much better in tissues than full-size IgGs, and their size allow them to label thinner and more distal segments. These advantages make sdAbs the best capture molecules to prepare QD-based fluorescent nanoprobes for biodetection and diagnostics.

We have recently developed a protocol for highly oriented conjugation of sdAbs with QDs, with all sdAb antigen-recognizing sites facing outwards, which considerably increases the nanoprobe sensitivity. These conjugates displayed excellent specificity and quantitative discrimination of tumor and normal cells in flow cytometry. Additionally, the quality of immunohistochemical labeling of biopsy samples with these conjugates was found to be comparable to the quality obtained with gold standard protocols of anatomopathology practice. Finally, we have analyzed the two-photon optical properties of highly oriented sdAb-QD conjugates of QDs with sdAbs, applied them to imaging of normal colon epithelium and pathological human colon carcinoma and determined the optimal conditions for two-photon tissue imaging with this nanoprobe and achieved the highest ratio of the fluorescence signal from sdAb-QD to the tissue autofluorescence ensuring clear discrimination of tumor areas from normal tissue.

Prof. Igor Nabiev, PhD, DSci

Igor Nabiev received his PhD in Physics and Mathematics in 1983 from Lomonosov Moscow State University and his DSc degree in Chemistry from the Shemyakin Institute of Bioorganic Chemistry of the Russian Academy of Sciences. After professorships in the USA and France, he was nominated, in 1994, as full Professor of Biophysics in the University of Reims Champagne-Ardenne, France. In 2008–2009 Prof. Nabiev was a recipient of the Walton Award from the Science Foundation of Ireland. Since 2010 he has been Director of Technological Platform Semiconductor Nanocrystals of the "Large" European Project NAMDIATREAM.

At the end of 2011, Prof. Nabiev received a MEGA-grant in the framework of the Program of Attraction of the World Leading Scientists to Russian Institutions of Higher Education and founded the Laboratory of Nano-Bioengineering in the National Research Nuclear University "Moscow Engineering Physics Institute.

Nanotechnology in advanced imaging

Daniel Ciepielewski; NIKON AG Instruments, Paris, France.

Light microscopy using fluorescent proteins has greatly advanced our understanding of many functional biological systems, however the precision at which cellular structures can be visualized has been limited by the spatial resolution imposed by the diffraction limit of light. Several imaging methods are known to go below the diffraction-limited resolution to smaller values, and novel super-resolution imaging methods using photoactivatable proteins or photoswitchable fluorophores bypass this limitation like STORM.

Super-resolution microscope systems N-SIM (structured illumination microscopy) and N-STORM (Stochastic Optical Reconstruction Microscopy) are presented as methods that challenge the diffraction limit. Super-resolution imaging allows for single molecule detection and localization, providing the ability to follow cellular mechanisms such as the dynamics of transcription factors, as an example.

Nonlinear optical nanocrystals have been recently introduced as a promising alternative to fluorescent probes for multiphoton microscopy collecting their second and third harmonic signal. The multiphoton confocal microscope enables excitation by simultaneous absorption of two photons by a single fluorescent molecule. The intensity of the laser beam converged by the objective lens decreases in inverse proportion to the square of the distance from the focal plane, so that only a fluorescent molecule located within the diffraction-limited volume of the objective lens is excited and emits fluorescence. As a result, two-photon confocal microscopy is suitable for the visualization of single or small aggregates of nanoparticles within cells, as we show here in experiments with nonlinear optical nanocrystals $KNbO_3$ nanocystals coated with or $KNbO_3$ SCHRIMP's coated with PEG in stem cell samples.

Daniel Ciepielewski

Daniel Ciepielewski is the Sales and Marketing Manager at NIKON AG Instruments (Switzerland), a role he has held since 2012. From 2002 to 2012 he worked at NIKON France SAS Instruments (Champigny sur Marne / France), where he covered several functions such as measuring product specialist, application engineer and product specialist in images analysis, specialist consultant speaker in Universities, technical and sales support for export business, European software coordinator for industrial and material sciences, technical and sales manager of the industrial and material sciences sales team, and interdisciplinary business engineer & Nanoscopy I-SPT/STORM product Specialist. Mr. Ciepielewski obtained his Engineer Diploma in Physics and Instrumentation at the Ecole Supérieure d'Ingénieur d'Annecy France in 2002, and his 2 years technical degree DUT in Physical Measures speciality Instrumental Techniques at the University Institute of Technology of Clermont Ferrand-France in 1999. At Nikon AG he presently manages the Instruments Department.

Evolving flow cytometry from cellular to sub-cellular analysis

Frans Nauwelaers, Line De Kimpe, Tina Van den Broeck; BD Biosciences Belgium, Erembodegem, Belgium.

The basic concepts of cytometry were explained: particle alignment, analysis and sorting. Each of these are essential elements in flow cytometry technology but can be utilized with some flexibility if required. Particle alignment can be controlled via sheath fluid or can be sheathless. Analysis can be achieved with a jet-in-air concept or can be accomplished via a closed cuvette system. Particle sorting can be omitted or included and can be dual beam, quadruple ox six-way. Particle purity achieved normally ranges in the 98 to 99.9%.

The evolution of the optical path took about 30 years to come to the integrated design available today. In essence, the original path designed reflected much of the fluorescence microscope setup, but this has been optimized systematically and gradually from collection lens to PMT to filter over the years. Light collection has been changing from open air space to gel-coupled (as in fluorescence microscopy) to including the use of pinholes and optical fibers. Filters have been used as combinations, bandpass, reflection filters and transmission filters. Dyes have evolved from the classic fluorescence stains (fluorescein and TM-rhodamin), to the phycobiliprotein dyes derived from algae, to most recently the synthetic dyes and the multi-layer nanobeads. Actual chemistry technologies can synthesize almost any type of dye based on specifications. Each dye could be associated with a cell characteristic through direct staining (nuclear, pH, protein) or could represent a membrane structure via coupling with a detecting (monoclonal or single domain) antibody.

Recently the analysis transition was managed from cells to sub-cellular vesicles where types and origin can be identified, resulting from vesicle composition and detection via scatter or fluorescence characterization. Micro-particles can be recognized by antibodies and their origin identified (cell membrane-type or intracellular type). Their content can also be investigated with the use of intra-cellular staining methods. Particles are thus classified as to their size in ectosomes (1000 down to 600 nm) and exosomes (300 nm down to 100 nm). Each type has its own markers and content. Content can differ by type and by status of the cellular system (in rest, inflammation, other). Some data were shown on current particle measurement and limitations. Both scatter and fluorescence intensities can be used to confirm the particles of interest. However, control experiments will be necessary initially to optimize the measurement conditions and to find out what the sensitivity specifications are for these different particles. Preparation methodologies will need to be worked out and pitfalls will need to be identified for each of these preparation methods. Some particles might be harmed by the procedural steps and might show differences in detectable signals based on the procedural conditions. These difficulties could lead to false conclusions and interpretations if the required checks are omitted. Some of the different areas that need to be worked on to further improve the present technologies were identified.

Frans Nauwelaers, PhD

Frans Nauwelaers is Director of Scientific Affairs at BD Biosciences Europe. His primary scientific interests are in the fields of: leukaemia, minimal residual disease, stem cells, auto-immune disorders, allergy research, platelet activation and blood bank QC assays.

Frans Nauwelaers was born in Mechelen, Belgium in 1948. He graduated from the University of Leuven where he earned his PhD in Physical Chemistry in 1974. He was research assistant at the laboratory of Thrombosis and Haemostasis and assistant at the Central Clinical Laboratory of the Academic Hospital St Raphael in Leuven until 1980. From 1980, he joined the flow cytometry division (BD FACS Systems) of Becton Dickinson where he held several management positions from 1980 to 1990. From 1990 until 1996 he was General Manager and Vice President of BD Image Cytometry Systems in Leiden, the Netherlands. He was General Manager of NeoPath Europe from 1997 through 1999 and became Director of Scientific Affairs for BD Biosciences in 1999, a position he maintained until the present time.

The use of NanoSight NTA to measure exosomes and other nanosized biological particles

Patrick Hole; Nanosight, Malvern Instruments Ltd., Minton Park, London Road, Amesbury, UK.

Here we discuss the Nanoparticle Tracking Analysis (NTA) technique that sizes nanoparticles in suspension, based on their Brownian motion on a particle-by-particle basis, and also yields directly a count/concentration measurement. This technique has been extensively applied to the measurement of a wide range of nanomaterials such as exosomes, viruses and VLPs, liposomes, protein aggregates, bacteria as well as a range of engineered nanoparticles for their use as drug delivery nanoparticles.

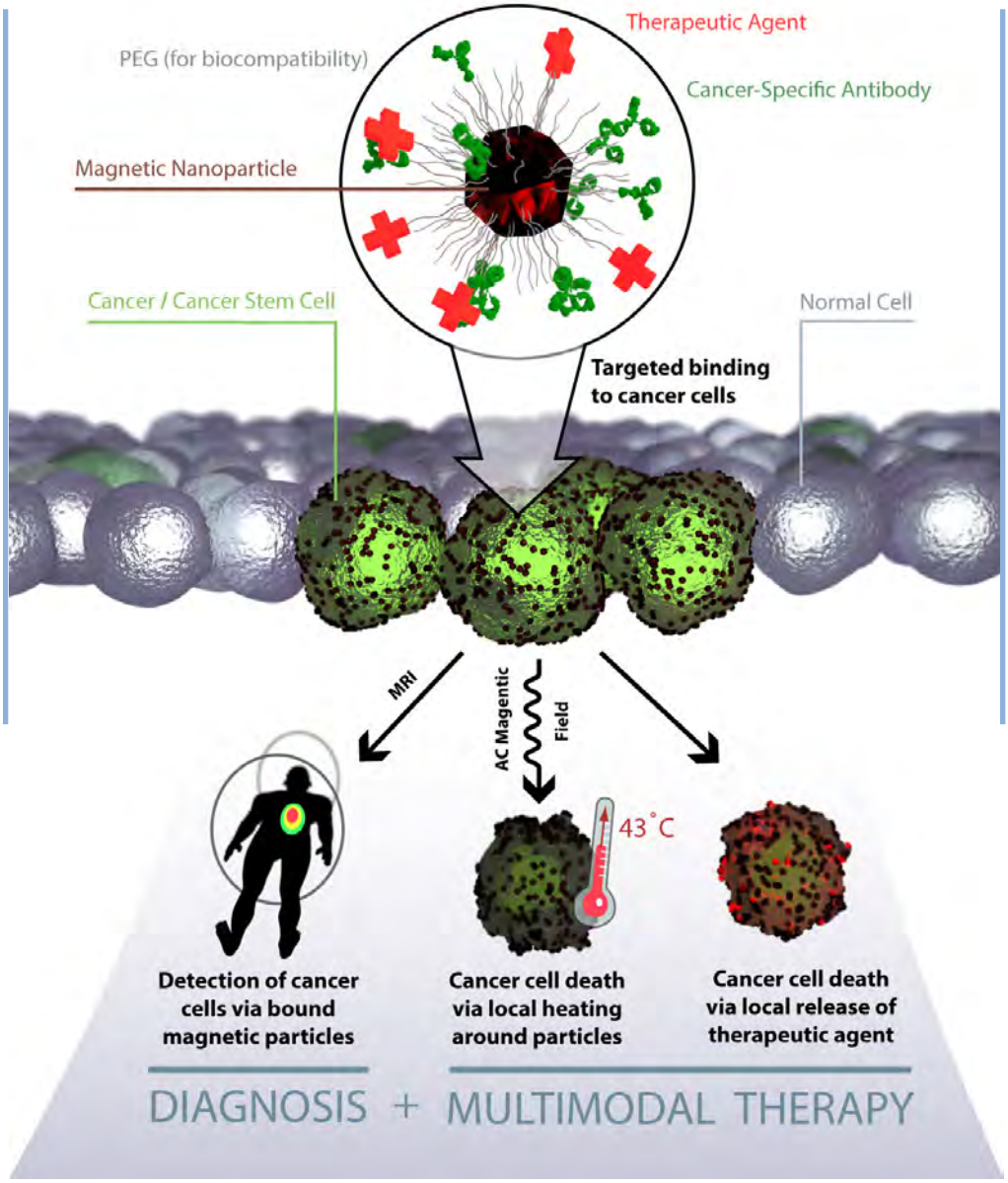
To date, exosomes research has been constrained by a lack of suitable methods for characterization. Nanoparticle Tracking Analysis (NTA) addresses this need. NTA allows specific exosomes and microvesicles in the range of 50—1000nm in liquid suspension to be directly and individually visualized and counted in real-time. The technique is easy to use, fast, robust, accurate and cost effective, representing an attractive alternative or complement to existing methods. Operation in fluorescence mode enables characterization and speciation of suitably labelled particles using a range of excitation wavelengths.

Examples of the characterization of several of these will be discussed highlighting the crucial parameters in each case (such as size distribution, concentration, fluorescence) along with sharing steps that have been taken to optimize such measurements.

Patrick Hole, PhD

Dr Patrick Hole is Head of Development for NanoSight at Malvern Instruments Ltd., a nanoparticle characterization company, providing instruments to both R&D and QA customers that has grown in size and reputation rapidly over the last five years. Patrick has worked with NanoSight for six years and now manages the development of both hardware and software and has brought to production two all-new systems. He is currently responsible for development, production and technical support quality within Malvern Instruments Ltd., managing a team of 14. Previously he completed his PhD at the Optoelectronics Research Centre (ORC) in Southampton in optics and electronics and has a Master's Degree from University of Oxford in Engineering.

(II) Advances in imaging and theranostics using nanotechnological tools



MULTIFUN: Project highlights

Rodolfo Miranda[1,3], Aitziber L. Cortajarena[1,2]; (1) IMDEA-Nanociencia, Universidad Autónoma de Madrid ,Cantoblanco, 28049 Madrid, Spain; (2) Centro Nacional de Biotecnología (CNB-CSIC) - IMDEA Nanociencia Associated Unit, Universidad Autónoma de Madrid, Cantoblanco, Madrid, Spain; (3) Departamento de Fısica de la Materia Condensada and Instituto Nicolás Cabrera, Universidad Autónoma de Madrid, Campus Universitario de Cantoblanco, Madrid, Spain.

The aim of the MULTIFUN consortium is to develop and validate a novel and minimally invasive nanotechnology system to improve cancer diagnosis and treatment. MULTIFUN nanotechnology is based on multifunctional zed magnetic nanoparticles to selectively target and eliminate breast and pancreatic cancer (stem) cells. The improved magnetic features of the MULTIFUN magnetic nanoparticles will lead to potential medical applications such as contrast agents and magnetic heating inductors.

Moreover, magnetic nanoparticles are functionalised with ligands, including targeting peptides and antibodies to increase their affinity towards cancer cells for selective drug delivery and to facilitate diagnosis of tumours by MRI means.

Multifunctional nanoparticles are used simultaneously as functional nanocarriers and heating inductors in order to provide a combined therapeutic modality. The synergistic effects of drugs, peptides and heat are evaluated to determine the effectiveness of different therapeutic combinations. Thus, MULTIFUN multimodal therapeutic approach is designed to efficiently remove cancer cells from the tumour site.

The toxicity of functionalised magnetic nanoparticles is assessed in vitro and in vivo to warrant a safe use and shed some light on the risks. The distribution and activity evaluation of functionalised nanoparticles is performed in human breast and pancreatic cancer xenograft models.

Finally, the use of novel magnetic nanoparticles for biomedical applications provides opportunities for development of new instrumentation for detection and quantification of magnetic nanoparticles in blood, urine and tissues and magnetic heating induction.

Rodolfo Miranda, PhD

Rodolfo Miranda graduated in Physics from the Universidad Autónoma de Madrid (UAM) in 1975. He obtained a PhD in Physics from the UAM in 1980 under the direction of Prof. Juan M. Rojo. Rodolfo Miranda was a Humboldt Fellow in Munich and Berlin (1981-1984) under the supervision of Prof. Dr. Gerhard Ertl, Nobel Prize in Chemistry 2007.

From March 1990, Prof. Miranda is Full Professor of Condensed Matter Physics at the UAM. He was elected Fellow of the American Physical Society in 2007, and, since December 2006, is Director of the Instituto Madrileño de Estudios Avanzados en Nanociencia (IMDEA-Nanociencia), an institution fostering Nanoscience and Nanotechnology in Spain.

His publications (more than 250 by now) have been steadily cited over more than 20 years, totalizing more than 7000 citations. The present h index of Prof. Miranda is 46. He has directed more than 40 projects as Principal Investigator, 30 Ph. D. Thesis and 30 Postdoctoral researchers. He has given more than 100 Invited Talks at international conferences.

Prof. Miranda´s interest has covered vastly different topics, from the mechanisms of epitaxial growth to the development of scanning tunnelling microscopy, from basic discoveries in low dimensional magnetism to fundamental studies on molecular self-organization or, more recently, to the growth and properties of graphene and biomedical applications of magnetic nanoparticles. He has developed surface physics and nanoscience in Spain through his students and co-workers. Prof. Miranda is the scientific coordinator of MULTIFUN project.

Magnetic nanoparticles for advanced MRI imaging and hyperthermia

Maria del Puerto Morales Herrero[1], Gorka Salas[2], Marzia Marciello[1], Lucía Gutiérrez[1], Sabino Veintemillas-Verdaguer[1], Carlos J. Serna[1]; (1) Institute of Materials Science of Madrid, ICMM/CSIC, Madrid, Spain; (2) IMDEA Nanociencia, Campus Universitario de Cantoblanco, Madrid, Spain.

Different approaches have been followed to optimize magnetic properties of iron oxide nanoparticles by controlling synthesis parameters. Thus, improvement of crystallinity and uniformity of large magnetite nanoparticles prepared by thermal decomposition of organic precursors can be achieved by controlling nucleation and growth rates by varying constituents and reaction time. Magnetite nanoparticles with diameters between 10 and 20 nm can be obtained with a size distribution lower than 10%, very good magnetic response and consequently high NMR relaxivity parameters and heating efficiency (100 W/g under 70 kHz, 40 mT), which makes then excellent candidates for cancer diagnosis and hyperthermia treatments [1].

On the other hand, magnetite nanoparticles have been synthesized by a simple aqueous route based on the precipitation of an Fe(II) salt in the presence of a mild oxidant, which leads to highly uniform and crystalline magnetic nanoparticles with sizes between 30 and 20 nm, saturation magnetization over 80 emu/g and heating capacities up to 200 W/g under 70 kHz, 40 mT [2]. An important effort has been paid on this preparation route to scale up the synthesis up to 20 grams of particles and reach colloidal stability for long time at high nanoparticle concentrations using biocompatible polymers such as dextran.

Optimization of magnetic properties of these nanoparticles also allows their detection even at low doses by magnetic methods. Detection and quantification of nanoparticles in tissues or organs is possible from the magnetization curves and AC susceptibility curves giving valuable information about nanoparticle pharmacokinetic and biodistribution [3].

1. G. Salas et al., J. Mater. Chem. 22 (2012), 21065.
2. M. Marciello et al., Submitted.
3. L. Gutiérrez et al., J. Phys. D: Appl. Phys. 44 (2011) 255002.

Maria del Puerto Morales, PhD

Maria del Puerto Morales is a senior scientist at the Institute of Material Science in Madrid, Spain (CSIC) since 2008. Her research activities are focused on the synthesis and characterization of magnetic nanoparticles for biomedicine.

Maria del Puerto was born in Plasencia (Cáceres, Spain) in 1966. She got her degree in Chemistry by the University of Salamanca in 1989 and her Ph.D. in Material Science from the Madrid Autonomous University in 1993. From 1994 to 1996, she worked as a postdoctoral fellow at the School of Electronic Engineering and Computer Systems of the University of Wales (UK). Her research activities are focused on the area of nanotechnology, in particular in the synthesis and characterization of magnetic nanoparticles for biomedicine. She has authored several book chapters in the field of nanoparticle synthesis and more than 135 articles in interactional scientific journals, has directed 3 doctoral theses and has given numerous conferences and seminars. She is now the principal investigator from the CSIC of the European-funded research project called MULTIFUN and responsible for the Work package on synthesis of multifuntional magnetic iron oxide nanoparticles.

Functionalization of magnetic nanoparticles for theranostics

Aitziber L. Cortajarena[1,3], Pierre Couleaud[1], Alfonso Latorre[1], Macarena Calero[1,2], Angeles Villanueva[1,2], Alvaro Somoza[1,3]; (1) IMDEA-Nanociencia, Universidad Autónoma de Madrid ,Cantoblanco, 28049 Madrid, Spain; (2) Departamento de Biología, Universidad Autónoma de Madrid, Madrid, Spain; (3) Centro Nacional de Biotecnología (CNB-CSIC) - IMDEA Nanociencia Associated Unit, Universidad Autónoma de Madrid, Madrid, Spain.

The presented work is part of a European Project called "MULTIFUN" for Multifunctionalization of magnetic particles for selective cancer detection and treatment.

Here, we present the multi-functionalization of dimercaptosuccinic acid (DMSA) coated iron oxide nanoparticles (NPs) with targeting molecules and chemotherapeutic drugs. The DMSA coated iron oxide NPs have been developed by ICMM-CSIC in collaboration with IMDEA-Nanoscience with specific properties for magnetic heating and/or magnetic resonance imaging. We have developed a general approach for the multifunctionalization of magnetic nanoparticles (MNPs) with drugs (Doxorubicin and Gemcitabine) and targeting moieties (Nucant pseudopeptide, antibodies) for controlled and selective release. The functionalization is achieved by the formation of (a) disulfide bonds between MNPs and drugs derivatives synthesized. This approach also allows the quantification of the covalently immobilized molecules. The linkers developed allow the release of drugs without any chemical modification. This process is triggered under highly reducing environment, such as that present inside the cells.

The detailed characterization of functionalised NPs will be presented and discussed. We will also present the *in vitro* studies that show the effective targeting and uptake of DMSA functionalized nanoparticles by breast-cancer cell lines.

Aitziber L. Cortajarena, PhD

Dr. Cortajarena earned her PhD in Biochemistry from the Universidad del País Vasco in 2002. Then, she joined the group of Dr. L.Regan at Yale University, USA, as a Postdoctoral Fellow. She worked on protein design, structure and function. In 2006 she was Visiting Scientist at the Weizmann Institute, Israel, with Dr. G.Haran working on single molecule spectroscopy. She continued her work at Yale University, as an Associate Research Scientist with Dr. Regan. She joined IMDEA Nanociencia as Group Leader in January 2010. Her research focuses on protein engineering and generation of biofunctional nanostructures for applications in nano-biotechnology and nano-biomedicine. Dr. Cortajarena is work-package leader (WP2-Multifunctionalization of MNPs) in the MULTIFUN project (FP7-MNP-2010-262943-2).

Nanoparticles and advanced MRI detection and imaging

Dirk Krüger[1], Gorka Salas[2], Macarena Calero[2], Silvia Lorrio González[1], Maria del Puerto Morales[3], Angeles Villanueva[2], René M. Botnar[1]; (1) Division of Imaging Sciences & Biomedical Engineering, King's College London, London, United Kingdom; (2) Instituto IMDEA Nanociencia, Madrid, Spain; (3) Instituto de Ciencia de Materiales de Madrid, CSIC, Madrid, Spain.

Limitations of current cancer therapies are nonspecific delivery and poor biodistribution of drugs as well as the lack of an effective modality for tracking delivery and monitoring treatment response. The aim of this project is therefore to develop and validate multifunctional zed magnetic nanoparticles (MF-MNP) to selectively target and monitor delivery and treatment response of MNPs by MRI. MNPs can be used as contrast agents and magnetic heating inductors and can be functionalised with targeting ligands to increase their affinity towards cancer [1]. Here we sought to investigate imaging properties of MNPs and validate these in breast and pancreatic cancer cell lines.

Subjects and Methods. We used a water phantom to investigate the longitudinal and transversal relaxation rates of various MNP alone and 24 hours post incubation with human pancreatic carcinoma cells (i.e. PANC-1). We used a 2D multi-gradient-echo sequence (echoes = 5, slice thickness = 3 mm, TE = 1.7 ms, TR = 11 ms, FA = 25°) to measure T2* and a 2D multi spin-echo sequence to measure T2. T1 was determined by using a sequence that employs two non-selective inversion pulses with inversion times ranging from 20 ms to 2000 ms, followed by eight segmented readouts for eight individual images [2]. All experiments were performed on a clinical 3T Philips Achieva scanner.

Results. Three MNPs (ADNH, ASi and OD15) that have been synthesized in the MULTIFUN consortium (Fig. 1) show excellent r1 and r2* values making them promising contrast agent prototypes for both T1 and T2* imaging (tab 1). We also showed that MRI can be used to monitor the dose dependent uptake of those nanoparticles (e.g. F1563) by PANC-1 cancer cells as demonstrated by T2* mapping.

Conclusions. We demonstrate that the investigated nanoparticles have good MR imaging properties and can be used to label carcinoma cell lines. The investigated MNPs show promising imaging and labelling properties and will now be investigated in nude tumour bearing mice (BT474, MDA-MB-231, BxPc-3 PANC-1) to evaluate biodistribution, specificity and suitability for in vivo imaging and therapy.

1. Peng, X.-H., et al., 2008. Int J of Nanomed, 3, pp.311-21.
2. Makowski, M.R., et al., 2011. Nature Med, 17, pp.383-88.

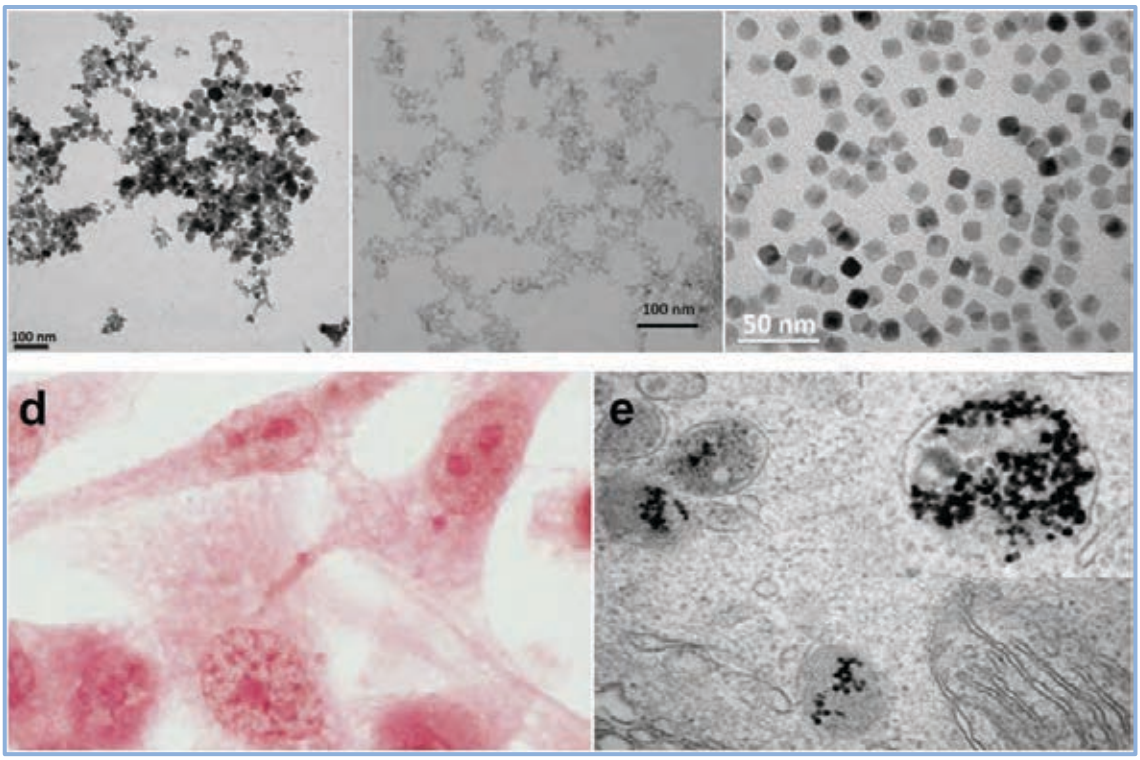


Fig. 1: Transmission electron microscopy (TEM) images of (a) ADNH, (b) ASi and (c) OD15. (d) Light microscopy image of MDA-MB-231 carcinoma cells without any nanoparticles and (e) TEM image after incubation with 15 nm particles (OD15).

René Botnar, PhD

Dr. Botnar received his PhD from the ETH Zurich. From 1996–97 he was a Research Associate in the Department of Radiology at the University Zurich. In 1997, he joined the Cardiac MR Center at the Beth Israel Deaconess Medical Center and Harvard Medical School where his research was focused on the development and clinical validation of novel MRI sequences and molecular contrast agents for coronary artery lumen and plaque imaging. In 2003, Dr. Botnar became the Scientific Director of the Cardiac MR Center at the Beth Israel Deaconess Medical Center and was appointed to Assistant Professor of Medicine at Harvard Medical School, Boston, USA. In 2005, Dr. Botnar accepted a Professorship of Biomedical Imaging at the Technische Universität München where he set up a cardiac MR program with a special focus on pre-clinical and translational multi-modality imaging. His work was funded by the German Ministry of Research and Education, by the German Excellence Program, and by industry. At the end of 2007, he joined the Imaging Sciences Division at King's College London where he is currently Chair of Cardiovascular Imaging. Dr. Botnar is a Fellow of the International Society of Magnetic Resonance Imaging in Medicine and was a board member of Society for Cardiovascular Magnetic Resonance from 2008-2011. He is on the scientific advisory board of the High Risk Plaque initiative and is also on the editorial board of the European Heart Journal: Cardiovascular Imaging. He has authored more than 175 peer-reviewed original papers, 25 review articles and 20 book chapters in the field of CMR. He also holds 5 patents and is an editor of a CMR textbook on Cardiovascular Magnetic Resonance Imaging.

Clinical use of magnetic nanoparticles

Ingrid Hilger; Institute for Diagnostic and Interventional Radiology I, Jena University Hospital, Jena, Germany.

Many different nanoparticles formulations have been developed for biomedical applications in diagnostics as well as therapy. In this context, a variety of different organic and inorganic materials are being employed. Among the inorganic formulations, iron oxide-based nanoparticles are very prominent, as they have already been introduced into clinical practice such as MRI applications. For therapeutic applications, researchers desire multifunctional nanoparticles, which combine selective targeting, diagnostics and therapy (with magnetic properties for hyperthermia treatment and/or selective drug release).

For therapeutic purposes, the utilization of magnetic nanoparticles have been proposed to effectively kill tumour cells and tumours by local thermal stress, e.g. by hyperthermia. In general, hyperthermia induces through cytotoxic temperatures pathophysiological changes on a cellular level that ultimately lead to cell death within the tumour tissue. Whole body hyperthermia is generally used for carcinomas with various metastases or heating sources are placed outside the body when employing electromagnetic waves (microwaves or radiowaves). For a more effective localized therapy within the tumour tissue, magnetic nanoparticles, consisting of a biocompatible iron oxide core (magnetite and maghemite) and a polysaccharide coating, can be deposited directly in the tumour via intratumoural application. There, their intrinsic magnetic properties are utilized to generate heat within the tumour during the exposure to an alternating magnetic field. The heating potential or specific absorption rate is defined as the amount of heat delivered per mass unit and time. Only homogeneous distributions of the injected magnetic material consistently induce hyperthermic temperatures above 43 °C within the tumour. Therefore, the control of the deposition of nanoparticle dosages and the handling of the intratumoural distribution patterns are key factors in determining the therapeutic outcome.

Ingrid Hilger, PhD

Prof. Hilger is head of the Department of Experimental Radiology at the University Hospital, Jena, Germany. Born in Argentina, she studied biology at the Christian Albrechts University in Kiel, Germany, and received her diploma in 1990. She performed several studies in biology in South America and Asia. Later on, she got interested in human biology and biochemistry received her PhD at the Medical High School Hannover, Germany, in 1996. Since then, she focused her research activities to the areas of therapeutic nanotechnology and in vivo meso/macroscopic near-infrared fluorescence molecular imaging and preclinical imaging in general. Since 2008, she is full professor at the University Hospital Jena, Germany. She was awarded with the Walter Friedrich Prize in 2003. She is the spokesman of the German molecular imaging network and the German representative of the European Society of Molecular Imaging.

New devices based on advanced imaging

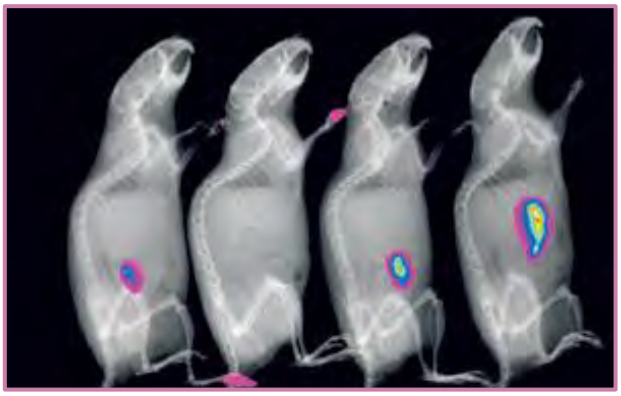
Stephanie Teughels, Peter Vaes, Joeri Verbiest; Pepric nv, Leuven, Belgium.

Quantitative detection of magnetic nanolabels enables the determination of the pharmacokinetics of magnetic particle therapies through longitudinal and quantitative monitoring of the magnetic nanolabel. Newly developed contrast agents for medical imaging have to prove a positive influence on the healing prognosis. In order to receive FDA approval for use in the clinic, a higher contrast in the MRI image is not sufficient. The contrast must enable an improved therapeutic treatment of the patient. Hereto magnetic nanoparticles are now being developed as diagnostic and therapeutic agents with ligands and drugs on the outer coating of the contrast particles such as antibodies and peptides for selective targeting and drugs for target specific drug delivery.

For validation of the developed particles and coatings, it is necessary to determine the pharmacokinetics of the therapeutic and diagnostic particles in the body fluids and tissues. Quantitative distribution of the particles is key to evaluate the specificity of the targeting and efficacy of the drug delivery. Quantitative distribution of therapeutic particles will allow to

- determine the maximal effective dose concentration and optimize the dose repetition time
- reduce cost in the pre-clinical trajectory of particle therapies
- obtain a predictive value for the design of the clinical trials reducing the patient studies

The developed particle spectrometer is based on a direct and selective detection method pEPR (particle Electron Paramagnetic Resonance). When combined with MRI, the PPS offers the solution for quantitative distribution studies.



Stephanie Teughels, PhD

Stephanie Teughels is the CEO and Founder of Pepric: she took the initiative to set-up the Pepric and now has 5 years' experience in the business development and financial aspects of the company. Previous to Pepric, she prepared and assisted spin-off projects at Imec as Venture Development Manager. During her PhD she worked at several international particle accelerator facilities (France, US, Japan) on nuclear magnetic resonance techniques. This offered her the necessary background for a clear understanding of the technological principles of MRI, PET and SPECT. Dr. Teughels has obtained postgraduate degrees in Corporate Finance (2006), Business Administration (2005), and nuclear physics (PhD, 2001).

(III) Cluster: Targeted nanopharmaceuticals and diagnostics

Images courtesy of the Targeted Nanopharmaceuticals and Diagnostics Cluster.

Overview of targeted nanopharmaceuticals and diagnostics cluster activity

Yuri Volkov; School of Medicine and CRANN, Trinity College Dublin, Ireland.

Nanomedicine promises to develop more effective, safe and cheaper therapies for patients, and to improve Europe's health system by supporting its sustainable and economic growth. This objective is mostly achieved by utilizing flexible and multifunctional approaches, technologies and tools that exploit the specific properties from the nanoworld.

Several EU funded projects are developing innovative therapies, detection and diagnosis methods in the field of nanomedicine and cover a wide variety of diseases. The 'Targeted Nano-Pharmaceuticals and Early Diagnostics' cluster brings together about 25 such research projects, funded by the EU Sixth and Seventh Framework Programmes for Research (FP6 and FP7), under the priorities NMP and HEALTH.

The projects in the cluster each have their own research objectives, but an important common element is the emphasis on the application of state-of-the-art nanotechnology. Each of them brings together specialists from 5 to 30 partner laboratories in different universities, research organizations, clinics, industries and SMEs from across Europe to collaborate on the common aim. The project consortia are also able to organise education activities, workshops and conferences in cooperation with the other initiatives. All the research teams involved have an interest in being kept up-to-date on future regulatory requirements and procedures, in order to anticipate the likely requirements to gain market authorization for their new therapies.

The cluster activities promote further networking on a European scale, enabling researchers in the various organizations and countries to get to know and work with each other, thus contributing to the European Research Area. Furthermore, the cluster activities help to increase the visibility of individual projects as well as the importance of the research field as a whole.

The present section will show the rationale and available updates of five projects that form the cluster and are focused on different research areas of nanomedicine: 3 Micron is focused on diagnosis, NAMDIATREAM on biomarkers, NANOFOL on inflammatory diseases, MULTIFUN on breast and prostate cancer, SAVEME on cancer diagnosis and treatment. For further information on the cluster, please visit the webpage at **http://ec.europa.eu/research/industrial_technologies/targeted-nano-pharmaceuticals_en.html**.

Yuri Volkov, MD, PhD

Yuri Volkov received his MD from the Moscow Medical University and subsequently a PhD in biomedical sciences from the Institute of Immunology, Moscow. He is a Professor at the Department of Clinical Medicine and former Director of Research at the School of Medicine, Trinity College Dublin (TCD). Prof. Volkov coordinates a large-scale EU FP-7 project, NAMDIATREAM, for early diagnostics and monitoring of malignant diseases, with 22 European academic, research, clinical and industrial partners. He is the lead TCD partner for the EU FP-7 project, MULTIFUN, and Principal Investigator on a number of other grants. Prof. Volkov is the author of over 100 scientific publications, including articles and book chapters, and he is an inventor on several patents.

Folate-based nanobiodevices for integrated diagnosis/therapy targeting chronic inflammatory diseases

Artur Cavaco-Paulo; Department of Biological Engineering, University of Minho, Braga, Portugal.

It is estimated that inflammatory diseases affect more than **80 million people** worldwide leading to untold suffering, economic loss and premature death. Considering life expectancy in Europe, these numbers are expected to increase in the next 20 years. Moreover, **studies** have shown that disorders such as **rheumatoid arthritis (RA)** can shorten **life span by 10** years. The treatment of chronic inflammatory disorders, including RA, remains a challenge for the medical and scientific community. The emergence of new drugs creates new options though it also entails **high costs, complicated drug administration**, **allergic reactions** and potentially **fatal side effects**. Therefore, more efficient strategies have to be identified in order to improve inflammatory disease treatment while decreasing the side effects with an improved cost-benefit ratio.

In this presentation are reported the last development achieved under the NANOFOL project. The consortium produced FBN (liposomal, protein-based nanoparticles) with encapsulated anti-inflammatory drugs that showed to be biologically active, non-cytotoxic and capable of specifically targeting folate receptor (FR)-positive cells. The consortium also showed that FRβ might be exclusively expressed on a certain subset of macrophages present in several types of inflammatory conditions. Special attention is being given to minimizing the use of animal testing by establishing adequate in vitro models.

Artur Cavaco-Paulo, PhD

Artur Cavaco-Paulo has been a Professor in the Department of Biological Engineering at the University of Minho since 2012. Previously, he was at the Department of Textile Engineering of the same University, where he worked on fibre bio-based process in the last 20 years. His current research interests are on application of bio-based molecules and materials in areas of pharma, cosmetics and fibre areas. He authored more than 200 research papers, with H factor of 32, he supervised more than 25 PhD students and he keeps a group with the size 13-15 persons. He was (and it is) involved in more than 20 EU funded project since the fourth Framework program in 1995.

SaveMe: A modular active nano-platform for advanced cancer management — Core nanosystems, tumour targeting and penetration, molecular imaging and degradome based therapy

Louis Shenkman; Tel Aviv University, Tel Aviv, Israel.

An estimated 3.2 million new cancer cases and 1.7 million deaths per year in Europe define cancer as a crucial public health problem. SaveMe is developing a novel modular nanosystems platform integrating advanced functionalized nano-core particles and active agents. The modular platform will enable the design of diverse active nanosystems for diagnostic or therapeutic applications. As a model system, SaveMe is developing and validating the platform for pancreatic cancer. Pancreatic cancer has the highest one-year mortality rate of any cancer and is Europe's sixth deadliest cancer. Most pancreatic tumours are detected late, at metastatic stage and 85% are unresectable at the time of detection. This is due, in part, to the limitation of current imaging systems in diagnostic accuracy, particularly in identifying sub-cm disease.

For early diagnosis, active nanosystems are being developed for MR, PET and gamma camera imaging. For that purpose, novel functionalized nano-core systems will be conjugated with semi-confluent active shell layer. Three types of shell layers will be designed (1) iron oxide nanoparticles as advanced MRI contrast agents; (2) DOTA complexes for MRI (with Gd3+), PET (with Ga-68) or gamma camera (with Ga-67); (3) both iron oxide nanoparticles and DOTA-Ga68 complexes for a sequential or simultaneous MR/PET imaging, as well as a novel hybrid PET/MRI prototype.

For therapeutics, active nanosystems are being developed to deliver antibodies or nucleic acids including: (1) anti-matrix metalloproteases (MMP)-inhibitory-scFv, and (2) therapeutic siRNAs. We have preliminary data in cell systems demonstrating the feasibility of our NPs as carriers of siRNA and their effectiveness in silencing selected genes.

The nanosystems will be designed for intravenous (IV) administration. Targeting moieties will consists of tumour cell targeting peptides, including novel somatostatin receptor analogue (SSTR) subtypes and targeting moieties such as the receptor/ligand system Gal-1/tPA. The later may allow selective targeting of pre-malignant pancreatic cancer. To enable specific tumour tissue penetration, a PEG–MMP-substrate-PEG agent for optimal tissue diffusion will be used.

To date, we have designed and screened novel generic core polymeric nanosystems for optimal nanomaterial properties (NC average size and low size dispersivity, functionality level, water compatibility, minimal aggregation level). Polymers used are non-toxic, biodegradable and biocompatible, classified as GRAS (Generally Recognized As Safe) like PLGA (poly[lactic-co-glycolic acid] co-polymer), PEG (polyethyleneglycol) derivatives including PLGA hybrids, human serum albumin (HSA), and polyacrylates. Various nanofabrication methodologies have been explored, including polyacrylate (PAs) Huisgen “Click” cycloadditions (Intramolecular polymer single chain cross-linking/collapse), NC nanoprecipitation (solvent deposition) with oil/water emulsion and desolvation methods.

Our preliminary results showing attachment of NPs to cancer cells and intracellular penetration and silencing of selected genes with siRNAs is promising and supports the validity of the concept.

Louis Shenkman, MD

Louis Shenkman is a professor of medicine at Tel Aviv University, Tel Aviv, Israel. He is the coordinator of the SaveMe project. Shenkman graduated from Neew York University School of Medicine and joined the faculty at Tel Aviv University in 1982. He was chair of the department of medicine at one of the affiliated hsopitals, and he has been engaged in biomedical research all of his professional career. He currently is the medical director of Elfi-Tech Ltd., a biomedical company specializing in non-invasive medical sensors.

3MICRON: Multimodality microballoons

Hans Hebert; School of Technology and Health, KTH, Sweden.

In vivo multimodality imaging is a fast growing field in medical research. Although the achievements at clinical level of this diagnostic method are recent, it is already one of the most promising approaches in the diagnosis of diseases at many research medical centres.

The 3MICRON project team gathers together scientists from some of the most advanced European medical and technical institutions to design a set of new diagnostics strategies based on advanced medical imaging, and to push the potential of this technology beyond the state-of-the-art.

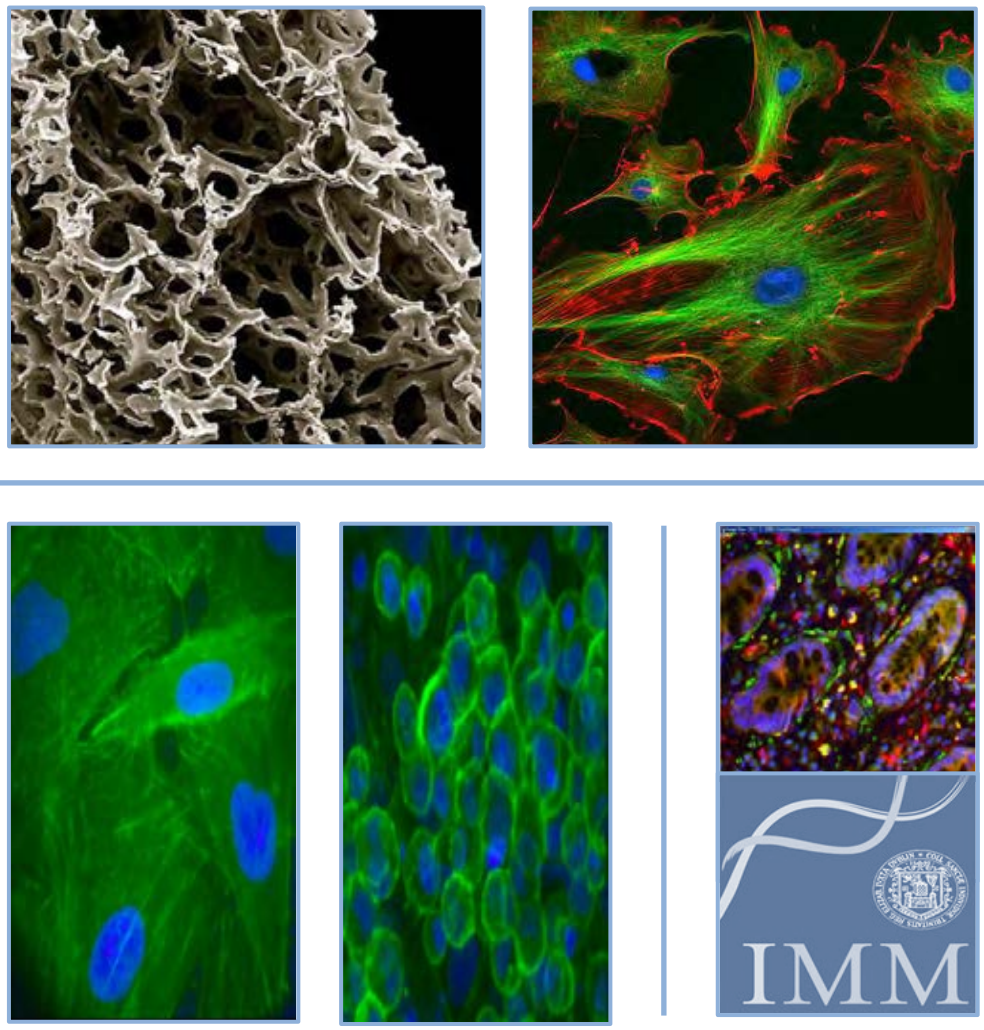
Multimodality imaging is currently viewed as a simple and powerful integration of two or more imaging methods (e.g. PET-CT). In the 3MICRON project, the multimodality approach being taken is supported by a class of next-generation micro/nanodevices called microballoons. In other imaging methods (SPECT, MRI), these subsystems at present provide the function of an ultrasound contrast agent. In the approach being taken by 3MICRON, they could act as a minimally invasive drug delivery method and hyperthermia device.

The project team will test these multi-functional microballoon devices both in vitro and in vivo in order to assess bioclearance and cytoxicity effects toward high impact diseases, e.g. vascular and inflammation pathologies. Finally, selected types of microballoons will undergo pre-clinical screening for a consolidated assessment of the “bench-to-bed” pathway for such new microdevices.

Hans Hebert, PhD

Prof. Hans Herbert is Head of Department Professor in Biotechnology at the School of Technology and Health at the KTH, a position he has held since 2005. He obtained his Master of Technology at Uppsala University in 1975, his PhD as Doctor of Technology at Karolinska Institutet (KI) in 1979, and Docent in medical physics at KI in 1981. He has several academic and industrial positions and merits: Professor in Cryo Electron Microscopy, Lund University in 2001-2004, Research position at Swedish Natural Science Research Council in 1995-2000. He is Member of Central Promotion Review Committee, KTH, since 2005; member of Faculty Assembly, KTH, since 2009; President of Scandinavian Society for Electron Microscopy, 1998–2002; Member of the Executive Board for the European Microscopy Society, 1998 – 2004; Technical platform manager, Swegene, Lund University, 2001-2004; VR-M assessment group member, since 2009; Member of international advisory board “Ion pump consortium” Århus University, since 2007; International Advisory Board for ISDSB2007, Tokyo; International Advisory Board for 14th European Microscopy Congress, Aachen, 2008; International Advisory Board for ISDSB2010, Paris member of organizing committee for SCANDEM2001 and member of SCANDEM2010. An overall aim of his current research is to characterize, under close to native conditions, membrane related processes in living organisms. Such processes are dependent on the interplay between the barrier function of the lipids and the mechanisms of membrane proteins. Another focus is to determine structures of large macromolecular complexes in their unconstrained, physiological states, using cryo-electron microscopy.

(IV) Imaging cell and tissue interaction with nanomaterials



Images courtesy of the Institute of Molecular Medicine, Trinity College Dublin.

Advanced models for imaging cancer

J.E. Mathejczyk[1], F. Ramos-Gomes[1]; A.K. Wege[2], W. Stuehmer[1], L. Pardo[3], T. Rakovich[4], Y. Volkov[4], A. Prina-Mello[4], P. Chames[5], D. Baty[5], A. Sukhanova[4,6], I. Nabiev[4,6], F. Alves[1,7]; (1) Max Planck Institute for Experimental Medicine, Department of Molecular Biology of Neuronal Signals; (2) Clinic of Gynecology and Obstetrics, Caritas Hospital St. Josef, University of Regensburg, Germany; (3) Max Planck Institute for Experimental Medicine Oncophysiology group, Göttingen, Germany; (4) Institute of Molecular Medicine, Trinity College Dublin, Dublin, Ireland; (5) INSERM Centre de Recherche en Cancérologie de Marseille, Inserm UMR 1068, CNRS UMR 7258, 13288 Marseille Cedex 09, France; (6) Laboratory of Nano-Bioengineering, Moscow Engineering Physics Institute, 115409 Moscow, Russian Federation; (7) Department of Hematology and Oncology, University Medical Center, Göttingen, Germany.

The aim of the study was to validate new generation nanoparticles (NP) based on quantum dots (QDs) or on second harmonic generation (SHG) properties conjugated either to single domain antibodies or to small molecules targeting tumour associated antigens for their ability to detect tumour cells in biological samples.

For this purpose human breast, lung and pancreatic metastatic tumour mouse models were established with tumour cells expressing either human epidermal growth factor receptor 2 (HER2), human epidermal growth factor receptor (EGFR) and carcinoembryonic antigen (CEA). Human tumour cells and biological samples from orthotopic tumour mouse models were analysed by western-blot and immunohistochemistry. Human tumour cells with high, moderate and low expression of the tumour-associated antigen were identified in order to validate the specific binding capacity of the NP-conjugates to tumour cells in vitro. For validation of NP-conjugates to detect tumour cells within tissue or body fluids the tumour cells were implanted orthotopically into nude mice.

In order to establish metastatic breast cancer models, HER2 positive tumour cells BT-474 cells were orthotopically implanted into nu/nu mice that had received hormone-pellets subcutaneously. HER2 negative tumour cells MDA-MB231 were used as negative controls. HER2 positive mammary carcinoma SKBR3 cells were implanted into the liver of neonatal immunodeficient NOD.Cg-Prkdcscid Il2rgtm1Wjl/SzJ mice (JAX®) that were irradiated with 1 Gy for 3 hours before transplantation. Both models resulted in a metastatic spread of tumour cells in different organs. Biological samples were collected from these tumour-bearing mice for further analysis.

By applying single domain anti-EGFR antibody conjugated to QDs we were able to stain specifically tumour cells in paraffin sections of biological samples from mice with breast tumours and metastatic spread. Furthermore, single tumours cells were detected in body fluids like ascites and blood.

Frauke Alves, MD

Frauke Alves is Professor of Internal Medicine at the University Medical Center Goettingen (UMG). She graduated from the University of Goettingen in medicine in 1989. She was a postdoctoral fellow in the Dept. Molecular Biology (Prof. A. Ullrich), at the Max-Planck-Institut for Biochemistry, Martinsried from 1991–1994, working on tyrosine kinases and cancer before moving to the Oncolgy and Haematology department at the UMG to establish her own scientific group alongside her clinical training. She heads a research group in the Dept. of Haematology and Oncology at the University Medicine Center, Goettingen. In addition to being a clinician, since 2008 she is leading a second research group at the Max-Planck-Institute for Experimental Medicine in Goettingen, in the Dept. of Molecular Biology of Neuronal Signals, with the aim of translating basic knowledge into clinical practice. The focus of her interdisciplinary tumour imaging group is the investigation of mechanisms of tumour progression, angiogenesis, and the development of novel anti-tumour therapies and diagnostic tools in orthotopic metastatic and transgenic tumour mouse models. Another goal is to establish novel diagnostic procedures by means of imaging in combination with new nanoparticle based probes, especially for oncology, and to optimize these for future use in the clinic.

Nanotoxicology implications in Nanomedicine: unanswered questions and future directions

Marcello G. Cacace; Institute for the Study of Nanostructured Materials, National Council of Research (CNR), Bologna, Italy.

Almost every industrialized country has largely invested in nanotechnology-related initiatives to foster R&D in this area, which encompasses ICT, health-, food- and materials-related themes. The European Commission's contribution to this effort is of the order of €1.5 billion, deployed mainly, but not exclusively, through the execution of almost 600 nanoscience projects. The nanosafety field has witnessed a sharp increase in interest and in ensuing R&D programmes, by national and international authorities and institutions to face the problem of possible risks posed to health and environment by the widespread diffusion of the new materials.

On the other hand, the scientific community and industry have long recognized the enormous potential of exploiting the multi-faceted properties of nanomaterials and nanoparticles for improving the available assortment of tools to tackle more and more pathophysiological challenges in medicine.

Both nanomedicine and nanotoxicology are inquiring on the new, sometimes-unforeseen properties emerging from the bio-nano interaction to exploit them for improving our health on one side and to prevent and control undesired effects, on the other. Undoubtedly, at this stage nanotoxicology and nanomedicine share the common quest of a thorough understanding of the details of the bio-nano interactions.

I will present some examples taken from the recent literature, which exemplify the innovative feature of biology-inspired nanotechnology research. In fact, exploitable consequences may emerge from situations where the mere fact of touching and sampling the targeted biological entities with new materials (because of their size and properties) evoke biophysical phenomena of intrinsic peculiarity.

Marcello G. Cacace, PhD

Besides his research activity in the field of nanotechnology, biophysics and nano-biotechnology, Dr. Cacace is currently Advisor to the European Commission, "Nano and converging Sciences and Technologies" G4 Unit, DG Research, for projects related to the fields of Nanomedicine, Nanomaterials and Nanotoxicology. He has supervised over 15 projects in the above-mentioned fields and is involved in currently running horizontal activities, such as the Nanosafety Cluster initiative, the Nanomedicine Cluster initiative and Support Actions in communication of nanotechnology research.

He has held various teaching appointments and was responsible partner in a CNR bilateral project with the Biozentrum, University of Basel, Switzerland, from 1995 to 1998, and responsible partner in a CNR bilateral project with the Institute of Biophysics, Biological Research Center, Hungarian Academy of Sciences, from 1998 to 2002. He has been the coordinator of the EU Project DNA Switch, FP5 "QUALITY OF LIFE ": QLK3-CT-2002, and has participated as a partner in the FP6 Integrated Project, CANAPE, and in two RTD-STREP Projects, NOCHEMFOOD and BIODOT. His current research interests are on the interactions of biomolecules at surfaces, functionalization and integration of cells into devices.

Imaging cell and tissue interaction with nanomaterials: From 2D to 3D models

Dania Movia[1], Adriele Prina-Mello [1,2], Yuri Volkov [1,2]; (1) CRANN and (2) School of Medicine, Trinity College Dublin, Ireland.

In vitro risk assessment of nanomaterials commonly relies on two-dimensional (2D) cell culture systems, which do not accurately reproduce the structure, function or physiology of living tissues. In vitro three-dimensional (3D) cell culture is about creating suitable surroundings for optimal cell growth, differentiation and function. 3D cell cultures allow individual cells to maintain their normal 3D shape and structure with minimal substrate interference (i.e., reducing stress and artefacts resulting from cell adaptation to 2D growth surfaces) and they enable close imitation of the native architecture found in tissues. The life sciences market is showing a substantial need for incorporating in vivo-like cell systems (such as 3D cell cultures) in the risk assessment of chemicals and other materials. Not only there is the drive to improve the quality of data generated from in vitro assays, but there is also the pressing demand for higher efficiency and decreased cost of the R&D process.

As part of the combined effort of the Nanosafety and Nanomedicine communities, our work is focused on the evaluation of the potential of 3D cell culture models in providing guidance on nanomaterials' safety assessment. Our studies clearly demonstrate the influence of the third dimension on cell responses when cells are exposed to nanomaterials. In particular, the simplicity, rapidity and reliability of our 3D cell model provide a breakthrough opportunity for the safety assessment of nanomaterials that can be translated into competitive market products. 3D cell models can therefore facilitate the bench-to-bedside translation of a range of new biomedically engineered biocompatible nanomaterials.

Dania Movia, PhD

Dania Movia is a post-doctoral researcher at the Centre for Research on Advanced and Adaptive Nanostructures (CRANN)/School of Medicine of Trinity College Dublin (Ireland). In 2007, she was awarded a BSc in Medicinal Chemistry at University of Trieste (Italy). In 2011, she completed her PhD in Chemistry at Trinity College Dublin with a thesis entitled "Single-walled carbon nanotubes as novel NIR fluorescent probes for biomedical optical imaging". Currently she is involved in an EU FP7 funded projects called NANoREG, aiming at delivering the answers needed by regulators and legislators on EHS (Environmental Health and Safety) of nanomaterials.

Raman spectroscopy in clinical diagnostics

Furio Gramatica; Fondazione Don Carlo Gnocchi ONLUS, Laboratory of Nanomedicine and Clinical Biophotonics (LABION), Milano, Italy.

Minimal Residual Disease (MRD) appears in acute leukemia patients when leukemia cells are not totally eradicated from the body even after about four weeks of chemotherapy, but the amount of malignant cells is beyond the sensitivity level of classical cytomorphologic methods. Follow-up of the presence of the residual leukemia cells during chemotherapy allows a better, personalized control during the treatment, and was proved to be a powerful prognostic tool in anticipating relapse..

There are molecular biological methods available for the detection of residual blasts, but sensitive, fast and reliable MRD detection is still a challenge in establishing the follow-up process. The overexpression of the Wilm's tumour (WT1) gene transcript has been proposed as a good MRD marker in acute myeloid leukemia. The mechanism of action in case of WT1 is not completely clarified, but it was shown that WT1 overexpression leads to uncontrolled proliferation and defective differentiation of leukemia cells and has effects on the expression of other genes involved in cell-cycle promotion, differentiation and apoptosis.

Here we present the synthesis and surface modification of star shaped gold nanoparticles dedicated to Surface Enhanced Raman Spectroscopic (SERS) application as analytical tool for WT1 transcript detection. A simple, gold nanoparticle synthesis method utilizing hydroquinone as reducing agent in water based reaction mixture at room temperature was optimized in order to produce gold nanoparticles for SERS application.

The Raman signal enhancing efficacy of the star shaped nanomaterial was compared with the efficacy of spherical particles. Well-known Raman reporter dyes were applied to evaluate the enhancement factor in the suspensions of star shaped and spherical gold nanoparticles with similar particle size and at the same particle concentration using various laser excitation wavelengths.

The star shaped particles synthesized with this method are easy to functionalize with thiols and also transferable into organic solvent based further modifications. The good physicochemical characteristics of these particles and the sensitivity observed in SERS experiments allow us to expect good performance in the further development steps of a novel, fast and reliable spectroscopic method for WT1 detection in MRD patients.

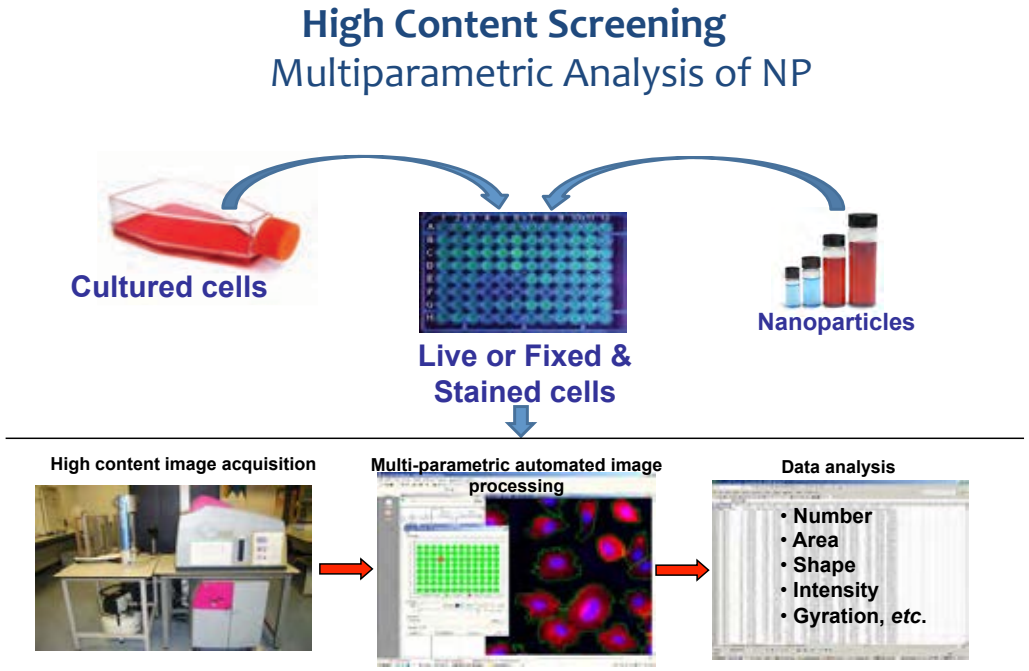
Furio Gramatica, PhD

Furio Gramatica, physicist, was born in Milano in 1964. He is the Chair of the Centre for Innovation and Technology Transfer (CITT) at Don Gnocchi Foundation – an Italian chain of 30 healthcare and research centres specialized in rehabilitation – where he also leads the Nanomedicine and Clinical Biophotonics Laboratory (LABION). For five years he served as Director of the Biomedical Technology Department "Polo Tecnologico" in the same Foundation. Formerly, he spent several years at CERN (Geneva), at Italian Institute of Nuclear Physics and in high-technology companies, with R&D management roles. Dr. Gramatica is a member of the Executive Board of the European Technology Platform of Nanomedicine (ETPN) and Chair of the ETPN Clinical Interface Group; member of the board of experts, evaluators and reviewers of the European Commission and of Wellcome Trust; nanomedicine Scientific Advisor of the Italian Ministry of Health; national representative of Italy at ETPN Mirror Group; member of Nanotechnology Commissions of Assobiotec and of Milano Engineers Association; fellow professor of physics at Milan University Medical School.

High content screening of cellular response to nanomaterials interaction as imaging and decision-making tool

Adriele Prina-Mello, Yuri Volkov; School of Medicine and CRANN, Trinity College Dublin, Ireland.

High Content Screening (HCS) has been extensively utilised in the pharmaceutical industry as decision-making tool for drug efficacy and lead-candidate titration. In the past decade, this technology has also enabled academia to embrace and develop this technique further into emerging biomedical applications. Since an increasing number of biomedical applications, both in diagnostics, therapeutics and regenerative medicine have been adopting engineered nanoparticles, there is therefore a pressing demand for quantitative tools that inform selection of the most efficient and cost-effective of the myriad potential solutions. HCS has been shown to improve such selective decision-making processes and such systems are based on high-resolution precision subcellular imaging, which enable detection of nanoparticles within identified organelles, tracking of molecular targets within the cell and quantification of selected targets.



Adriele Prina-Mello, PhD, MSc

Dr. Adriele Prina-Mello is a Senior Research Fellow at the School of Medicine, a CRANN Investigator and a part-time lecturer at Trinity College Dublin. He is also a member of the executive board of the European Technology Platform on Nanomedicine, a Nanosafety Cluster member and has recently become the National Coordinator of the NANoREG project concerning regulatory testing of nanomaterials.

Dr Prina-Mello has published extensively across many fields, including nanomedicine, nanotechnology, nanotoxicology and bioengineering. Dr Prina-Mello's main research interest is the development of future applications for nanoparticles and nanomaterials in clinically relevant biomedical and bioengineering fields.

Dr Prina-Mello is involved in developing and advancing several multidisciplinary research projects between the university, research hospital and industry partners for future applications in medicine and the nanotechnology industry. Dr. Prina-Mello is also involved in several EU FP7 funded projects: Deputy Coordinator of NAMDIATREAM (NMP), dissemination coordinator of MULTIFUN (NMP), National coordinator of NANoREG (NMP), and Science Foundation Ireland CRANN Pathfinder project.

Workshops at EuroNanoForum 2013

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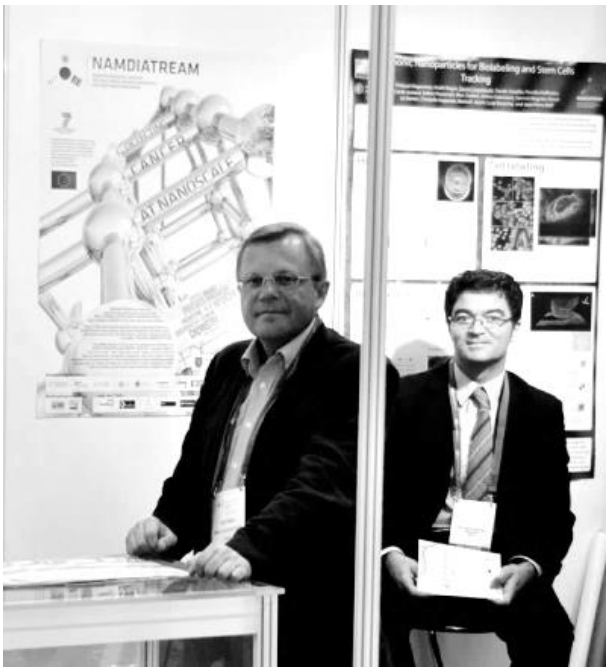


EuroNanoForum 2013

At the 2013 EuroNanoForum (ENF) conference, the MULTIFUN project made a major contribution to the event, through the Irish project partner Trinity College Dublin, to bridging the gap between the world of medical research/innovation and that of the nanotechnology stakeholders, by collaborating to organize two workshops entirely dedicated to the nanomedicine. The ENF was a great opportunity for the nanomedicine community to emphasize to a large panel of research, industry and public authority representatives, the huge potential of nanotechnology as an enabling technology for medical applications. With participation at ENF supported by the European Technology Platform on Nanomedicine (ETP Nanomedicine), the nanomedicine field was widely represented and specific needs and requirements for the successful implementation of nanotechnologies in the health sector were highlighted.

The ENF 2013 conference, organized by Enterprise Ireland, was held in Dublin in June 2013 during the Irish presidency of the European Union under the motto "Nanotechnology Innovation: From research to commercialization, the bridge to Horizon 2020". The event was a tremendous success, attracting nearly 1,500 active members of the nanotechnology community from 50 countries around the world. Over 140 high-level speakers presented at the plenaries, sessions and workshops. Nanotechnology developments and commercialization success stories were also visible at the coinciding Nanotech Europe exhibition. The event was supported by the European Commission and its Industrial Technologies programme, and co-organised by Enterprise Ireland and Spinverse Ltd.

The focus of the conference was the commercialization of nanotechnology, exploiting its potential for new applications, pushing it from an enabling technology through to use in end products. Industrial and research organizations such as BASF, Shell, Ottobock, Nanobiotix, Intel, Philips Healthcare, Airbus, VTT, Fraunhofer, Max-Planck Institute, CRANN, Tyndall, MSSI and NanoStart, initiated a thriving discussion about the future of nanotechnology, its economic and technological impact on European growth and the commercialization challenges of nanoproducts. Speakers agreed that understanding of industrial needs, focused R&D and suitable funding instruments are required, as is the identification of areas where nanotechnology is most likely to have an impact.

The Nanotech Europe exhibition completed the conference. Here, 70 innovative organizations from 20 countries like Austria, the UK, Germany, Denmark, Finland, France, the Czech Republic, Switzerland and the Netherlands showcased their ground-breaking innovations. In the meantime, a brokerage day on June 20th saw 50 parallel meetings take place, sparking new businesses, project consortia and cooperative activities for technology transfer.

With Horizon 2020 beginning in 2014, the conference offered a unique chance to look at how nanotechnology will fit into the new structure's key priority areas of *excellent science*, *industrial leadership* and *societal challenges*. "Today, as demonstrated in ENF 2013, nanotechnology applications can be found in all areas of industrial technologies", commented Dr. Herbert von Bose, Director of the Industrial Technologies Programme of DG Research and Innovation at the European Commission. "Still, a lot more needs to be done, especially in fastening the pace of industrial innovation. We are looking forward to continuing our work within the next seven-year programme of Horizon 2020, following the principles of safe, responsible and sustainable nanotechnology development and ensuring the competitiveness and growth of European industries".

The central role of nanomedicine within the sphere of applied nanotechnology was one of the key topics at the conference; and this point was emphasized by the award for Best Research Project going to the EU nanomedicine project, SONODRUGS. The project aims to develop novel technologies for drug delivery to enable localized treatment of cardiovascular disease and cancer. For patients, this could mean a treatment with fewer side effects and burdens, in addition to reduced post-intervention recovery times.

Nanomedicine at EuroNanoForum 2013

Nanomedicine research and applications were the focus of two workshops and two of the life science sessions. They received endorsement and direct contributions from ETP Nanomedicine

During the first day, the "Healthcare Session" dealt with technology transfer and commercialization of nanotechnology-based healthcare innovations, focusing on industry needs, with presentations, among others, by Dr. Hans Hofstraat, Vice President of Philips Research, and Dr. Patrick Boisseau, Chair of the ETP Nanomedicine.

The "Session on Advances in Bionanotechnology" was held the second day. Here, nanobiotechnology was shown to provide tools for the study of living matter at the nanoscale, and to generate scientific and technological advances in the fields of medicine and biology. In addition, biomimetic approaches to creating new crystalline nano-materials was illustrated as a further example of bionanotechnology, highlighting its impact beyond the biological sciences.

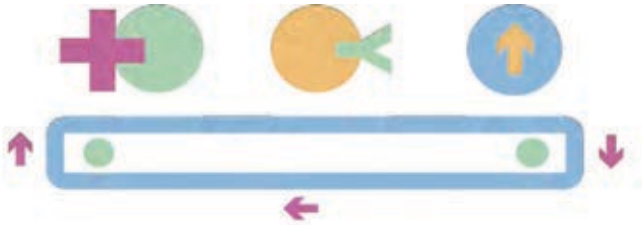
During the third day, the two nanomedicine-focused workshops took place with the direct involvement of the MULTIFUN and NAMDIATREAM projects, which contributed to defining the workshop's mission and program. The main objectives were to describe, with the interests of all stakeholders in mind, the current action plan and measures taken to foreground the field of nanomedicine, and to highlight the policy actions, led by the ETP Nanomedicine, to integrate nanomedicine as a horizontal topic in Horizon 2020 (H2020).

Workshop 7, titled, "Nanomedicine: Technology Platforms and Breakthrough Projects", centred around ongoing EU nanomedicine projects and initiatives, the White Paper contribution of the ETP Nanomedicine to H2020, as well as current actions to support initiatives of transnational multidisciplinary research in nanomedicine by fostering the competitiveness of the European nanomedicine actors.

The clinical translation of nanomedical technologies requires the stakeholders to understand and apply the process of open innovation, which is essential for translation into the clinic. As stated in the ETP Nanomedicine "White Paper", the best option for successfully implementing the open innovation model in Europe involves establishing a supply chain providing nanomedicine products compatible with industrial processes and strategies. The ETP Nanomedicine has a track record as a neutral communication platform and think tank for academia, SMEs and industry; this has allowed it to foster interaction between key players, leading to translation of scientific inventions into healthcare products. Based on this unique European experience, the ETP Nanomedicine and its FP7-funded projects are working on collaborative platforms that contribute to the development of the associated healthcare-industry organizations and a number of the roadmaps and visions of the European Commission.

The session was chaired by Dr. Patrick Boisseau (ETP Nanomedicine and CEA Grenoble, France) and co-chaired by Dr. Laurent Levy (Nanobiotix, Paris, France) and Dr. Adriele Prina-Mello (Trinity College Dublin, Ireland). The panel of experts was composed of ETP Nanomedicine Executive board members and EC representatives. The sessions were organized by Dr. Adriele Prina-Mello (NAMDIATREAM Deputy Coordinator).

Workshop 8 was titled, "Market Strategies in Medical Device Sector". The starting point of this workshop was the realization that nanotechnology and nanomaterials have majorly contributed to innovation within the medical device industry. This has led to tremendous advances in the provision of care for patients worldwide, but continued progress will require effective innovation solutions taking advantage of the most advanced scientific breakthroughs.



Highlights I: Nanomedicine: Technology Platforms and Breakthrough Projects (ENF Workshop 7)

Nanotechnology has already provided several medical solutions for both therapeutics and diagnostics, but progress in clinical translation requires the stakeholders to understand and apply the process of open innovation. As stated in the previous section, establishment of a supply chain of industry-compatible nanomedical products would be an important part of this open innovation model. Such a task would necessitate major changes in the thinking of all stakeholders and the empowering of an organization to actively manage the translation effectiveness of its members (academics and SMEs) to help revitalize industry in Europe.

Key tasks to foster open innovation in nanomedicine, to be put in place by the ETP Nanomedicine, have been identified as the creation of a Translation Advisory Board (TAB) and new infrastructures supporting the translation of nanomedical products into the clinic. The TAB will need to include experienced industrial experts, who will apply horizontal innovation filters to R&D proposals from academics and SMEs to select, guide and push the best translatable concepts towards funding and clinical proof of concept. The new infrastructures supporting the translation of nanomedical materials will include a European Nano-Characterization Laboratory (EU-NCL), a European Pilot line for GMP manufacturing of batches for clinical trials (GMP Pilot Lines); a European network of preclinical centres of excellence; and coordination of the efforts of European clinical organizations in nanomedicine. The TAB, together with the new infrastructures and an active support towards the setting-up of a dedicated NanoMed SME instrument will create an umbrella of complementary actions and initiatives within the **Translational Hub**. The four key elements composing the Translation Hub (EU-NCL, GMP Pilot Lines, the SME Instrument and TAB) aim to be the most relevant concepts for ensuring the competitiveness of European Research and Innovation. More details are available on the ENF website where it is possible to download the presentation given by Dr. Patrick Boisseau (CEA, France), chair of ETP Nanomedicine:

http://www.euronanoforum2013.eu/wp-content/uploads/2013/07/Boisseau-Nanomedicine-Workshop.pdf

"Small but heading to a big future" is the motto of **Nanobiotix**, but this ambition could be extended to the entire nanomedicine field. Founded in 2003, the company has already established itself as a pioneer in nanomedicine, developing a pipeline of first-in-class products targeting cancer. The first product, NBTXR3, has now achieved a clinical milestone, reaching proof-of-concept in a phase I trial for the treatment of soft tissue sarcoma. Local procedures like surgery and radiotherapy play an important role in the treatment of solid tumours, and they also increase the probability of success for systemic treatments. The innovation in Nanobiotix lies in their NanoXray technology, which comprises three products to selectively increase the efficacy of radiotherapy against tumour cells, promising to ovecome some of the limitations of standard radiotherapy. During the workshop, NanoBiotix CEO, Dr. Laurent Levy, described the impressive achievements of this company; more details can be found in the presentation itself:

http://www.euronanoforum2013.eu/wp-content/uploads/2013/07/L-Levy-Nanomedicine-Workshop.pdf

The first breakthrough project to be presented at the workshop was **NAMDIATREAM** and this was done by its coordinator Prof. Yuri Volkov (Trinity College Dublin). The NAMDIATREAM project, which targets development of nanotechnological toolkits for multi-modal cancer diagnostics and treatment monitoring, is an excellent example of a strong interdisciplinary effort in nanomedicine. It is a pan-European consortium built around 7 high-tech SMEs, 2 multinational corporations and 13 academic institutions. The breakthrough innova-

tions that NAMDIATREAM brings to medicine centre on the concepts of super-sensitive "lab-on-a-bead", "lab-on-a-chip" and "lab-on-a-wire" nano-devices, which are tackled from the standpoints of both academic and industrial science. Prof. Volkov's presentation is available from the ENF website:

http://www.euronanoforum2013.eu/wp-content/uploads/2013/07/Y-Volkov-Nanomedicine-Workshop.pdf.

Multifunctional Nanotechnology for selective detection and Treatment of cancer, **MULTIFUN**, is an FP7 large-scale collaborative nanomedicine project. The MULTIFUN consortium focuses on the development and validation of new nanotechnological systems for the early, selective detection and elimination of breast and pancreatic cancer with reduced side effects. The project will deploy a strategy based on the multifunctionalization of magnetic iron oxide nanoparticles (MNPs) to produce a potential "theranostic" tool, combining diagnostic and therapeutic features against cancerous breast, pancreatic and stem cells. To improve the translation of the research outcomes and their economic potential, a major part of the project is focused on studying the scale-up of the production methods for the main components More information is available from the presentation of Prof. Bonifacio Vega (IMDEA, Spain) at this link:

http://www.euronanoforum2013.eu/wp-content/uploads/2013/07/B-Vega-Nanomedicine-Workshop.pdf

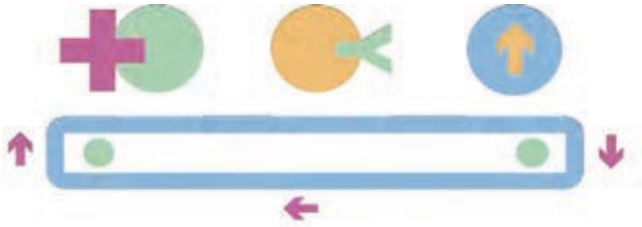
TRANS-INT is a large-scale collaborative FP7 project devoted to the development of nanocarriers for the transport of therapeutic macromolecules across the intestinal barrier. The administration of medical macromolecules via the oral route will greatly increase patient's compliance and quality of life, especially for chronic diseases that require daily administration of macromolecular drugs, such as diabetes. Yet the oral administration route poses a big challenge due to the gastrointestinal environment, which degrades most macromolecules. Nanoparticles are able to overcome this problem by protecting the drug and allowing it transport through the intestinal barrier. The concept behind TRANS-INT is the rational design of oral nanomedicines based on safety, mechanistic, bioengineering and pharmaceutical technology criteria. The consortium is composed of 16 partners from industry and academia with complementary leading expertise in chemistry, chemical engineering, pharmaceutical technology, molecular physiology, biopharmaceutics, toxicology and drug development. Prof. David Brayden (University College Dublin) presented this project as deputy coordinator. Further details can be found at the link below:

http://www.euronanoforum2013.eu/wp-content/uploads/2013/07/D-Brayden-Nanomedicine-Workshop.pdf

ALEXANDER is another large-scale collaborative FP7 project, with the aim of identifying novel strategies for the efficient transport of nanocarriers through the mucus gel layer (e.g. intestinal, ocular), while also optimizing existing strategies. In particular, R&D activities are focused on the synthesis of functionalized nanocarriers capable of permeating the mucus gel layer and delivering their therapeutic payload to the epithelium. Such nano-delivery systems are expected to open the door to oral versions of currently injected macromolecular drugs, such as insulin and EPO-mimetics. It is hoped that they will also lead to radical improvements in ocular treatments and in non-viral gene therapy in mucosal tissues, e.g. treatment of cystic fibrosis with the CFTR-gene. The interdisciplinary ALEXANDER consortium consists of 14 partners from 8 European countries, with 5 universities, 2 research institutes, 3 large industrial partners and 3 SMEs. More details can be found in the presentation of the coordinator, Prof. Silke Megelski (DECHEMA e.V.) at the following link:

http://www.euronanoforum2013.eu/wp-content/uploads/2013/07/S-Megelski-Nanomedicine-Workshop.pdf

The last presentation of this Workshop was dedicated to **EuroNanoMed II** (ENM II), an ERA-NET under the FP7 NMP programme 2012–2016, presented by the



coordinator, Prof. Natalia Martin, of the French National Research agency (ANR). ENM II is based on the foundations of ENM I (ERA-NET under the FP7 NMP programme, 2009–2011), which laid the ground for a transnational research programme that responds to the needs of the nanomedicine community (ENM I strategic agenda, ETP Nanomedicine). ENM II has 20 partners in 17 countries, and its main objectives are to

- develop innovative diagnostic and therapeutic solutions for the patient
- solve unmet medical needs and improve the efficacy of current medical solutions
- contribute to maturation of the nanomedicine field in Europe
- allow multidisciplinary collaboration between the best researchers/teams in nanomedicine, bringing together teams from academic/public research and the clinical/public health industry
- increase the attractiveness of multidisciplinary and transversal nanomedicine research to the younger generation of scientists
  - provide a platform dedicated to young scientists' information and networking
  - use specific measures to stimulate the participation of young scientists in the ENM II calls

Other objectives of the ENM II are to continue the European coordination of research programmes in nanomedicine, integrating new members, keeping close relations with the relevant stakeholders (especially the ETP Nanomedicine) and the research community in order to react flexibly to needs and new developments in nanomedicine. In addition, ENM II aims to collaborate with other ERA-NETs to create synergies that integrate lessons/solutions learned from others (JTCs, young researchers, sustainability, etc.) and implement common activities (calls, workshops, etc.). Further details of the presentation on ENM II are available at this link:

http://www.euronanoforum2013.eu/wp-content/uploads/2013/07/N-Martin-Nanomedicine-Workshop.pdf.

Highlights II: Market Strategies in the Medical Device Industry (ENF Workshop 8)

Nanomedicine has established a substantial presence in today's markets, with engineered nanomaterials already providing medical enhancements that, otherwise, would not have been achievable. Furthermore, Europe has a competitive medical device industry, producing a great variety of devices and offering patients timely access to innovative products. Nevertheless, a recent analysis on nanomedicine commercialization by Etheridge and colleagues (published in *Nanomedicine: Nanotechnology, Biology, and Medicine 9 (2013) 1–14*) highlighted the infancy of the field, pointing to the fact that a large portion of the nanomedicine applications identified are still in the research and development stage.
The study reveals two clear needs that should be addressed for nanomedicine to succeed: 1) development of a clear and effective of the field and 2) creation of a standardized approach for gathering, sharing and tracking relevant information on nanomedical applications and products, without the addition of new barriers to medical innovation.

The **Nanotechnology Industries Association (NIA)** is a committed partner of industry that seeks to achieve these solutions. NIA also supports market intelligence in nanomedical applications and products through a nanodata service (a tender for data-providing services in support of research and policy in the field of nanosciences and nanotechnologies), commissioned by DG Research & Innovation from 2013 to 2017. The aim is to provide "systematic insights into the whole nano-value chain, from scientific research to market" and medical devices will be the first sector analyzed. Further information on NIA's support for nanomedicine commercialization can be found in the presentation of Dr. Frederick Ntow (NIA) on the ENF website at this link:

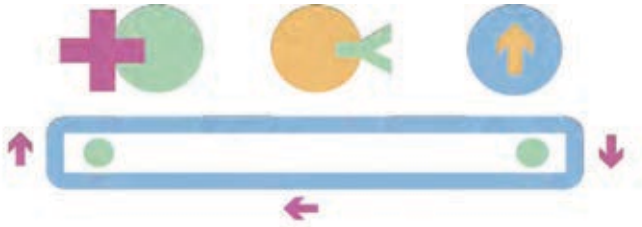
http://www.euronanoforum2013.eu/wp-content/uploads/2013/07/F-Ntow-Market-Strategies-in-the-Medical-Device-Industry.pdf

DISC REGENERATION is a project, which aims to treat intervertebral disc (IVD) degeneration via a tissue engineering (TE) approach leading to the regeneration of the different histological compartments of the disc. The project objectives are as follows: (1) Regeneration of a healthy IVD and restoration of a physiological disc-vertebral system through appropriate bio-mimicking of the anatomy, physiology, cell biology and metabolism of the relevant natural structures; (2) Development of novel biomaterials capable of controlling angiogenesis such that it can proceed to the different extents required by different regions of the disc structure; and (3) Enhanced integration of the IVD with the adjacent vertebral body upon implantation. The presentation by Dr. Luigi Ambrosio and Dr. Luisa Tondelli can be found at the link below:

http://www.euronanoforum2013.eu/wp-content/uploads/2013/07/L-Tondelli-Market-Strategies-in-the-Medical-Device-Industry.pdf

The exploitation of nanotechnology for point-of-care diagnostics at the Biomedical Diagnostics Institute was presented by Dr. Conor Burke (Dublin City University). The Institute was established in 2005 to work on point-of-care diagnostics as one of the nine SFI CSETs. It consists of a team numbering about 150 people and is based on a broad partnership between academia, business and clinical organziations. The institute includes embedded industry researchers and has a strong focus on innovative platforms and translational research activities.

AmBeR is a point-of-care prototype device developed at the Institute; it allows for monitoring breath ammonia via printed sensor technology that uses nanoparticulate polyaniline (NanoPANI). It is a low-cost, disposable and mass-manufacturable sampler/sensor component, with a custom-designed breath sampling system. Clinical applications are Kidney/liver dysfunction and H.pylori detection. Other examples of activities are antibody optimization for biochip applications (C-reactive protein, CRP), glassy surface deposition by PECVD for polymer biochip functionalization and DiCAST (direct clone analysis and selection technologies). Further details can be found in Dr. Burke's presentation on



the ENF website:

http://www.euronanoforum2013.eu/wp-content/uploads/2013/07/C-Burke-Market-Strategies-in-the-Medical-Device-Industry.pdf

iONE, Implantable Organic Nano-Electronics, is an FP7 project that aims to build the knowledge and technology required to regenerate nerves at the site of injury. The use of flexible, organic, electronic devices (using ultra-thin film organic field effect transistors [FETs], organic electrochemical transistors or nanoparticle, organic memory FETs) will advance the state-of-the-art for implantable devices from passive to active layouts that will promote nerve regeneration by a combination of local stimuli delivered on demand. In addition, they will sense inflammation and control the immune-/nflammatory response. Prof. Fabio Biscarini (Università di Modena e Reggio Emilia & CNR-ISMN) presented the project, and further details are available at the following link:

http://www.euronanoforum2013.eu/wp-content/uploads/2013/07/F-Biscarini-Market-Strategies-in-the-Medical-Device-Industry.pdf

Translating research to industrial innovation: Veneto Nanotech involvement in the medical sector was the title of Dr. Pozzi Mucelli's presentation (from Veneto Nanotech). Veneto Nanotech is a cluster for nanotechnology based in the Veneto Region. Nanomedical research carried out within Veneto Nanotech includes development of innovative bio-sensing techniques, plasmonics, enhanced gene- and protein-array platforms, and antimicrobial treatments for wound dressings. It also offers support with regulatory compliance and acts as secretariat for ENM II. The presentation can be downloaded from the ENF webste :

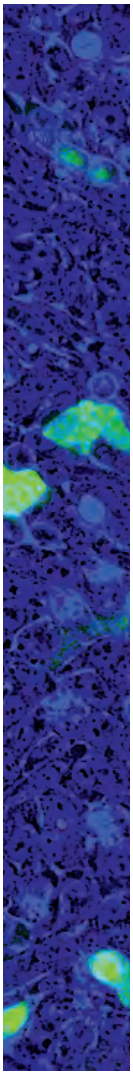
http://www.euronanoforum2013.eu/wp-content/uploads/2013/07/S-P-Mucelli-Market-Strategies-in-the-Medical-Device-Industry.pdf

Final Multifun Workshop 2015

instituto imdea nanociencia

february
23-25
2015

final
multifun
2015 workshop



Final Multifun Workshop 2015

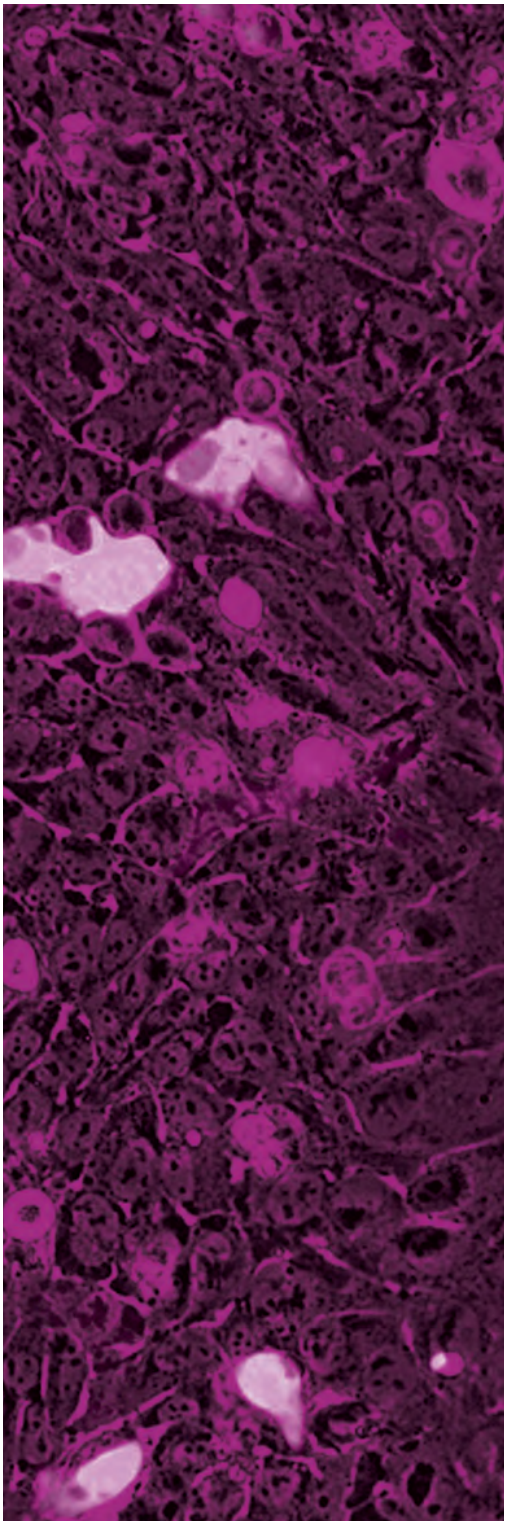
During the last four years the MULTIFUN consortium has focused its activity on the development and validation of new systems based upon minimal invasive nanotechnology for the early and selective detection and elimination of breast and pancreatic cancer. The project has successfully produced multifuntionalised magnetic iron oxide nanoparticles (MNP) that combine diagnostic and therapeutic features against these two types of cancer.

The therapeutic approach developed within the project includes a synergistic effect between the therapeutic effect produced by magnetic hyperthermia and that due to the intracellular drug delivery selectively targeted to tumour cells.

Some of the designed formulations have proven their efficiency, safety and non-toxicicty in *in vivo* models, making them promising candidates to produce new nanomedicines against breast and pancreatic cancer.

The Madrid Institute for Advanced Studies in Nanoscience (IMDEA Nanociencia) is responsible of the scientific coordination of the project. Atos Spain S.A is the Administrative Coordinator and Contract Manager.

Prof. Rodolfo Miranda,
MULTIFUN Scientific Coordinator &
IMDEA Nanociencia Director



Workshop program

Monday 23 February

08.30 - 09.30 Registration

09.30 - 10:00 Opening session

Prof. Rodolfo Miranda, *(MULTIFUN Scientific Coordinator & IMDEA Nanociencia Director)*

Session 1: Introduction and Perspectives: Cancer Science

Chairperson: **Prof. Rodolfo Miranda**, *IMDEA Nanociencia*

10:00 - 10:45 Keynote speaker:
Dr. Manuel Hidalgo, *Centro Nacional de Investigaciones Oncológicas*
"Nanodrugs: Development and Applications to Pancreas Cancer Treatment"

10:45 - 11:30 Keynote speaker:
Dr. José Antonio López, *Instituto de Investigación Hospital 12 de Octubre*
"An overview of cancer and (druggable-) immune response"

11:30 - 12:00 COFFEE BREAK

Session 2: Nanotoxicity, Biodistribution, Biodegradation

Chairperson: **Prof. Yuri Volkov**, *Centre for Research on Adaptive Nanostructures, Trinity College Dublin*

12:00 - 12:40 Keynote speaker: **Prof. Florence Gazeau**, *Université Paris-Diderot-CNRS*
"Ageing and biotransformation of nanomagnets in the body: How to conciliate therapeutic efficiency and safe life-cycle?"

12:40 - 13:30 Round table on regulation for manufacturing and biomedical applications of nanomaterials: safety on nanohandling.

Members: **Prof. África González-Fernández** *Instituto de Investigaciones Biomédicas de Vigo - Universidad de Vigo*, **Prof. Florence Gazeau** *Université Paris-Diderot-CNRS,* **Prof. Yuri Volkov** *Trinity College Dublin*

Moderator: **Dr. Pilar Calvo** *Pharmamar*

13:30 - 15:00 LUNCH BREAK

15:00 - 16:40 Poster session

Chairperson: **Dr. Domingo F. Barber**, *Centro Nacional de Biotecnología-CSIC*

16:40 - 17:10 Invited speaker: **Prof. África González-Fernández**, *(Instituto de Investigaciones Biomédicas de Vigo - Universidad de Vigo)*
"Safety of nanomaterials: Immunotoxicology and interaction between Nanomaterials and human proteins"

17:10 - 17:30 **Mr. Vladimir Mulens,** *Centro Nacional de Biotecnología-CSIC*
"Effect of Polyethylenimine-coated SPIONs on macrophage activation and podosome dynamics"

17:30 - 17:50 **Dr. Lucía Gutiérrez,** *Instituto de Ciencia de Materiales de Madrid-CSIC*
"Tracking magnetic nanoparticles in tissues by AC magnetic susceptibility: Recent achievements and future challenges"

17:50 - 18:10 **Dr. Francisco Javier Chichón,** *Centro Nacional de Biotecnología-CSIC*
"Soft X-ray tomography as quantitative tool to decipher the interaction between DMSA-SPION and MCF7 cancer cells"

18:10 - 18:30 **Mr. K. Crosbie-Staunton,** *Trinity College Dublin*
"Multiparametric High Throughput Methods for Magnetic Iron Oxide Nanoparticle Lead Selection"

Tuesday 24 February

Session 3: NanoDiagnostic

Chairperson: **Prof. René Botnar**, *King's College London*

09:30 - 10:15 Keynote speaker: **Prof. Kannan Krishnan**, *University of Washington*
"Tracer development critical for translational medical applications of Magnetic Particle Imaging"

10:15 - 10:35 **Prof. Yung-Ya Lin,** *University California, Los Angeles*
"Early Detection of Pancreatic Cancers & Brain Tumors by MR Molecular Imaging"

10:35 - 10:55 **Dr. F. Herranz,** *Centro Nacional de Investigaciones Cardiovasculares*
"Parallel multifunctionalisation of Nanoparticles: A One-Step Modular Approach for *in vivo* Imaging"

10:55 - 11:15 **Dr. Rocío Costo,** *Instituto de Ciencia de Materiales de Madrid-CSIC*
"Improving magnetic properties of ultrasmall magnetic nanoparticles by biocompatible coatings"

10:15 - 11:30 **Ms. Yurena Luengo,** *Instituto de Ciencia de Materiales de Madrid-CSIC*
"Synthesis and characterization of bimetallic magnetic oxides obtained by oxidative precipitation"

11:30 - 12:00 COFFEE BREAK

Chairperson: **Dr. Jose Courty**, *CRRET-CNRS*

12:00 - 12:20 **Dr. Marzia Marciello,** *Instituto de Ciencia de Materiales de Madrid-CSIC*
"Coating strategies for the application of magnetic nanoparticles in cancer diagnosis and therapy"

12:20 - 12:40 **Dr. Wim Busing,** *FEI*
"Beyond the frontiers of the cell: unveiling the wonders of nature at a molecular level"

12:40 - 13:30 Round table on entrepreneurship: from results to industry.

Members: **Dr. Stephanie Teughels** *(CEO at Pepric)*, **Dr. Rafael Ferritto** *(Co-founder and General Manager*

at NanoInnova Technologies), **Ms. Cecilia Hernández** *(Head of Department Health, Bioeconomy, Climate and Natural Resources, Centre for Industrial Technological Development)*; **Mr. Eduardo Díaz** *(Head of Unit New Technology Based Firms Unit, Fundación para el Conocimiento madri+d)*

Moderator: **Mr. Bonifacio Vega** *(Technology Transfer and Business Development Manager at IMDEA Nanociencia)*

13:30 - 15:00 LUNCH BREAK

15:00 - 16:40 Poster session

Chairperson: **Dr. Aitziber L. Cortajarena**, *IMDEA Nanociencia*

16:40 - 17:10 Invited speaker: **Dr Gray Kueberuwa**, *Institute of Cancer Sciences, University of Manchester*
"Chimeric Antigen Receptor T-cells for Cancer Therapy"

17:10 - 17:30 **Dr. Antonio Benayas,** *University of Quebec*
"At the Fluorescent Nanoprobes´ Frontiers: Multifunctionality for Bio-Imaging and T-Sensing"

17:30 - 17:50 **Dr. Ángel Millán,** *Instituto de Ciencias Materiales de Aragón*
"Joining time-resolved thermometry and magnetic-induced heating in a single nanoparticle"

20:30 NETWORKING DINNER

Wednesday 25 February

Session 3: NanoTherapy

Chairperson: **Dr. Mª del Puerto Morales**, *Instituto de Ciencia de Materiales de Madrid-CSIC*

09:30 - 10:15 Keynote speaker: **Prof. Ingrid Hilger**, *University Hospital Jena*
"Magnetic hyperthermia, a promising tool for the minimal-invasive treatment of tumors"

10:15 - 10:35 **Dr. A. Roig,** *Instituto de Ciencia de Materiales de Barcelona-CSIC*
"Encapsulation of VEGF165 in magnetic PLGA nanocapsules for potential local delivery and bioactivity into human brain endothelial cells"

10:35 - 10:55 **Mr. D. Cabrera,** *IMDEA Nanociencia*
"Influence of nanoparticle size and field frequency on the concentration dependence of magnetic heating"

10:55 - 11:15 **Ms. Blanca del Rosal,** *Universidad Autónoma de Madrid*
"Intratumoral thermal reading during photothermal therapy by multifunctional fluorescent nanoparticles"

11:15 - 11:35 **Prof. Nguyen TK Thanh,** *University College London*
"Magnetic nanoparticle-based therapeutic agents for thermo-chemotherapy treatment of cancer"

11.35 -12:00 COFFEE BREAK

Chairperson: **Dr. Sara Trabulo,** *Barts Cancer Institute*

12:00 - 12:20 **Dr. Cristina Fornaguera,** *Instituto de Química Avanzada de Cataluña-CSIC*
"Nano-emulsion templating: a versatile technology to prepare multifunctional polymeric nanoparticles for advanced biomedical applications"

12:20 - 12.40 **Dr. Anna Aviñó,** *Instituto de Química Avanzada de Cataluña-CSIC*
"G-quadruplex aptamers with therapeutic applications"

12:40 - 13:30 Round table on nanotherapy: small treats different.

Members: **Prof. Ingrid Hilger** *(University Hospital of Jena)*, **Dr. Domingo F. Barber** *(Centro Nacional de Biotecnología-CSIC)*, **Dr. Petra Gener** *(Hospital Vall d'Hebron)*

Moderator: **Dr. Aitziber L. Cortajarena**, *IMDEA Nanociencia*

13:30-15:00 LUNCH BREAK

Chairperson: **Dr. Pilar Calvo,** *Pharmamar*

15:00 - 15:30 Invited Speaker: **Dr. Petra Gener**, *Hospital Vall d'Hebron*
"Targeted Drug Delivery Systems against Cancer Stem Cells"

15:30 - 15:50 **Ms. Alicia Soler Cantón,** *Delft University of Technology*
"Development of gene-expressing liposomes as drug delivery systems"

15:50 - 16:10 **Dr. Laura Gallego-Yerga,** *Universidad de Sevilla*
"Self-assembling nanoparticles based on calixarene-cyclodextrin heterodimers for targeted delivery and controlled release of docetaxel"

16:10 - 16:30 **Mr. Gustavo da Silva,** *Instituto de Ciencia de Materiales de Madrid-CSIC*
"Design of biocompatible magnetic nanoplatforms for potential platinum-based drug delivery applications"

16:30 - 17:00 CLOSING REMARKS

ORAL contributions

Monday 23rd (morning session)

1. Nanodrugs Development and Applications to Pancreas Cancer, **Dr. Manuel Hidalgo**, *Centro Nacional de Investigaciones Oncológicas*

2. An overview of cancer and (drugabble-) Immune response, **Dr. José Antonio López,** *Instituto de Investigación Hospital 12 de Octubre*

3. Ageing and biotransformation of nanomagnets in the body: How to conciliate therapeutic efficiency and safe life-cycle, **Prof. Florence Gazeau,** *U. Paris Diderot-CNRS*

Monday 23rd (afternoon session)

4. Safety of Nanomaterials: Immunotoxicology and interaction between Nanomaterials and human proteins, **Prof. Africa González Fernández,** *Instituto de Investigaciones Biomédicas de Vigo (UVIGO)*

5. Effect of Polyethylenimine-coated SPIONS on macrophage activation and podosome dynamics, **Dr. Vladimir Mulens,** *Centro Nacional de Biotecnología-CSIC*

6. Tracking magnetic nanoparticles in tissues by AC magnetic susceptibility: Recent achievements and future challenges, **Dr. Lucía Gutiérrez,** *Instituto de Ciencia de Materiales de Madrid-CSIC*

7. Soft X-ray tomography as quantitative tool to decipher the interaction between DMSA-SPION and MCF7 cancer cells, **Dr. Francisco Javier Chichón,** *Centro Nacional de Biotecnología-CSIC*

8. Multiparamagnetic High Throughput Methods for Magnetic Iron Oxide Nanoparticle Lead Selection, **Mr. K. Crosbie-Staunton,** *Trinity College Dublin*

Tuesday 24th (morning session)

9. Tracer development critical for translational medical applications of Magnetic Particle Imaging, **Prof. Kannan Krishnan,** *University of Washington*

10. Early detection of Pancreatic Cancers & Brain Tumors by MR Molecular Imaging, **Prof. Yung-Ya Lin,** *University California, Los Angeles*

11. Parallel multifuncionalisation of nanoparticles: A One-Step Modular Approach for *in vivo* Imaging, **Dr. Fernando Herranz,** *Centro Nacional de Investigaciones Cardiovasculares*

12. Improving magnetic properties of ultrasmall magnetic nanoparticles by biocompatible coatings, **Dr. Rocío Costo,** *Instituto de Ciencia de Materiales de Madrid-CSIC*

13. Synthesis and characterization of bimetallic magnetic oxides obtained by oxidative precipitation, **Ms. Yurena Luengo,** *Instituto de Ciencia de Materiales de Madrid-CSIC*

14. Coating strategies for the application of magnetic nanoparticles in cancer diagnosis and therapy, **Dr. Marzia Marciello,** *Instituto de Ciencia de Materiales de Madrid-CSIC*

15. Beyond the frontiers of the cell: unveiling the wonders of nature at molecular level, **Dr. Wim Busing,** *FEI*

Tuesday 24th (afternoon session)

16. Chimeric Antigen Receptor T-cells for Cancer Therapy, **Dr. Gray Kueberuwa,** *Institute of Cancer Sciences, University of Manchester*

17. At the Fluorescent Nanoprobes' Frontiers: Multifuncionality for Bio-Imaging and T-sensing, **Dr. Antonio Benayas,** *University of Quebec*

18. Joining time-resolved thermometry and magnetic-induced heating in a single nanoparticle, **Dr. Ángel Millán,** *Instituto de Ciencias de Aragón*

Wednesday 25th (morning session)

19. Magnetic hyperthermia, a promising tool for the minimal-invasive treatment of tumors, **Prof. Ingrid Hilger,** *University Hospital Jena*

20. Encapsulation of VEGF165 in magnetic PLGA nanocapsules for potential local delivery and bioactivity into human brain endothelial cells, **Dr. A. Roig,** *Instituto de Ciencia de Materiales de Barcelona-CSIC*

21. Influence of nanoparticle size and field frequency on the concentration dependence of magnetic heating, Mr. David Cabrera, Fundación IMDEA Nanociencia

22. Intratumoral thermal reading during photothermal therapy by multifunctional fluorescent nanoparticles, **Ms. Blanca del Rosal,** *Universidad Autónoma de Madrid*

23. Magnetic nanoparticle-based therapeutic agents for thermo-chemotherapy treatment of cancer, **Prof. Nguyen TK Thanh,** *University College London*

24. Nano-emulsion templating: a versatile technology to prepare multifunctional polymeric nanoparticles for advanced biomedical applications, **Dr. Cristina Fornaguera,** *Instituto de Química Avanzada de Cataluña-CSIC*

25. G-quadruplex aptamers with therapeutic applications, **Dr. Anna Aviñó,** *Instituto de Química Avanzada de Cataluña-CSIC*

Wednesday 25th (afternoon session)

26. Targeted Drug Delivery Systems against Cancer Stem Cells, **Dr. Petra Gener,** *Hospital Vall d'Hebron*

27. Development of gene-expressing liposomes as drug delivery systems, **Ms. Alicia Soler Cantón,** *Delft University of Technology*

28. Self-assembling nanoparticles based on calixarene-cyclodextrin heterodimers for targeted delivery and controlled release of docetaxel, **Dr. Laura Gallego Yerga,** *Universidad de Sevilla*

29. Design of biocompatible magnetic nanoplatforms for potential platinum-based drug delivery applications, **Mr. Gustavo da Silva,** *Instituto de Ciencia de Materiales de Madrid-CSIC*

POSTER contributions

23rd and 24th afternoon session

P1. Interaction of multifunctional magnetic nanoparticles with biological membranes, **Ms. María José Rodríguez,** *Centro Nacional de Biotecnología-CSIC*

P2. Biodistribution of magnetic nanoparticles evaluated by AC magnetic susceptibility in a murine model, **Mr. Eduardo Lorente-Sorolla,** *Instituto de Ciencia de Materiales-CSIC*

P3. *C. elegans*: an *in vivo* model to evaluate nanoparticles?, **Dr. A. Roig,** *Instituto de Ciencia de Materiales de Barcelona-CSIC*

P4. Iron quantification inside cells incubated with SPION by Soft-Xray Absorption Spectro-Tomography (SXAST), **Mr. José Javier Conesa,** *Centro Nacional de Biotecnología-CSIC*

P5. Following the transformation of magnetic nanoparticles over time in macrophages by AC magnetic susceptibility, **Ms. Vanesa del Dedo,** *Instituto de Ciencia de Materiales de Madrid-CSIC*

P6. pH-Dependent Switch for Anticancer Metallodrugs, **Mr. Francisco Martínez,** *Fundación IMDEA Nanociencia*

P7. Superparamagnetic properties of hydrothermally prepared $CoFe_2O_4$ as a function of size (6–10 nm) and coating (oleic/citric acid or TiO_2), **Dr. Daniel Nižnanský,** *Charles University in Prague*

P8. Targeting cancer cells with photoactive silica nanoparticles, **Ms. Wioleta Borzecka,** *University of Aveiro*

P9. Combinational sensitization of tumor cells with two photosensitizers synergistically enhances their photodynamic inactivation *in vitro*, **Ms. Andrea Tabero,** *Universidad Autónoma de Madrid*

P10. Synthesis Strategies of Single-Core Magnetic Nanoparticles, **Ms. Helena Gavilán,** *Instituto de Ciencia de Materiales de Madrid-CSIC*

P11. Silica encapsulation of magnetic nanoparticles, **Ms. Leonor de la Cueva,** *Fundación IMDEA Nanociencia*

P12. Synthesis of hybrid magneto-plasmonic nanostructures based on Au nanorods and iron oxides nanoparticles, **Mr. Jesús G. Ovejero,** *Universidad Complutense de Madrid*

P13. Functionalized magnetic nanoparticles for cancer therapy, **Dr. Antonio Aires,** *Fundación IMDEA Nanociencia*

P14. Detection and Inhibition of Mutated GNAQ Gene using Spherical Nucleic Acids, **Ms. Ana Latorre,** *Fundación IMDEA Nanociencia*

Workshop highlights

Nowadays there is a significant gap between Nanomedicine research and the clinical practice. The **Final Multifun Workshop** attempted to overcome this gap, and leveraged the translation from the laboratory to the hospital.

The main aim of this workshop was to create a suitable environment to favour interactions and discussion among researchers, medical doctors, engineers, entrepreneurs and stakeholders from different disciplines. Thereby, the above reported different sessions, roundtables and key speakers allowed sharing, experience and fostering collaborations across the different disciplines that the nanomedicine research field entailed.

The workshop involved outstanding international speakers and stakeholders in Nanomedicine and Oncology, public authorities and researchers around Europe. The programme included comprehensive talks, special sessions in clinical translation and entrepreneurship, debates, and oral and poster presentations (of MULTIFUN researchers and researchers outside the consortium) of peer-reviewed contributions.

Oral and Poster contribution details can be found in purposely prepared and publicly available final MULTIFUN workshop available online at the MULTIFUN project website. For convenience we have also attached this as appendix at the end of the document.

Online Web address: http://www.multifun-project.eu/content/final-multifun-2015-workshop

Final MULTIFUN workshop: example of industry driven round table discussion around the technological development of Nanomedicine-based products

Workshop organising committee

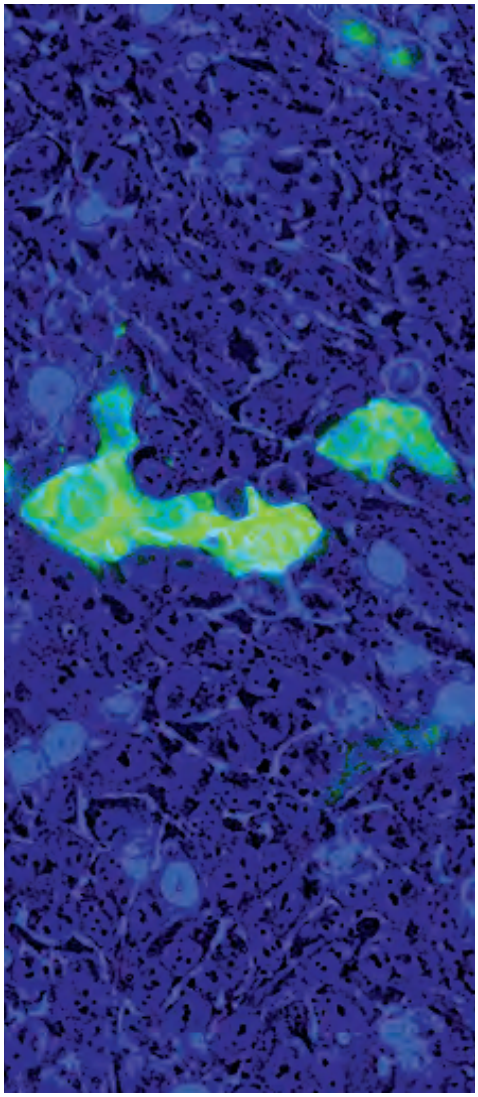
organizing committee

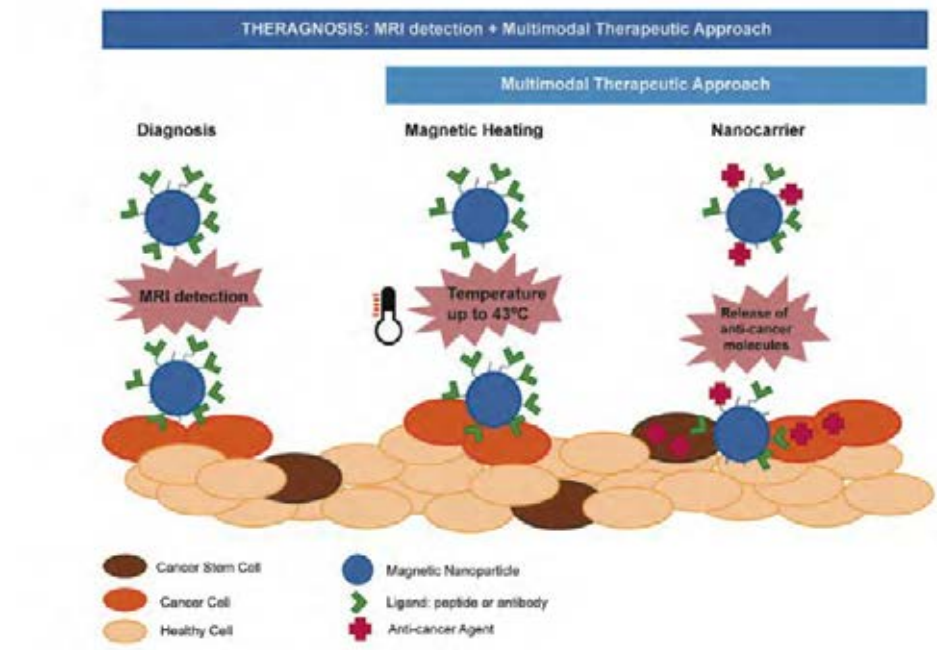
Chairperson:

Prof. Rodolfo Miranda *(IMDEA Nanociencia)*
Dr. Francisco J. Terán *(IMDEA Nanociencia)*
Dr. Adriele Prina Mello *(TCD-CRANN)*
Dr. César Mediavilla *(ATOS)*

advisory board committee

Prof. Rene Botnar *(KCL)*
Dr. Pilar Calvo *(Pharmamar)*
Dr. Robert Clarke *(UNIMAN)*
Dr. Jose Courty *(CRRET)*
Dr. Domingo F. Barber *(CSIC-CNB)*
Prof. Christopher Heeschen *(QMUL)*
Dr. Aitziber L. Cortajarena *(IMDEA Nanociencia)*
Dr. Farouk Markos *(UCC)*
Dr. Mª del Puerto Morales *(CSIC-ICMM)*
Dr. Gorka Salas *(IMDEA Nanociencia)*
Prof. Yuri Volkov *(TCD-CRANN)*





Public project website: http://www.multifun-project.eu/content/public-results

Acknowledgements

The Project Coordinator and Scientific Coordination committee would like to acknowledge the efforts of all parnters involved in the MULTIFUN consortium.

Special thanks to the workshop organising committees of the three events and participating partners for their dedication and hard work at the core activities of the MULTIFUN project.

We express our sincere gratitude to the MULTIFUN Dissemination committee for their work.

We would also like to personally thank the TCD NANOMEDICINE team involved in MULTIFUN project and Dr. Darragh Crotty from TCD for the work and dedication to produce this booklet.

Funding

This work was supported by the European Commission under the Seventh Framework Programme focused on Nanosciences, Nanotechnologies, Materials and New Production Technologies (NMP), as the MULTIFUN project (FP7 NMP4-LA-2011-ref. 262943).

Appendix 1 – Final Multifun workshop 2015, complete booklet

Online Web address: http://www.multifun-project.eu/content/final-multifun-2015-workshop

welcome

#multifunworkshop

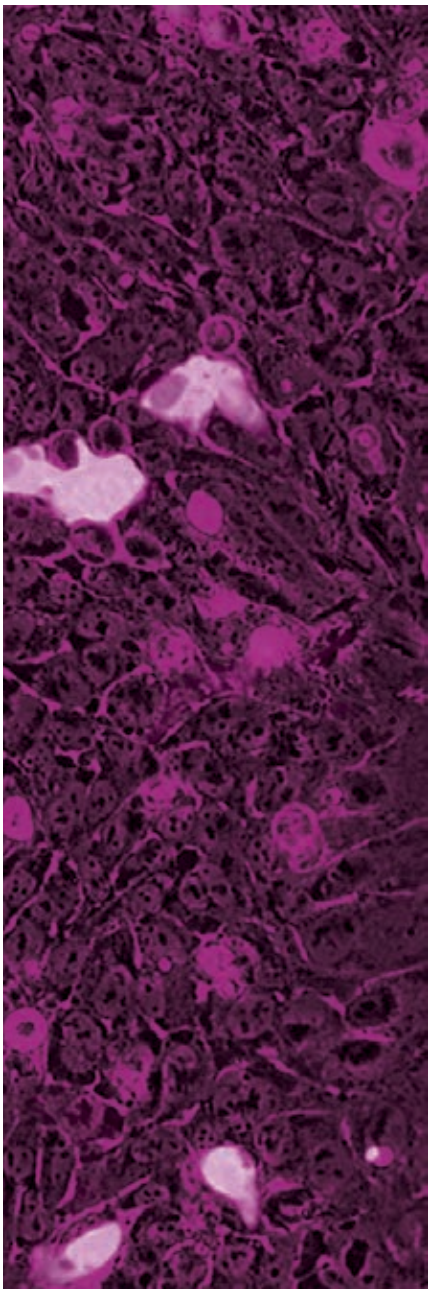
During the last four years the MULTIFUN consortium has focused its activity on the development and validation of new systems based upon minimal invasive nanotechnology for the early and selective detection and elimination of breast and pancreatic cancer. The project has successfully produced multifuntionalised magnetic iron oxide nanoparticles (MNP) that combine diagnostic and therapeutic features against these two types of cancer.

The therapeutic approach developed within the project includes a synergistic effect between the therapeutic effect produced by magnetic hyperthermia and that due to the intracellular drug delivery selectively targeted to tumour cells.

Some of the designed formulations have proven their efficiency, safety and non-toxicicty in *in vivo* models, making them promising candidates to produce new nanomedicines against breast and pancreatic cancer.

The Madrid Institute for Advanced Studies in Nanoscience (IMDEA Nanociencia) is responsible of the scientific coordination of the project. Atos Spain S.A is the Administrative Coordinator and Contract Manager.

Prof. Rodolfo Miranda,
MULTIFUN Scientific Coordinator & IMDEA Nanociencia Director



organizing committee

Chairperson:

Prof. Rodolfo Miranda *(IMDEA Nanociencia)*
Dr. Francisco J. Terán *(IMDEA Nanociencia)*
Dr. Adriele Prina Mello *(TCD-CRANN)*
Dr. César Mediavilla *(ATOS)*

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Dr. Farouk Markos *(UCC)*
Dr. Mª del Puerto Morales *(CSIC-ICMM)*
Dr. Gorka Salas *(IMDEA Nanociencia)*
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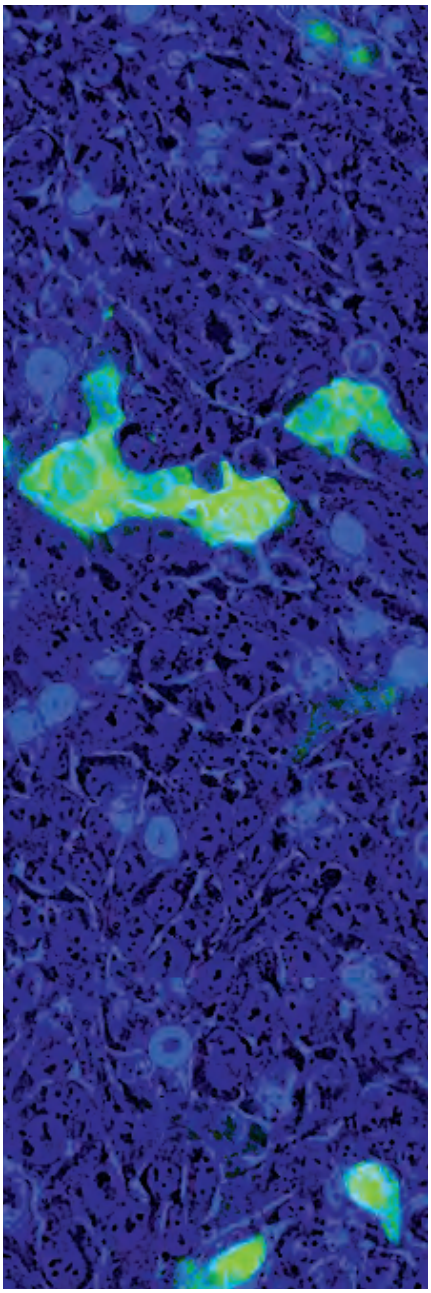

Monday 23 February

08.30 - 09.30 Registration

09.30 - 10:00 Opening session

Prof. Rodolfo Miranda, *(MULTIFUN Scientific Coordinator & IMDEA Nanociencia Director)*

Session 1: Introduction and Perspectives: Cancer Science

Chairperson: **Prof. Rodolfo Miranda**, *IMDEA Nanociencia*

10:00 - 10:45 Keynote speaker:
Dr. Manuel Hidalgo, *Centro Nacional de Investigaciones Oncológicas*
"Nanodrugs: Development and Applications to Pancreas Cancer Treatment"

10:45 - 11:30 Keynote speaker:
Dr. José Antonio López, *Instituto de Investigación Hospital 12 de Octubre*
"An overview of cancer and (druggable-) immune response"

11:30 - 12:00 COFFEE BREAK

Session 2: Nanotoxicity, Biodistribution, Biodegradation

Chairperson: **Prof. Yuri Volkov**, *Centre for Research on Adaptive Nanostructures, Trinity College Dublin*

12:00 - 12:40 Keynote speaker: **Prof. Florence Gazeau**, *Université Paris-Diderot-CNRS*
"Ageing and biotransformation of nanomagnets in the body: How to conciliate therapeutic efficiency and safe life-cycle?"

12:40 - 13:30 Round table on regulation for manufacturing and biomedical applications of nanomaterials: safety on nanohandling.

Members: **Prof. África González-Fernández** *Instituto de Investigaciones Biomédicas de Vigo - Universidad de Vigo*, **Prof. Florence Gazeau** *Université Paris-Diderot-CNRS,* **Prof. Yuri Volkov** *Trinity College Dublin*

Moderator: **Dr. Pilar Calvo** *Pharmamar*

13:30 - 15:00 LUNCH BREAK

15:00 - 16:40 Poster session

Chairperson: **Dr. Domingo F. Barber**, *Centro Nacional de Biotecnología-CSIC*

16:40 - 17:10 Invited speaker: **Prof. África González-Fernández**, *(Instituto de Investigaciones Biomédicas de Vigo - Universidad de Vigo)*
"Safety of nanomaterials: Immunotoxicology and interaction between Nanomaterials and human proteins"

17:10 - 17:30 **Mr. Vladimir Mulens,** *Centro Nacional de Biotecnología-CSIC*
"Effect of Polyethylenimine-coated SPIONs on macrophage activation and podosome dynamics"

17:30 - 17:50 **Dr. Lucía Gutiérrez,** *Instituto de Ciencia de Materiales de Madrid-CSIC*
"Tracking magnetic nanoparticles in tissues by AC magnetic susceptibility: Recent achievements and future challenges"

17:50 - 18:10 **Dr. Francisco Javier Chichón,** *Centro Nacional de Biotecnología-CSIC*
"Soft X-ray tomography as quantitative tool to decipher the interaction between DMSA-SPION and MCF7 cancer cells"

18:10 - 18:30 **Mr. K. Crosbie-Staunton,** *Trinity College Dublin*
"Multiparametric High Throughput Methods for Magnetic Iron Oxide Nanoparticle Lead Selection"

Tuesday 24 February

Session 3: NanoDiagnostic

Chairperson: **Prof. René Botnar**, *King's College London*

09:30 - 10:15 Keynote speaker: **Prof. Kannan Krishnan**, *University of Washington*
"Tracer development critical for translational medical applications of Magnetic Particle Imaging"

10:15 - 10:35 **Prof. Yung-Ya Lin,** *University California, Los Angeles*
"Early Detection of Pancreatic Cancers & Brain Tumors by MR Molecular Imaging"

10:35 - 10:55 **Dr. F. Herranz,** *Centro Nacional de Investigaciones Cardiovasculares*
"Parallel multifunctionalisation of Nanoparticles: A One-Step Modular Approach for *in vivo* Imaging"

10:55 - 11:15 **Dr. Rocío Costo,** *Instituto de Ciencia de Materiales de Madrid-CSIC*
"Improving magnetic properties of ultrasmall magnetic nanoparticles by biocompatible coatings"

10:15 - 11:30 **Ms. Yurena Luengo,** *Instituto de Ciencia de Materiales de Madrid-CSIC*
"Synthesis and characterization of bimetallic magnetic oxides obtained by oxidative precipitation"

11:30 - 12:00 COFFEE BREAK

Chairperson: **Dr. Jose Courty**, *CRRET-CNRS*

12:00 - 12:20 **Dr. Marzia Marciello,** *Instituto de Ciencia de Materiales de Madrid-CSIC*
"Coating strategies for the application of magnetic nanoparticles in cancer diagnosis and therapy"

12:20 - 12:40 **Dr. Wim Busing,** *FEI*
"Beyond the frontiers of the cell: unveiling the wonders of nature at a molecular level"

12:40 - 13:30 Round table on entrepreneurship: from results to industry.

Members: **Dr. Stephanie Teughels** *(CEO at Pepric)*, **Dr. Rafael Ferritto** *(Co-founder and General Manager*

at NanoInnova Technologies), **Ms. Cecilia Hernández** *(Head of Department Health, Bioeconomy, Climate and Natural Resources, Centre for Industrial Technological Development)*; **Mr. Eduardo Díaz** *(Head of Unit New Technology Based Firms Unit, Fundación para el Conocimiento madri+d)*

Moderator: **Mr. Bonifacio Vega** *(Technology Transfer and Business Development Manager at IMDEA Nanociencia)*

13:30 - 15:00 LUNCH BREAK

15:00 - 16:40 Poster session

Chairperson: **Dr. Aitziber L. Cortajarena**, *IMDEA Nanociencia*

16:40 - 17:10 Invited speaker: **Dr Gray Kueberuwa**, *Institute of Cancer Sciences, University of Manchester*
"Chimeric Antigen Receptor T-cells for Cancer Therapy"

17:10 - 17:30 **Dr. Antonio Benayas,** *University of Quebec*
"At the Fluorescent Nanoprobes´ Frontiers: Multifunctionality for Bio-Imaging and T-Sensing"

17:30 - 17:50 **Dr. Ángel Millán,** *Instituto de Ciencias Materiales de Aragón*
"Joining time-resolved thermometry and magnetic-induced heating in a single nanoparticle"

20:30 NETWORKING DINNER

Wednesday 25 February

Session 3: NanoTherapy

Chairperson: **Dr. Mª del Puerto Morales**, *Instituto de Ciencia de Materiales de Madrid-CSIC*

09:30 - 10:15 Keynote speaker: **Prof. Ingrid Hilger**, *University Hospital Jena*
"Magnetic hyperthermia, a promising tool for the minimal-invasive treatment of tumors"

10:15 - 10:35 **Dr. A. Roig,** *Instituto de Ciencia de Materiales de Barcelona-CSIC*
"Encapsulation of VEGF165 in magnetic PLGA nanocapsules for potential local delivery and bioactivity into human brain endothelial cells"

10:35 - 10:55 **Mr. D. Cabrera,** *IMDEA Nanociencia*
"Influence of nanoparticle size and field frequency on the concentration dependence of magnetic heating"

10:55 - 11:15 **Ms. Blanca del Rosal,** *Universidad Autónoma de Madrid*
"Intratumoral thermal reading during photothermal therapy by multifunctional fluorescent nanoparticles"

11:15 - 11:35 **Prof. Nguyen TK Thanh,** *University College London*
"Magnetic nanoparticle-based therapeutic agents for thermo-chemotherapy treatment of cancer"

11.35 -12:00 COFFEE BREAK

Chairperson: **Dr. Sara Trabulo,** *Barts Cancer Institute*

12:00 - 12:20 **Dr. Cristina Fornaguera,** *Instituto de Química Avanzada de Cataluña-CSIC*
"Nano-emulsion templating: a versatile technology to prepare multifunctional polymeric nanoparticles for advanced biomedical applications"

12:20 - 12.40 **Dr. Anna Aviñó,** *Instituto de Química Avanzada de Cataluña-CSIC*
"G-quadruplex aptamers with therapeutic applications"

12:40 - 13:30 Round table on nanotherapy: small treats different.

Members: **Prof. Ingrid Hilger** *(University Hospital of Jena)*, **Dr. Domingo F. Barber** *(Centro Nacional de Biotecnología-CSIC)*, **Dr. Petra Gener** *(Hospital Vall d'Hebron)*

Moderator: **Dr. Aitziber L. Cortajarena**, *IMDEA Nanociencia*

13:30-15:00 LUNCH BREAK

Chairperson: **Dr. Pilar Calvo,** *Pharmamar*

15:00 - 15:30 Invited Speaker: **Dr. Petra Gener**, *Hospital Vall d'Hebron*
"Targeted Drug Delivery Systems against Cancer Stem Cells"

15:30 - 15:50 **Ms. Alicia Soler Cantón,** *Delft University of Technology*
"Development of gene-expressing liposomes as drug delivery systems"

15:50 - 16:10 **Dr. Laura Gallego-Yerga,** *Universidad de Sevilla*
"Self-assembling nanoparticles based on calixarene-cyclodextrin heterodimers for targeted delivery and controlled release of docetaxel"

16:10 - 16:30 **Mr. Gustavo da Silva,** *Instituto de Ciencia de Materiales de Madrid-CSIC*
"Design of biocompatible magnetic nanoplatforms for potential platinum-based drug delivery applications"

16:30 - 17:00 CLOSING REMARKS

ORAL contributions

Monday 23rd (morning session)

1. Nanodrugs Development and Applications to Pancreas Cancer, **Dr. Manuel Hidalgo**, *Centro Nacional de Investigaciones Oncológicas*

2. An overview of cancer and (drugabble-) Immune response, **Dr. José Antonio López,** *Instituto de Investigación Hospital 12 de Octubre*

3. Ageing and biotransformation of nanomagnets in the body: How to conciliate therapeutic efficiency and safe life-cycle, **Prof. Florence Gazeau,** *U. Paris Diderot-CNRS*

Monday 23rd (afternoon session)

4. Safety of Nanomaterials: Immunotoxicology and interaction between Nanomaterials and human proteins, **Prof. Africa González Fernández,** *Instituto de Investigaciones Biomédicas de Vigo (UVIGO)*

5. Effect of Polyethylenimine-coated SPIONS on macrophage activation and podosome dynamics, **Dr. Vladimir Mulens,** *Centro Nacional de Biotecnología-CSIC*

6. Tracking magnetic nanoparticles in tissues by AC magnetic susceptibility: Recent achievements and future challenges, **Dr. Lucía Gutiérrez,** *Instituto de Ciencia de Materiales de Madrid-CSIC*

7. Soft X-ray tomography as quantitative tool to decipher the interaction between DMSA-SPION and MCF7 cancer cells, **Dr. Francisco Javier Chichón,** *Centro Nacional de Biotecnología-CSIC*

8. Multiparamagnetic High Throughput Methods for Magnetic Iron Oxide Nanoparticle Lead Selection, **Mr. K. Crosbie-Staunton,** *Trinity College Dublin*

Tuesday 24th (morning session)

9. Tracer development critical for translational medical applications of Magnetic Particle Imaging, **Prof. Kannan Krishnan,** *University of Washington*

10. Early detection of Pancreatic Cancers & Brain Tumors by MR Molecular Imaging, **Prof. Yung-Ya Lin,** *University California, Los Angeles*

11. Parallel multifuncionalisation of nanoparticles: A One-Step Modular Approach for *in vivo* Imaging, **Dr. Fernando Herranz,** *Centro Nacional de Investigaciones Cardiovasculares*

12. Improving magnetic properties of ultrasmall magnetic nanoparticles by biocompatible coatings, **Dr. Rocío Costo,** *Instituto de Ciencia de Materiales de Madrid-CSIC*

13. Synthesis and characterization of bimetallic magnetic oxides obtained by oxidative precipitation, **Ms. Yurena Luengo,** *Instituto de Ciencia de Materiales de Madrid-CSIC*

14. Coating strategies for the application of magnetic nanoparticles in cancer diagnosis and therapy, **Dr. Marzia Marciello,** *Instituto de Ciencia de Materiales de Madrid-CSIC*

15. Beyond the frontiers of the cell: unveiling the wonders of nature at molecular level, **Dr. Wim Busing,** *FEI*

Tuesday 24th (afternoon session)

16. Chimeric Antigen Receptor T-cells for Cancer Therapy, **Dr. Gray Kueberuwa,** *Institute of Cancer Sciences, University of Manchester*

17. At the Fluorescent Nanoprobes' Frontiers: Multifuncionality for Bio-Imaging and T-sensing, **Dr. Antonio Benayas,** *University of Quebec*

18. Joining time-resolved thermometry and magnetic-induced heating in a single nanoparticle, **Dr. Ángel Millán,** *Instituto de Ciencias de Aragón*

Wednesday 25th (morning session)

19. Magnetic hyperthermia, a promising tool for the minimal-invasive treatment of tumors, **Prof. Ingrid Hilger,** *University Hospital Jena*

20. Encapsulation of VEGF165 in magnetic PLGA nanocapsules for potential local delivery and bioactivity into human brain endothelial cells, **Dr. A. Roig,** *Instituto de Ciencia de Materiales de Barcelona-CSIC*

21. Influence of nanoparticle size and field frequency on the concentration dependence of magnetic heating, Mr. David Cabrera, Fundación IMDEA Nanociencia

22. Intratumoral thermal reading during photothermal therapy by multifunctional fluorescent nanoparticles, **Ms. Blanca del Rosal,** *Universidad Autónoma de Madrid*

23. Magnetic nanoparticle-based therapeutic agents for thermo-chemotherapy treatment of cancer, **Prof. Nguyen TK Thanh,** *University College London*

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25. G-quadruplex aptamers with therapeutic applications, **Dr. Anna Aviñó,** *Instituto de Química Avanzada de Cataluña-CSIC*

Wednesday 25th (afternoon session)

26. Targeted Drug Delivery Systems against Cancer Stem Cells, **Dr. Petra Gener,** *Hospital Vall d'Hebron*

27. Development of gene-expressing liposomes as drug delivery systems, **Ms. Alicia Soler Cantón,** *Delft University of Technology*

28. Self-assembling nanoparticles based on calixarene-cyclodextrin heterodimers for targeted delivery and controlled release of docetaxel, **Dr. Laura Gallego Yerga,** *Universidad de Sevilla*

29. Design of biocompatible magnetic nanoplatforms for potential platinum-based drug delivery applications, **Mr. Gustavo da Silva,** *Instituto de Ciencia de Materiales de Madrid-CSIC*

POSTER contributions

23rd and 24th afternoon session

P1. Interaction of multifunctional magnetic nanoparticles with biological membranes, **Ms. María José Rodríguez,** *Centro Nacional de Biotecnología-CSIC*

P2. Biodistribution of magnetic nanoparticles evaluated by AC magnetic susceptibility in a murine model, **Mr. Eduardo Lorente-Sorolla,** *Instituto de Ciencia de Materiales-CSIC*

P3. *C. elegans*: an *in vivo* model to evaluate nanoparticles?, **Dr. A. Roig,** *Instituto de Ciencia de Materiales de Barcelona-CSIC*

P4. Iron quantification inside cells incubated with SPION by Soft-Xray Absorption Spectro-Tomography (SXAST), **Mr. José Javier Conesa,** *Centro Nacional de Biotecnología-CSIC*

P5. Following the transformation of magnetic nanoparticles over time in macrophages by AC magnetic susceptibility, **Ms. Vanesa del Dedo,** *Instituto de Ciencia de Materiales de Madrid-CSIC*

P6. pH-Dependent Switch for Anticancer Metallodrugs, **Mr. Francisco Martínez,** *Fundación IMDEA Nanociencia*

P7. Superparamagnetic properties of hydrothermally prepared $CoFe_2O_4$ as a function of size (6–10 nm) and coating (oleic/citric acid or TiO_2), **Dr. Daniel Nižnanský,** *Charles University in Prague*

P8. Targeting cancer cells with photoactive silica nanoparticles, **Ms. Wioleta Borzecka,** *University of Aveiro*

P9. Combinational sensitization of tumor cells with two photosensitizers synergistically enhances their photodynamic inactivation *in vitro*, **Ms. Andrea Tabero,** *Universidad Autónoma de Madrid*

P10. Synthesis Strategies of Single-Core Magnetic Nanoparticles, **Ms. Helena Gavilán,** *Instituto de Ciencia de Materiales de Madrid-CSIC*

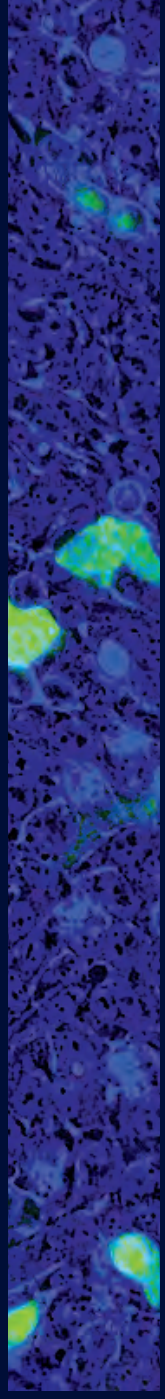
P11. Silica encapsulation of magnetic nanoparticles, **Ms. Leonor de la Cueva,** *Fundación IMDEA Nanociencia*

P12. Synthesis of hybrid magneto-plasmonic nanostructures based on Au nanorods and iron oxides nanoparticles, **Mr. Jesús G. Ovejero,** *Universidad Complutense de Madrid*

P13. Functionalized magnetic nanoparticles for cancer therapy, **Dr. Antonio Aires,** *Fundación IMDEA Nanociencia*

P14. Detection and Inhibition of Mutated GNAQ Gene using Spherical Nucleic Acids, **Ms. Ana Latorre,** *Fundación IMDEA Nanociencia*

ORAL contribution



2.

An overview of cancer and (druggable-) immune response

Dr. José Antonio López
Translational Oncology Group co-Director,`12 de Octubre' Research Institute Attending Physician, '12 de Octubre' University Hospital

Abstract: The immune system has a great potential for the specific destruction of tumours and for long-term memory that can prevent cancer recurrence. The probability of an effective tumour immune response depends on 1- the existence of tumour antigens and 2- the possibility of modulating the mechanisms of suppression of tumour immune response.

Tumour escape from immunosurveillance be achieved either by changes in the tumour cells (loss of tumour antigens, loss of human leukocyte antigen molecules, loss of sensitivity to complement, or T cell or natural killer (NK) cell lysis) or by tumour subvertion of the normal immune regulation. Tumour immunity can be enhanced by blocking inhibitory pathways and inhibitory cells in the tumour microenvironment, or by increasing the specificity of antitumour immunity, inducing the expansion of T cells and antibodies directed to well-defined tumour antigens (e.g. cancer vaccines, potent adjuvants, immunostimulatory cytokines). These strategies can be pursued either alone or as part of a multimodal therapy.

3.

Ageing and biotransformation of nanomagnets in the body: How to conciliate therapeutic efficiency and safe life-cycle?

Florence Gazeau
Matière et Systèmes Complexes, CNRS / Université Paris Diderot
75205 Paris Cedex 13, FRANCE
Florence.gazeau@univ-paris-diderot.fr

The increasing exposure of living organism to nanoparticles calls for a comprehensive assessment of the processing of nanomaterials *in vivo*. Understanding nanoparticles distribution, transformations, persistence, ageing and degradation will help to predict exposure risks as

well as therapeutic efficiency of nanoparticles. The challenge is to establish how the synthetic identity of nanoparticles can determine their whole life cycle in the body.

We have developped a multiscale methodology to examine the influence of biological environment on the structure and physical properties of magnetic nanoparticles that show outstanding properties for magnetothermal therapy and MRI detection.

Our approach, which is based on materials sciences, is combining nanoscale electron microscopy observations of nanoparticle with the follow-up of magnetic properties in biological environments. From several examples of magnetic nanostructures – iron oxide nanospheres, nanocubes, cooperative nanoflowers and iron oxide/gold dimers with different coating – we will examine how cell-induced transformations critically alter the nanoparticles magnetic properties, heating power and Magnetic Resonance relaxivity over time and determine the long term fate of the nanoparticles in the organism. In the research for safe and efficient nanoparticles for nanomedecine, one should control the balance between short term efficacy in the relevant biological context and long term degradability and clearance from the body.

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3. Javed Y, Lartigue L,Hugounenq P, Vuong QL, Gossuin Y, Bazzi R, Wilhelm C, Ricolleau C, Gazeau F*, Alloyeau A*. Biodegradation mechanism of iron oxide monocrystalline nanoflowers and tunable shield effect of gold coating, *Small*, 2014 Aug 27;10(16):3325-37

4.

Safety of nanomaterials: Immunotoxicology and interaction between Nanomaterials and human proteins.

Prof. África González-Fernández
MD, PhD, Immunologist
Professor of Immunology
Director of the Biomedical Research Center (CINBIO)
Coordinator of BIOCAPS (EU 7º FW program)

UNIVERSITY OF VIGO
Campus Lagoas Marcosende
36310 Vigo, Pontevedra
Spain
phone: +34-986812625
fax: +34-986812556
email: dircinbio@uvigo.es; africa@uvigo.es

The use of nanomaterials (NMs) is increasing in the last years because of their potential to improve a wide range of applications, especially in the biomedical field. However, NMs can interact with the body inducing undesirable or desirable effects. It is crucial to understand their potential harmful effects, and the success of the NMs on biomedical applications will partially depend on their interactions with blood and immune components. The immune system has the role, between other functions, of defending the body from foreign materials. The study of the interaction between the components of the immune system and NMs is, therefore, an area of great interest. Moreover, NMs can be used in biomedicine to target different cells to modulate their function towards a desired response, for example in vaccines. The talk will focus on how the interactions between NMs and biological systems could affect to the normal function of these systems.

5.

Effect of Polyethylenimine-coated SPIONs on macrophage activation and podosome dynamics

Authors: **Vladimir Mulens[1], José Manuel Rojas[1], Sonia Pérez-Yagüe[1], María del Puerto Morales[2], Domingo F. Barber[1*]**

1. *Department of Immunology and Oncology and NanoBiomedicine Initiative, Centro Nacional de Biotecnología (CNB)/CSIC, Darwin 3, Cantoblanco, 28049 Madrid, Spain*
2. *Department of Biomaterials and Bioinspired Materials, Instituto de Ciencia de Materiales de Madrid (ICMM)/CSIC, Sor Juana Inés de la Cruz 3, Cantoblanco, 28049 Madrid, Spain*

Mulens V. email address: vmulens@cnb.csic.es

Huge efforts have been made to develop multifunctional platforms for cancer gene therapy. One of the current strategies is the combination of SPIONs (superparamagnetic iron oxide nanoparticles) and polyethylenimine (PEI), due to their unique chemical and physical properties. PEI appears to activate different immune cells toward an inflammatory response (M1/T_H1), whereas the response induced by SPIONs seems to be controversial. Nonetheless,

the immunoreactivity of PEI-coated SPIONs has not been addressed so far. In the present study, we proved that PEI-coated SPIONs (PMag) induce an M1 phenotype. PMag induced phosphorylation of p38 MAPK, p44/p42 MAPK and JNK, and upregulation of CD40, CD80, CD86 and I-A/I-E activation markers. Importantly, PMag-induced macrophage activation depended partially on TLR4 (Toll-like receptor 4) and ROS (reactive oxygen species) signaling, and was different from that induced by LPS. Intriguingly, PMag promoted podosome formation in murine macrophages, but inhibited gelatin degradation. This effect seems to be due to the overexpression of genes related with MMP inhibitors and downmodulation of active MMP-2. In conclusion, PMag induced an M1-like phenotype that was partially dependent on both TLR4 and ROS, and modulated the dynamics of podosomes.

6.

Tracking magnetic nanoparticles in tissues by AC magnetic susceptibility: Recent achievements and future challenges

Lucía Gutiérrez[a], Raquel Mejías[b,c], Domingo F. Barber[b], Francisco J. Lázaro[d] and M. Puerto Morales[a]

a. *Dept. Biomaterials and Bioinspired Materials, Instituto de Ciencia de Materiales de Madrid (ICMM)/CSIC, Sor Juana Inés de la Cruz 3, Cantoblanco, 28049 Madrid, Spain*
b. *Dept. Immunology and Oncology, and NanoBiomedicine Initiative, Centro Nacional de Biotecnología (CNB)/CSIC, Darwin 3, Cantoblanco, 28049 Madrid, Spain*
c. *Department of Biomedical Engineering, Vanderbilt University, 5824 Stevenson Center, Nashville, Tennessee 37235-1631, USA*
d. *Dept. de Ciencia y Tecnología de Materiales y Fluidos, Universidad de Zaragoza, Maria de Luna 3, 50018, Zaragoza, Spain*

lucia@icmm.csic.es

The quantitative determination of magnetic nanoparticles in tissue samples from animal models using magnetic measurements is helpful but not free of difficulties. Once the particles enter the body, they become surrounded by a wide variety of proteins and other molecules, altering their environment, possibly their degree of aggregation, and therefore the magnetic interparticle interactions. Also, due to ultrastructural differences, each organ may present a characteristic way of packing the particles forfurther processing that can also result in an alteration of the magnetic properties of the material. Furthermore, these systems suffer a continuous transformation process, where degradation of the particles also affects the magnetic properties of the whole tissue. Due to these complex factors, the comparison

between the magnetic properties of the administered compound and those of the tissue samples containing the particles is not always straightforward.

Based on AC magnetic susceptibility measurements of freeze-dried tissue samples, we have developed a protocol to quantify magnetic nanoparticles in animal organs [1]. This characterization protocol has been recently used to study the biodistribution of magnetic nanoparticles in mouse models, and the effects of the particle coating on the biodistribution [2,3]. It has also been used to follow particle transformations over long time periods [4]. The advantages of this technique include the possibility to distinguish the particles from other endogenous species such as the ferritin iron cores, and that no prior isolation procedures, potentially altering the results, are required. Nevertheless, especial attention has to be paid to the influence of interparticle dipolar interactions in the different tissues and the possible reduction of the particle size due to degradation processes, as both parameters affect the magnetic susceptibility results. We will describe the developed quantification protocol and our recent results on the quantification of magnetic nanoparticles in tissue samples. Future possible approaches to improve this characterization technique will be discussed.

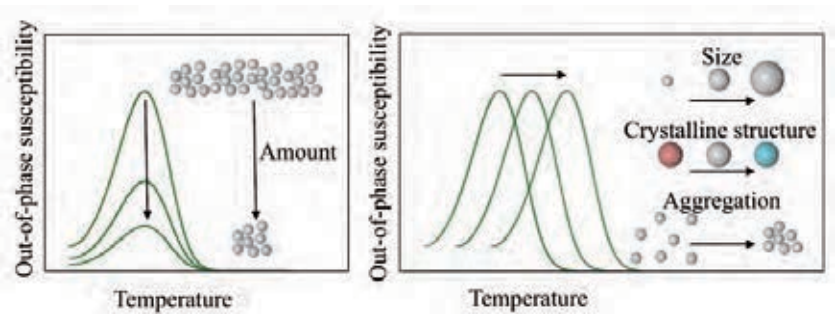

Figure 1. Schematic representation of some parameters that affect the out-of-phase susceptibility used for the quantitative determination of magnetic nanoparticles in tissue samples.

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7.

Soft X-ray tomography as quantitative tool to decipher the interaction between DMSA-SPION and MCF7 cancer cells

Francisco Javier Chichón[a], José Javier Conesa1[a], Michele Chiappi[a], Eva Pereiro[b], Maria Josefa Rodriguez[a], and José L. Carrascosa[a,c]

a. *Centro Nacional de Biotecnología (CNB-CSIC), Cantoblanco, 28049 Madrid, Spain.*
b. *ALBA Synchrotron Light Source. MISTRAL Beamline - Experiments division. 08290 Cerdanyola del Vallès, Barcelona, Spain.*
c. *Instituto Madrileño de Estudios Avanzados en Nanociencia (IMDEA Nanociencia), Cantoblanco, 28049 Madrid, Spain*

fjchichon@cnb.csic.es

In the last years the use of nanotechnology in medicine opened a broad field for studying cell-nanoparticle interaction. Due to their relatively easy generation and their low toxicity, iron magnetic nanoparticles are nowadays very promising. Superparamagnetic nanoparticles (SPION) could be coated with specific agent to target cells for drug delivery. Their magnetic properties also allow the manipulation of threated cells for cellular sorting or to induce cellular death by processes, such as hyperthermia. Those are feasible and important objectives in nanobiotechnology. In this frame, we have used Soft-X-ray microscopy to decipher and quantify the interaction of superparamagnetic iron oxide nanoparticles with average diameter of 15 nm (DMSA-SPION) with MCF-7 human breast cancer cells [1]. SPION were incubated from 0 to 24 h with cells and cryo-preserved by plunge freezing. We also performed correlative microscopy to locate by cryo-epifluorescent microscopy the Internalized SPION accumulations in the endocytic pathway and therefore, the samples were submitted to cryo Soft X-ray tomography (cSXT) to get whole cell volume maps. This is an emerging three-dimensional technique that exploits Soft X-rays high penetration into the biological matter to imaging vitrified cells at nanometric 3D resolution without chemical fixation or staining agents [2]. The three-dimensional reconstructions of whole cells allowed us to perform statistical analysis of SPION accumulation. Our results enlighten parameters such the average vesicles size, distance between them, distance to the nucleus and accumulation rates that characterize this type of accumulation. This new approach opens a new door to study in a quantitative way and with high resolution the interaction of nanoparticles and cells.

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8.

Multiparametric High Throughput Methods for Magnetic Iron Oxide Nanoparticle Lead Selection

K. Crosbie-Staunton[1*], G. Salas[2,3], O. Gobbo[4], M. Marciello[2,3], P. Couleaud[3], B. M Mohamed[1], T. Rakovich[1], R. Miranda[5,6], M. del Puerto Morales[2], A. Prina-Mello[1,7], Y. Volkov[1,7]

1. *School of Medicine, Trinity College Dublin, Ireland.*
2. *Instituto de Ciencia de Materiales de Madrid/CSIC, Madrid, Spain.*
3. *IMDEA Nanociencia, Madrid, Spain.*
4. *School of Pharmacy and Pharmaceutical Sciences, Trinity College Dublin, Ireland.*
5. *Instituto Madrileño de Estudios Avanzados, IMDEA Nanociencia, Madrid, Spain.*
6. *Departamento de Física de la Materia Condensada, Universidad Autónoma de Madrid, Spain.*
7. *CRANN and AMBER Research Centres, Trinity College Dublin, Ireland.*

* *Corresponding author, kcrosbie@tcd.ie*

Introduction: The application of magnetic nanoparticles (MNPs) can be tailored on a case by case basis by coupling them simultaneously with targeting moieties and medicinal drugs, which make multifunctional (MF) MNPs suitable for theranostic applications. Their potential role as magnetic resonance imaging (MRI) contrast agents, in drug and gene delivery platforms and hyperthermia are being intensely researched [4]. This study, as part of the MULTIFUN FP7 project, focuses on the multiparametric biocompatibility testing of a range of MNPs to select lead formulations. Further testing of the selected leads following functionalization with a targeting ligand (NUCANT 6L, N6L) and chemotherapeutic drugs (Doxorubicin and Gemcitabine) in breast cell lines was conducted to identify suitable theranostic MF-MNPs. Experimental procedure: Physicochemical characterisation of MNPs was carried out by transmission electron microscopy, dynamic light scattering and nanoparticle tracking analysis prior to cell exposure. A high throughput approach to determine biocompatibility was implemented with a range of MNP concentrations tested in breast-derived cell lines. Multiparametric analysis was conducted to determine the changes in the total cell count, cell membrane permeability and lysosomal mass/pH while flow cytometry was employed to further investigate the effect on cell cycle progression with one lead MNP. Main results: OD15 and MF66 MNPs below 100 μg/mL did not reduce cell viability or induce damage to the cell membrane, and were selected as the lead formulations for functionalization. Cell cycle analysis by flow cytometry with cells exposed to a range of MF66 MNP concentrations was conducted to ensure that MF66 was suitable to bring forward. It was determined that MF66 did not alter the normal cell cycle at concentrations below 100 ug/mL and was considered a suitable lead formulation. N6L was previously shown to target nucleolin and nuclophosmin on the cell surface and inhibit malignant tumour growth, angiogenesis and cancer cells invasion alike [5]. We demonstrate that free N6L exposure at 72 h timepoint

reduces cell count in a dose dependent manner and is most pronounced in breast cancer cell lines compared to the normal-like breast-derived cell line, which is consistent with previously published data. However, functionalised OD15 and MF66 MNPs containing N6L did not demonstrate similar efficacy compared to the free ligand. Therefore, we utilised N6L as a targeting moiety to facilitate greater MNP uptake into the MCF-7 and MCF-10A cells with chemotherapy drug-functionalised MNPs. We found that N6L-functionalised MF-MNPs had enhanced uptake and chemotherapeutic drug delivery efficacy compared to N6L-free MF-MNPs. Conclusion: This study demonstrates that by implementing an in-depth investigation of physicochemical properties and multiparametric cytotoxicity analysis, it is possible to select MNP formulations and subsequently select targeted MF-MNP leads for transfer to in vivo animal models and clinical exploitation.

Acknowledgement: This work is partially funded by the European Commission under the FP7 programme (MULTIFUN project: NMP4-LA-2011-262943)

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9.

Tracer development critical for translational medical applications of Magnetic Particle Imaging

Kannan M. Krishnan
Department of Materials Science & Engineering
University of Washington, 323 Roberts Hall,
Box 352120, Seattle, WA 98195

The performance of Magnetic Particle Imaging (MPI), a new whole-body medical imaging technology with promise in vascular angiography and molecular imaging, depends largely on the structural, chemical, magnetic, and biological characteristics of the tracers. In fact, MPI is the first biomedical imaging technique that truly depends on nanoscale materials properties; in particular, their response to alternating magnetic fields in a true biological environment needs to be optimized.

I will describe the development of our highly optimized nanoparticle tracers with magnetic properties tailored for the unique physics of MPI using a sound theoretical framework, an organic synthetic route producing phase-pure magnetite cores with controlled shape and narrow size-distribution. Their subsequent phase-transfer without agglomeration, even in biologically relevant media, and functionalization for targeting, biocompatibility, adequate in vivo circulation (tailorable between 4-80 minutes in mice models) and tailorable biodistribution (no uptake in the kidneys, critical for imaging patients with chronic kidney disease) will be presented.

In MPI phantom images, reconstructed in either real or Fourier space, these optimized tracers showed significant improvement compared to existing commercial particles (Resovist®), in both normalized signal intensity (6x) and spatial resolution (50% better) approaching sub-mm at 6 T/m/μ0 field gradients. Further, their MPI signal is linear with concentration, with minimal contribution from Brownian relaxation, making them suitable for molecular imaging.

Finally, we have established a flexible platform for functionalization of the magnetic cores with PEG (to increase water solubility, enhance circulation time, and improve shelf life), dyes (to turn them into multimodal -- MRI, MPI and NIRF -- contrast agents and used to further enhance studies of their biodistribution with significant anatomical detail in rodent models) and ligands (such as Lactoferrin with specific affinity for glioma cells) tailored for targeted molecular imaging.

These critical results, that pave the way for improved clinically relevant MPI performance, will be discussed with emphasis on the tracer response to both the alternating magnetic field and the biological environment.

This work was supported by NIH grants 1RO1EB013689-01 (National Institute of Biomedical Imaging and Bioengineering, NIBIB) and 1R41EB013520-01.

10.

Early Detection of Pancreatic Cancers & Brain Tumors by MR Molecular Imaging

Yung-Ya Lin[a], Zhao Li[a], and Chaohsiung Hsu[a]

a. *Department of Chemistry and Biochemistry, University of California, Los Angeles*

yylin@chem.ucla.edu

Introduction: Early detection of high-grade malignancy, such as pancreatic cancers (PC) and glioblastoma multiforme (GBM), using enhanced MRI techniques significantly increases not only the treatment options available, but also the patients' survival rate. For this purpose, a conceptually new approach, termed "Active-Feedback Controlled MR", was developed. An active feedback electronic device was homebuilt to implement active-feedback pulse sequences to generate avalanching spin amplification, which enhances the weak field perturbations from magnetic nanoparticles in targeted PC or malignant physiological conditions in GBM.

Theory and Methods: The general principles of the "Active-Feedback Controlled MR" can be found in our publications [1-6] (and references therein). Here, its specific applications to early tumor detection were developed and demonstrated. (i) First, an active-feedback electronic device was home-built to generate feedback fields from the received FID current. The device is to filter, phase shift, and amplify the signal from the receiver coils and then retransmit the modified signal into the RF transmission coil, with adjustable and programmable feedback phases and gains. The MR console computer can execute the active-feedback pulse sequences to control the trigger signal, feedback phase/gain, and the duration of the feedback fields, allowing us to utilize the active feedback fields in novel ways. (ii) Next, an active-feedback pulse sequence was developed for early tumor detection and was statistically tested on in vivo mice tumor models. In essence, the enhanced tumor contrast arises from "selective self-excitation" and "fixed-point dynamics" generated by the bulk water 1H under active feedback fields. Use the sensitive detection of magnetic nanoparticles as an example. A small flip-angle (q=5-10°) RF pulse tilts the sample equilibrium magnetization. Since the averaged transverse magnetization is mainly contributed from the bulk water ^{1}H spins, the resulting active feedback field possesses a frequency closer to that of the bulk water ^{1}H spins which are distant from the dipole center. By "selective self-excitation", the feedback field tilts the bulk water ^{1}H spins more effectively towards the stable fixed-point, –z-axis (assume feedback phase 180°), while the ^{1}H spins near the dipole center are less affected due to resonance mismatch. This "selective self-excitation" process continues and enlarges the contrast between the longitudinal magnetization of the ^{1}H spins in bulk water

and those near the dipole center. Maximum contrast in the longitudinal magnetization can be achieved and locked when all spin magnetizations evolve to the fixed point: all align along –z in this case.

Results (1): Early Detection of Pancreatic Cancers: Anti-CA 19-9 antibodies were conjugated to NH2-PEG-coated magnetic nanoparticles. The antigen binding capacity to CA 19-9 over-expressing cell lines (BxPC3) was confirmed by in vitro MR cellular images. In vivo images of human pancreatic tumors from nude mouse xenografts show that, while T2-weighted image cannot clearly locate the magnetic nanoparticles, the active-feedback images successfully highlight the magnetic nanoparticles distribution with a close correlation with iron-stained histopathology.

Results (2): Early Detection of Glioblastoma Multiforme: Stage-1 orthotopic GBM mouse models infected with human U87 cell line were imaged. While both T2 parameter images and T2-weighted images by spin echo successfully locate the GBM tumor, our active-feedback images and decay constant mapping provide 4-5 times of improvements in GBM tumor contrast through sensitively imaging the susceptibility variations due to irregular water contents and deoxyhemoglobin.

Discussion and Conclusion: In vivo PC and GBM mouse models validated the superior contrast/sensitivity and robustness of the "Active-Feedback MR" for early tumor detection. Statistical results (N>10) for PC and GBM mouse models at various cancer stages, alternative active feedback pulse sequences with further improved performance, and active feedback pulse sequences for enhanced R1/R2-weighted images will also be presented.

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11.

Parallel Multifunctionalisation of Nanoparticles: A One-Step Modular Approach for *in vivo* Imaging

Fernando Herranz[a], Hugo Groult[a], Juan Pellico[a], Ana V. Lechuga-Vieco[a], Riju Bhavesh[a], Jesús Ruiz-Cabello[a]

a. *Advanced Imaging Unit, Department of Atherotrombosis and Imaging, Fundación Centro Nacional de Investigaciones Cardiovasculares (CNIC) and CIBER de Enfermedades Respiratorias (CIBERES), Melchor Fernández-Almagro, 3, 28029 Madrid, Spain*

fherranz@cnic.es

Multifunctional nanoparticles are usually produced by sequential synthesis, with long multistep protocols.[1] Until now additional functionality meant additional synthetic steps and costs, lower yields and a complex mixture on the surface of the particles.[2] Our study reports a generic modular strategy for the parallel one-step multifunctionalisation of different hydrophobic nanoparticles.[3] The aim was to apply the concept of parallel synthesis, developed in organic chemistry, for the synthesis of multifunctional inorganic nanoparticles. The method was designed and developed by taking advantage of the natural non-covalent interactions between the fatty acid binding sites of the bovine serum albumin and the aliphatic surfactants on different inorganic nanomaterials. As a general example of the approach, three different nanoparticles – iron oxide, upconverting nanophosphors and gold nanospheres – were nanoemulsified in water with albumin. To support specific applications, multifunctional capability was incorporated with a variety of previously modified albumin modules. These modules include different conjugated groups, such as chelating agents for ^{68}Ga or ^{89}Zr and ligand molecules for enhanced in vivo targeting. A large library of 13 multimodal contrast agents was developed with this convergent strategy. This platform allows a highly versatile and easy tailoring option for efficient incorporation of functional groups. Finally, as demonstration of this versatility, a bimodal probe (PET/MRI) including a maleimide-conjugated BSA was selectively synthesized with an RGD peptide for in vivo imaging detection of tumour angiogenesis.

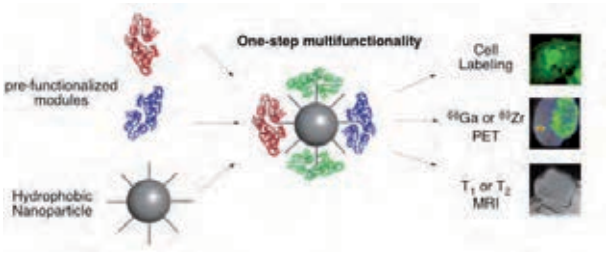

Figure 1. General scheme for the parallel functionalisation of hydrophobic nanoparticles with applications in cell labelling, PET imaging (^{68}Ga or ^{89}Zr) and MRI (T_1 and T_2 contrast).

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12.

Improving magnetic properties of ultrasmall magnetic nanoparticles by biocompatible coatings

Rocio Costo,[a] M. Puerto Morales,[a] and Sabino Veintemillas-Verdaguer[a]

a. *Instituto de Ciencia de Materiales de Madrid (CSIC). Sor Juana Ines de la Cruz, 3. 28049 Madrid*

rcosto@icmm.csic.es

During last decades much has been written about the use of magnetic iron oxide nanoparticles for applications in biomedicine as contrast agents for magnetic resonance imaging, drug delivery or hyperthermia cancer treatment [1]. In any case, particles should be coated with biocompatible molecules to avoid aggregation, improve the colloidal stability and guarantee the biocompatibility of the final product. However, as a result of the interactions between the particle surface and the coating, magnetic properties can be modified [2]. Understanding these changes in magnetic behavior is critical for developing magnetic nanoparticles for biomedical applications since the properties of the final product may be greatly different from those of the initial iron oxide cores.

To study this phenomenon, ultrasmall particles were synthesized by laser pyrolysis and fully oxidized to maghemite by acid treatment. The surface of the magnetic nanoparticles was

systematically coated with either phosphonate (phosphonoacetic acid or pamidronic acid) or carboxylate-based (carboxymethyl dextran) molecules and the binding to the nanoparticle surface was analyzed. Magnetic properties at low temperature show a decrease in coercivity and an increase in magnetization after the coating process. Hysteresis loop displacement after field cooling is significantly reduced by the coating, in particular for particles coated with pamidronic acid, which show a 10% reduction of the displacement of the loop. We conclude that the chemical coordination of carboxylates and phosphonates reduces the surface disorder and enhances the magnetic properties of ultrasmall maghemite nanoparticles.

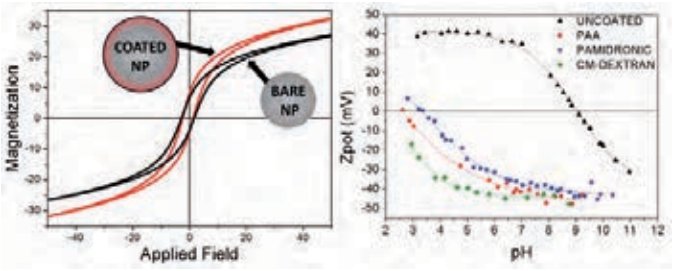

Figure 1. Left: Magnetization curves at 5 K of the coated and un-coated particles. Right: Colloidal stabilization of the particles with the three studied coatings: phosphonoacetic acid (red dots), pamidronic acid (blue squares) and carboxymethyl-dextran (green diamonds)

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13.

Synthesis and characterization of bimetallic magnetic oxides obtained by oxidative precipitation

Y. Luengo[a], M.A. Roldán[b], M. P. Morales[a], M. Varela[b,c] and S. Veintemillas-Verdaguer[a]

a. *Instituto de Ciencia de Materiales de Madrid, ICMM-CSIC, Sor Juana Inés de la Cruz 3, Cantoblanco, Madrid, Spain*
b. *Universidad Complutense de Madrid, Departamento de Fisica Aplicada III, Madrid 28040, Spain*
c. *Materials Science and Technology Division, Oak Ridge National Laboratory, Oak Ridge, TN 37831, United States*

yluengo@icmm.csic.es

Bimetallic magnetic oxides in nanoparticulated form have plenty of potential applications in many fields such as biomedicine (MRI contrast agents, magnetic hyperthermia agents, drug delivery, biosensors), technology (magnetic recording), environmental remediation (removal of pollutants with magnetic nanoparticles), etc. due to the combination of properties of both oxides. In the biomedical field, iron oxide is the most usual material because of its low toxicity, high biocompatibility and good magnetic properties. However, there is still plenty of room for research; for instance special features could be obtained by combination of iron oxide nanoparticles with another metal oxide in a single entity.

Thus, bimetallic nanoparticles consisting of magnetite and other metal oxide, Fe3O4@MOx (M: Bi, Gd, Co), were obtained by oxidative precipitation in aqueous medium. The different metals were chosen due to their special properties: the presence of Bi may confer TC contrast while Gd could be used as T1 contrast agent in MRI and Co will enhance the magnetic properties of the iron oxide particles. The maximum amount of metal oxide incorporation without segregation and the type of distribution (solid solution, core-shell, decoration with oxide particles, etc) were studied by scanning transmission electron microscopy (STEM) and electron energy loss spectroscopy (EELS). The materials were characterised by X-Ray diffraction, thermogravimetric analysis, infrared spectroscopy and chemical analysis. The saturation magnetization, initial susceptibility and coercivity of all the samples were measured at room temperature and 5 K to study the effect of these metal oxides on the magnetic properties of magnetite.

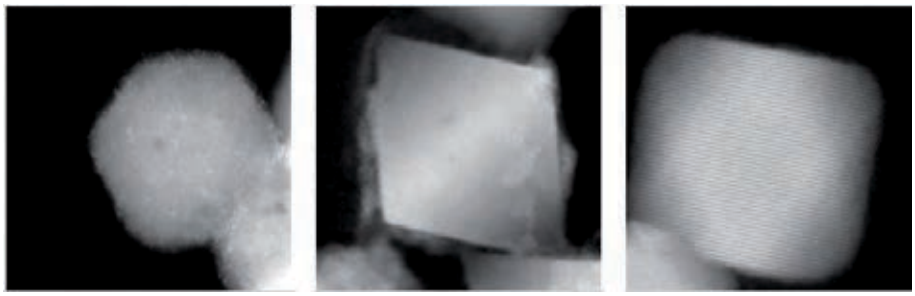


Figure 1. HAADF STEM micrographs of nanoparticles doped with: a) Bi, b) Gd and c) Co

14.

Coating strategies for the application of magnetic nanoparticles in cancer diagnosis and therapy

Marzia Marciello,[a] Marlène Branca,[b,c] Susana I.C.J. Palma,[d] Maryam Zahraei,[e] Diana Ciucu-Iescu-Pradines,[b,c] Behshid Behdadfara,[e] Ana Cecilia A. Roque,[d] Catherine Amiens[b,c] and M. Puerto Morales[a]

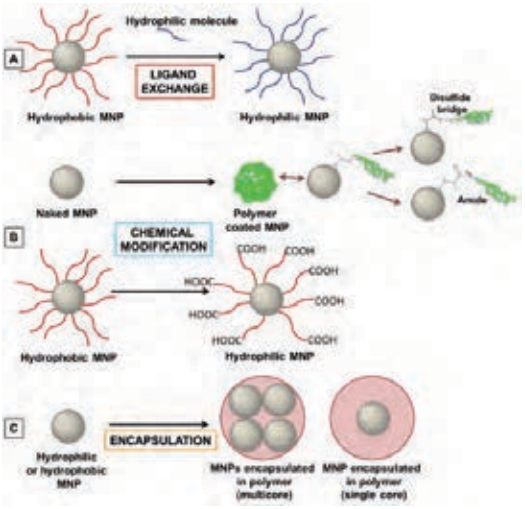
a. *Biomaterials and Bioinspired Materials Dpt., Instituto de Ciencia de Materiales de Madrid (ICMM-CSIC), Sor Juana Inés de la Cruz 3, Cantoblanco, 28049 Madrid, Spain;*
b. *CNRS, LCC (Laboratoire de Chimie de Coordination), 205, route de Narbonne, F-31077 Toulouse, France;*
c. *Université de Toulouse, UPS, INPT; LCC; F-31077 Toulouse, France;*
d. *UCIBIO, REQUIMTE, Chemistry Dpt., Universidade Nova de Lisboa, 2829-516 Caparica, Portugal;*
e. *Materials Engineering Dpt., Isfahan University of Technology, Isfahan 84156-83111, Iran.*

marziamarciello@icmm.csic.es

In the last decades the use of magnetic nanoparticles (MNPs) for cancer diagnosis and therapy has quickly increased. An important requirement for MNPs in vivo application is the presence of an opportune coating that stabilizes them in physiological conditions, and provides multifunctionality, biocompatibility and protection of magnetic core against degradation.

In this work MNPs synthesized by different routes (aqueous and organic medium) [1-4] have been coated using some opportune strategies: ligand exchange [2,3,4] superficial chemical modification [1,3,4] and encapsulation [4] (Scheme 1).

Small molecules (DMSA, citric acid, TEOS, APTS) or biocompatible polymers (dextran, polyethylene glycol, chitosan, gum arabic) have been strongly attached to MNPs surface to avoid coating loss in physiological conditions. The objective was to make these particles suitable as contrast agents for MRI or therapeutics for hyperthermia. All the coated MNPs were characterized by good stability in physiological conditions and displayed opportune size for biomedical application.



Scheme 1: Different coating strategies used to enable MNPs bioapplication: A) Ligand exchange, B) Chemical modification and C) Encapsulation.

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15.

Chimeric Antigen Receptor T-cells for Cancer Therapy

Dr David Gilham
Institute of Cancer Science - University of Manchester, Manchester, UK

One mechanism employed by tumors to avoid immune medicated destruction is reduced or loss of expression of Major Histocompatibility Complex (MHC) proteins which are essential for T cell recognition processes. To overcome this avoidance strategy, CAR technology has been developed that exploits antibody-based target binding thereby allowing the T-cell to recognise cell surface tumor associated antigen directly thereby circumventing loss of MHC expression. The CAR consists of four key elements; firstly, the antigen binding domain that is most commonly a single chain antibody fragment (scFv) derived from an antibody but can be derived from any potential ligand. Secondly, an extracellular spacer domain that allows

for optimal binding of the scFv to its cognate target. Thirdly, a transmembrane domain that tethers the CAR to the surface of the T cells and finally, an intracellular signalling domain that engages with downstream pathways that initiate T-cell effector function upon ligation of the CAR with target antigen. However, to date there has been consensus single optimal configuration. Consequently, many different CARs are currently in clinical testing. These will be discussed as well as recent developments in the CAR T cell field.

17.

At the Fluorescent Nanoprobes´ Frontiers: Multifunctionality for Bio-Imaging and T-Sensing

Antonio Benayas,[a] Fuqiang Ren[a], Blanca del Rosal[b],Wagner F. da Silva[c], Elisa Carrasco[b], Francisco Sanz-Rodríguez[b], Vicente Marzal[2], Karla Santacruz-Gómez[d], Gustavo A. Hirata[e], Dongling Ma[a], Daniel Jaque[b], Fiorenzo Vetrone[a].

a. *Univ Quebec, INRS-EMT, 1650 Blvd. Lionel Boulet, Varennes, PQ J3X 1S2, Canada.*
b. *UAM, Cantoblanco 28049, Madrid, Spain.*
c. *UFAL, Maceió, 57072-900 Alagoas, Brazil.*
d. *Univ Sonora, Hermosillo 83000, Sonora, Mexico.*
e. *CNyN, Ensenada, 22800 Baja California, Mexico*

antonio.benayas@emt.inrs.ca

Nowadays, new and better light-emitting probes are being demanded to fulfill the needs and specific requirements of nanomedicine, especially at the cancer diagnosis and therapy battleground. There is an intense appetite for multifunctional, non-invasive and biocompatible platforms to be designed and implemented.

In this work on fluorescent nanoparticles (NPs) for bio-imaging, we present our last outstanding results obtained throughout a research lines promoted at Vetrone group (INRS-EMT, Canada). Starting with dielectric hosts, a remarkable outcome has been reached using $NaGdF_4$ lanthanide-doped NPs for cell imaging. They provide an extended colour-palette within both visible and near-infrared spectral ranges, using two alternative excitation wavelengths (793 and 980nm). It should also be highlighted the pivotal role that fluorescent nanothermometry has been playing within our investigations. Thus, the second project here explained is constituted by the development of efficient Nd:YAG near infrared fluorescent nanothermometers[1,3], that have demonstrated remarkable performance on a broad range of temperature-monitoring applications (electrical circuits, sub-tissue measurements and optical trapping context). Finally, our most standing platform PbS/CdS quantum dots (QDs)

for in vivo imaging, which wavelength-wise design (they emit ~ 1.3μm) increases penetration depth[2], and their high-brightness allows a remarkable low dose of them to provide fluorescence images, upon injection in live animals. This system has also shown some promissory nanothermometry features, demonstrated through ex-vivo experiments.

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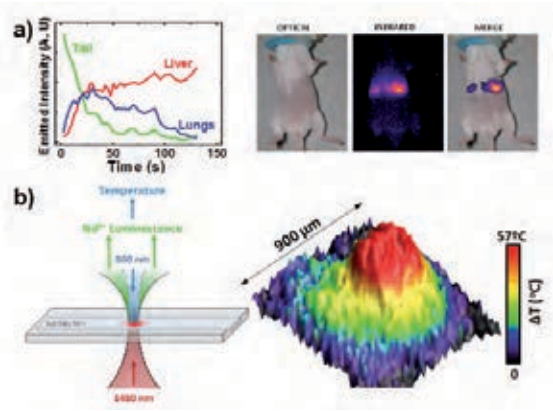

Figure 1: a) Left: graph showing the evolution of the QDs emitted intensity from three different parts of the mouse body (tail/green, liver/red and lungs/blue) as a function of time after injection inside the mouse´s body. Right: Combination of optical and fluorescence images taken from the live specimen. b) Left: Schematic representation of an optical trapping setup. A 1480 nm laser is focused into a microchannel filled with Nd:YAG NPs. Right: Thermal image of a 900 μmx900 μm area around the laser focus. Laser power was set to 62 mW. Figure taken from the author's recently published Ref. [3].

18.

Joining time-resolved thermometry and magnetic-induced heating in a single nanoparticle

Angel Millan,[a] Rafael Piñol,[a] Carlos D. S. Brites,[b] Rodney Bustamante,[a] Abelardo Martínez,[c] Nuno J. O. Silva,[b] José L. Murillo,[a] Rafael Cases,[a] Julian Carrey,[d] Carlos Estepa,[a] Cecilia Sosa,[e] Fernando Palacio,[a] Luis D. Carlos,[b]

a. *ICMA, CSIC–Universidad de Zaragoza, 50009 Zaragoza (Spain).*
b. *Departamento de Física and CICECO, Universidade de Aveiro, 3810–193 Aveiro (Portugal)*
c. *Departamento de Electrónica de Potencia. I3A. Universidad de Zaragoza 50018 Zaragoza (Spain)*
d. *Université de Toulouse, INSA, UPS, CNRS (UMR 5215), F-31077, Toulouse (France)*
e. *Departamento de Toxicología, Universidad de Zaragoza, 50013 Zaragoza (Spain)*

amillan@unizar.es

Magnetic- and light-induced thermal heating of nanoparticles are powerful non-invasive techniques for bio and nanotechnology applications, such as hyperthermia therapy of cancer and other diseases. To be effective this local heating requires an adequate monitoring of the nanoheaters local temperature. However, efficient and sensitive single nanoparticles integrating heaters and thermometers are a real synthetic challenge. Here we present a successful realization of this challenge. The nanoheaters are magnetic nanoparticles coated with amphiphilic polymers that had been already used in magnetic hyperthermia [1]. The ratiometric thermometric system was also developed in our group a few years ago [2]. The temperature readout is optical and the thermometric probes are dual-emissive Eu^{3+}/Tb^{3+} lanthanide complexes. These complexes are encapsulated in the hydrophobic inner part of the nanoparticle coating, around the iron oxide magnetic core. The resulting heater/thermometer nanoplatform shows an outstanding performance in terms of: low thermometer heat capacitance ($0.021 \cdot K^{-1}$) and heater/thermometer resistance ($1\ K \cdot W^{-1}$); high temperature sensitivity ($5.8\% \times K^{-1}$ at 296 K) and uncertainty (0.5 K), physiological working temperature range (295-315 K), readout reproducibility (>99.5%), and fast time response (0.250 s). Experiments of time-resolved thermometry under an AC magnetic field reveal the existence of an unexpected temperature gradient between nanoheaters and surrounding media for relatively long time intervals ($t > 10$ s) and relatively low heat powers (10^{-16} W/heater). The fitting of the data to simple heat models evidences the failure of using macroscopic thermal parameters to describe heat diffusion at the nanoscale. A proof of concept of temperature mapping has been realized on cells that were incubated with the nanoparticles. The fluorescence microscopy in two different wavelengths simultaneously after beam splitting permits the mapping of the intracellular local temperature using the pixel-by-pixel ratio of the Eu^{3+}/Tb^{3+} intensities.

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19.

Magnetic hyperthermia, a promising tool for the minimal-invasive treatment of tumors

Ingrid Hilger

Institute for Diagnostic and Interventional Radiology, Jena University Hospital, Erlanger Allee 101. D-07747 Jena, Germany

Early detection of tumours has been distinctly improved in the last years, which favours the development of minimal-invasive therapeutic methods promising a high therapeutic efficacy. Herein, the treatment of small tumours via magnetic heating was proposed, meaning the selective accumulation of magnetic materials, magnetite, at the tumour area and the exposure of the breast to an alternating magnetic field for several minutes. This will produce a selective heating spot which allows for a localized elimination of tumour cells. Even though the principle seems to be simple at a first glance, the efficacy of the methodology will strongly depend on how the different biomedical, physical and technical parameters are being combined and adjusted between each other. Therefore, in the present study we will consider the different aspects that have to be taken into account to fulfil these requirements.

20.

Encapsulation of VEGF165 in magnetic PLGA nanocapsules for potential local delivery and bioactivity into human brain endothelial cells

Roig,[1] E. Carenza[1], O. Jordan[2], P. Martínez-San Segundo[3], R. Ji ík[4], Z. Star uk jr.[4], G. Borchard[2], A. Rosell[3]

1. *Nanoparticles and Nanocomposites Group (www.icmab.es/nn), Institut de Ciència de Materials de Barcelona, Consejo Superior de Investigaciones Científicas (ICMAB-CSIC), Campus de la UAB, Bellaterra, Catalunya, E-08193 Spain.*
2. *School of Pharmaceutical Sciences, University of Geneva, University of Lausanne, Quai Ernest Ansermet 30, 1205 Geneva, Switzerland.*
3. *Neurovascular Research Laboratory and Neurovascular Unit, Vall d'Hebron Institut de Recerca, Universitat Autònoma de Barcelona; Passeig Vall d'Hebron, Barcelona, Catalunya, E-08035 Spain.*
4. *Institute of Scientific Instruments, Academy of Sciences of the Czech Republic, Královopolská 147, 612 64 Brno, Czech Republic.*

roig@icmab.es

The administration of pro-angiogenic proteins is an attractive therapeutic strategy to enhance angiogenesis after an ischemic event [1,2]. Nevertheless a labile structure and short circulation time in vivo are the main obstacles that reduce protein bioactivity and dose at the target site. Biodegradable magnetic nanomaterials, intravenously administrated and accumulated at the diseased zone under an external magnetic field, may circumvent these limitations.

We report on the synthesis of poly(D,L-lactic-co-glycolic acid) (PLGA) nanocapsules (diameter less than 200 nm) loaded with the vascular endothelial growth factor-165 (VEGF165) confined into the inner core and superparamagnetic iron oxide nanoparticles (SPIONs) embedded in the polymeric shell (Fig. 1 inset). The system showed high encapsulation efficiencies and sustained protein release over 14 days (Fig. 1). In vitro studies confirmed protein bioactivity after encapsulation. Moreover, through MRI measurements we demonstrated excellent T_2 contrast image properties. We therefore suggest the nanoconstruct as new targeted protein delivery carrier useful in pro-angiogenic treatments, especially after an ischemic event [3].

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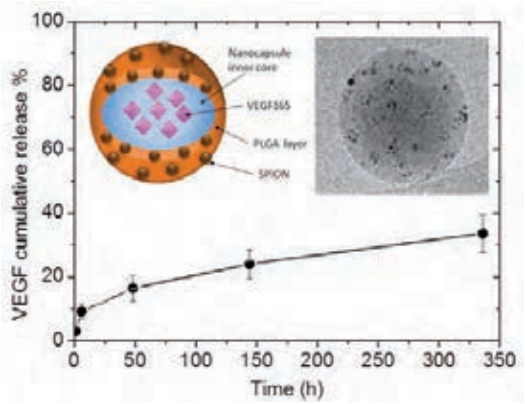

Figure 1: Protein release over a period of 2 weeks. Inset: scheme and cryo-TEM image of a PLGA nanocapsule dispersed in water

21.

Influence of nanoparticle size and field frequency on the concentration dependence of magnetic heating

David Cabrera,[a] Gorka Salas,[a] Robert Ludwig,[d] Julio Camarero,[a,b], Rodolfo Miranda [a,b], Ingrid Hilger,[d] and Francisco J. Terán[a, c]

a. *IMDEA Nanociencia, Ciudad Universitaria de Cantoblanco, 28049 Madrid, Spain ;*
b. *Dpto. Física de la Materia Condensada, Universidad Autónoma de Madrid, 28049 Madrid, Spain*
c. *Unidad Asociada de Nanobiotecnología, CNB-CSIC & IMDEA Nanociencia, 28049 Madrid, Spain;*
d. *Institute for Diagnostic and Interventional Radiology I, Jena University Hospital, Friederich Schiller University of Jena, Bachstraße 18, D-07740 Jena, Germany;*

david.cabrera@imdea.org

Iron oxide nanoparticles (IONP) have attracted much attention for their potential in biomedical applications in the last years. Their precise size control and tailored chemical features favor their internalization into cancer cells acting as drug-delivery nanocarriers without cytotoxicity drawbacks. In addition, their size-driven magnetic properties allow IONP to efficiently act as contrast agents, or intracellular hyperthermia mediators.

Recent works have shown that the internalization of IONP into cells and/or tissues lead to significant changes of their magnetic properties, resulting in a remarkable reduction of their heating efficiency [1]. The underlying reasons are yet to be clarified. At a first glance, IONP processing into biological matrix (cells or tissues) increases IONP clustering favoring magnetic dipolar interactions, which may be altered by viscosity. Hence, understanding the role of dipolar interactions is of great importance towards the use of IONP as reliable heating mediators.

Here, we report on the study of specific absorption rate (SAR) as a function of IONP concentration at different field frequencies and particle sizes. Our results indicate that both parameters (size and frequency) lead to distinct concentration dependences matching within the single picture described by Martinez-Boubeta et al [1]. While 19 nm size IONP show a non-monotonic behaviour with a relative SAR maximum the 12 nm size IONP shows a progressive decrease of SAR values until reaching a value which maintains constant in the overall concentration range studied. When varying filed frequency, the variety of behaviours becomes richer, resulting in the observation of an evolution from non-monotonic behaviour of SAR vs concentration to the progressive SAR decay (at low frequencies). However, we observe a shift of the relative SAR maximum which is clearly frequency-dependent and has not been considered by recent theoretical models [2]. Our data provide new experimental evidences which require novel theoretical models for understanding the role of magnetic

dipolar interactions in determining the heat dissipation processes of IONP under alternating magnetic fields.

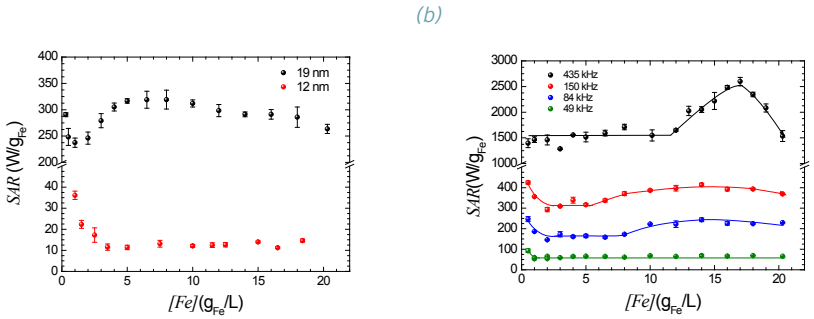

Figure 1. *(a) Concentration dependence of SAR values obtained IONP water dispersions of different sizes (19 and 12 nm) at given field conditions (104 kHz and 50mT), (b) Concentration dependence of SAR values obtained in 19±2 nm IONP water dispersions at different field frequencies and given field amplitude (40 mT)*

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22.

Intratumoral thermal reading during photothermal therapy by multifunctional fluorescent nanoparticles

Blanca del Rosal,[a] Elisa Carrasco,[b] Francisco Sanz-Rodríguez,[a,c,d] Ángeles Juarranz de la Fuente,[b,c] Patricia Haro-Gonzalez,[1] Ueslen Rocha,[e] Kagola Upendra Kumar,[e] Carlos Jacinto,[e] José García Solé,[a] and Daniel Jaque.[a]

a. *Fluorescence Imaging Group. Departamento de Física de Materiales, Facultad de Ciencias. Campus de Cantoblanco, Universidad Autónoma de Madrid. Madrid 28049, Spain*
b. *Instituto de Investigaciones Biomédicas "Alberto Sols", CSIC-UAM. Madrid 28029, Spain*
c. *Departamento de Biología, Facultad de Ciencias. Campus de Cantoblanco. Universidad Autónoma de Madrid. Madrid 28049, Spain*
d. *Instituto Ramón y Cajal de Investigación Sanitaria, Hospital Ramón y Cajal, 28034 Madrid, Spain*
e. *Grupo de Fotônica e Fluidos Complexos. Instituto de Física, Universidade Federal de Alagoas 57072-970, Maceió, Alagoas, Brazil*

blanca.rosal@uam.es

Photothermal therapy, which relies on light-induced heating to irreversibly damage cancer cells is nowadays attracting a great deal of attention as an effective and low cost technique for treating malignant tumors.[1] An adequate temperature monitoring during the treatment is key to minimizing damage to the surrounding healthy tissues while ensuring the effectiveness of the therapy. However, measuring intratumoral temperature constitutes a challenge that cannot be solved by traditional thermometry techniques.

Multifunctional nanoparticles, acting simultaneously as temperature sensors and photothermal agents upon excitation with a single light source are excellent candidates for this task. In this work, neodymium-doped LaF_3 nanocrystals have been successfully used for continuous intratumoral temperature monitoring during photothermal therapy in mice. These infrared-emitting nanoparticles double as efficient in vivo heating agents and temperature sensors. The use of this kind of nanoparticles for temperature-controlled therapy opens the way for highly efficient and minimally invasive photothermal treatments of cancer tumors.

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23.

Magnetic nanoparticle-based therapeutic agents for thermo-chemotherapy treatment of cancer

Prof Nguyen TK Thanh
UCL Healthcare Biomagnetic and Nanomaterials Laboratories
21 Albemarle Street, London W1S 4BS &
Department of Physics & Astronomy
University College London
Gower Street, London WC1E 6BT, UK
Email: ntk.thanh@ucl.ac.uk
http://www.ntk-thanh.co.uk

Magnetic nanoparticles have been widely investigated for their great potential as mediators of heat for localised hyperthermia therapy. Nanocarriers have also attracted increasing attention due to the possibility of delivering drugs at specific locations, therefore limiting systematic effects. The enhancement of the anti-cancer effect of chemotherapy with application of concurrent hyperthermia was noticed more than thirty years ago. However, combining magnetic nanoparticles with molecules of drugs in the same nanoformulation

has only recently emerged as a promising tool for the application of hyperthermia with combined chemotherapy in the treatment of cancer. We will present some of our recent work in the development of nanoparticles and effort in developing multifunctional therapeutic nanosystems incorporating both magnetic nanoparticles and drugs.

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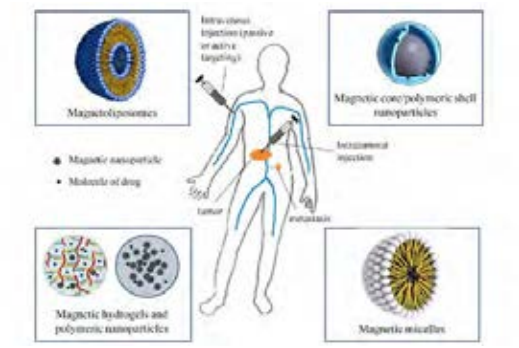

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24.

Nano-emulsion templating: a versatile technology to prepare multifunctional polymeric nanoparticles for advanced biomedical applications

Cristina Fornaguera, Gabriela Calderó, Conxita Solans

Institute of Advanced Chemistry of Catalonia (IQAC-CSIC) and CIBER of Bioengineering, Biomaterials and Nanomedicine (CIBER-BBB)

cristina.fornaguera@iqac.csic.es

Nano-emulsions are fine emulsions with droplet sizes between 20 – 200nm, showing high kinetic stability and transparent to translucent aspect. Since they are thermodynamically unstable systems, an energy input is required for their formation. Among the methods for nano-emulsion preparation, low-energy methods, which take advantage of the chemical energy of nano-emulsion components, are advantageous for pharmaceutical applications, since they enable obtaining nano-emulsions with smaller and less polydisperse droplets. Specifically, the phase inversion composition method (PIC), in which phase inversion is produced by changing the composition during the emulsification process at a constant temperature, is adequate for thermolabile compounds because it can be performed at mild temperature and process conditions [1]. Polymeric nanoparticles (pNP) can be obtained from nano-emulsions with an oil phase consisting on a preformed polymer dissolved in an organic volatile solvent followed by solvent evaporation [2]. In this context, the objective of this work was to obtain pNP, from nano-emulsion templating, as advanced pharmaceutical agents for diagnosis and/or therapeutic specific uses. O/W polymeric nano-emulsions were firstly obtained by the PIC method, using poly-(lactic-co-glycolic acid) (PLGA) as the preformed polymer [3,4]. They showed hydrodynamic radii between 20 – 140 nm, negative surface charge and enough stability to prepare nanoparticles. Nano-emulsions with 90wt% W, 70/30 O/S ratio were selected for further uses because they represent a compromise between small sizes (around 40nm) and low surfactant concentration. pNP were prepared from these nano-emulsions by solvent evaporation, resulting in hydrodynamic radii of around 20 nm, negative surface charge and stability higher than 3 months. These pNP were loaded and /or functionalized to achieve different purposes. Fluorescent dyes (e.g. coumarin 6) or inorganic nanoparticles (e.g. magnetic nanoparticles) were encapsulated in the pNP to use them as diagnosis carriers, obtaining very high encapsulation efficiency. pNPs were also designed as therapeutic agents following a pharmacological approach, in parallel to a gene therapy approach. Model analgesic and antiapoptotic drugs were successfully encapsulated to develop the pharmacological therapy, achieving also high encapsulation efficiencyiy and sustained drug release, maintaining the pharmacological activity of the drugs. To use nanoparticles as non-viral gene delivery systems, they were cationized to further electrostatically bind antisense oligonucleotides. Very high gene transfection efficiencies were obtained in cell cultures, with the advantage of pNPs over commercial vectors of a lower toxicity and over viral vectors of lower immune detection. In addition, with the purpose to actively target a specific organ, pNP surface was functionalized with a model monoclonal antibody targeting the Blood-Brain Barrier (BBB). In vivo studies of the BBB crossing evidenced that antibody functionalized pNP were able to cross the BBB in a similar extent than positive controls. Therefore, it can be concluded that the preparation of polymeric nano-emulsions by low-energy methods, followed by solvent evaporation to obtain polymeric nanoparticles is a very versatile technology that enables tailoring their properties rendering them useful for both, therapeutic and diagnosis purposes, achieving also an active targeting to the desired organ.

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25.

G-Quadruplex aptamers with therapeutic applications

Anna Aviñóa, Santigo Grijalvoa, Carme Fàbregaa, Maria Tintoréa, Stefania Mazzinib, Juan Carlos Moralesc and Ramon Eritjaa

a. *Networking Center on Bioengineering, Biomaterials and Nanomedicine (CIBER-BBN), Institute for Advanced Chemistry of Catalonia (IQAC), CSIC, Jordi Girona 18-26 08034 Barcelona, Spain*
b. *Department of Food, Environmental and Nutritional Sciences (DEFENS), Section of Chemical and Biomolecular Sciences, University of Milan, Via Celoria 2, 20133 Milan, Italy.*
c. *Instituto de Investigaciones Químicas, CSIC - Universidad de Sevilla, Americo Vespucio, 49, 41092 Sevilla, Spain.*

aaagma@cid.csic.es

Aptamers are short DNA- or RNA-based oligonucleotides selected from large combinatorial pools of sequences for their capacity to efficiently recognize targets, ranging from small molecules to proteins or nucleic acid structures. Like antibodies, they exhibit high specificity and affinity for target binding. Aptamers can be considered as antibodies, but, unlike antibodies they can be chemically derivatized to extend their lifetimes and/or their bioavailability and are non-immunogenic. As a result, they may display effective interference in biological processes, which renders them not only valuable diagnostic tools, but also promising therapeutic agents. Among several architectures, the polymorphic G-quadruplex structure is adopted by multiple aptamers. G-quadruplexes are very stable structures generated by the association of steps of four guanines (G-quartets) held together by hydrogen bonds. G-quadruplexes acting as aptamers can be powerful inhibitors of different proteins. Particularly, large research efforts have been focused on the 15-base long thrombin binding aptamer (TBA) sequence, which was derived as an inhibitor of -thrombin, a key enzyme in the blood clotting cascade. In addition, several anti-HIV aptamers also adopt DNA quadruplex structures. Among them, “Hotoda’s aptamer” has significant affinity to the HIV protein gp120. These aptamers are potentially prone to be modified in order to improve biological properties.

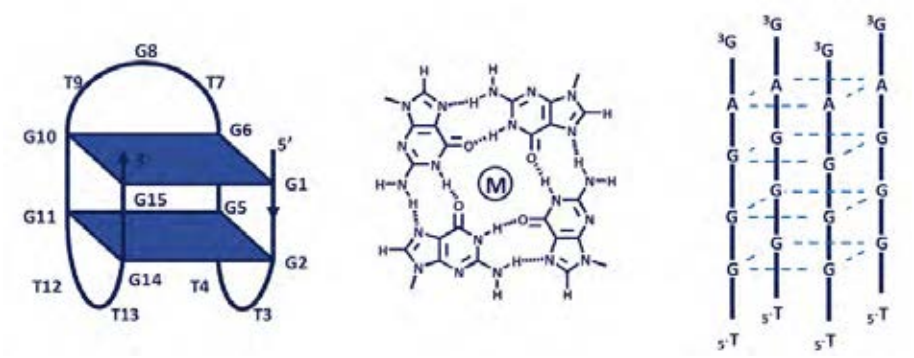

Figure 1. Struture of the TBA, G-tetrad and Hotoda.

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26.

Targeted Drug Delivery Systems against Cancer Stem Cells

Dr. Petra Gener
Vall d'Hebron Research Institute,
Barcelona, Spain

Despite the progress in understanding the mechanism of carcinogenesis, and the development of more effective therapies against cancer, advanced cancer is still remaining to be an incurable disease. The cure of advanced cancer is hampered because of metastatic spread to distal organs and resistance to therapy. Both phenomena are sustained by the presence of cancer stem cells (CSC) within the tumor. This minor cell population retains the capacity of spreading the tumor, while being insensitive to conventional anticancer therapies,

antimitotic agents or radiation. Specific delivery of chemotherapeutic compounds to CSC by nanotechnology based drug delivery systems (DDS) has great potential to increase CSC sensitivity. To be able to study efficacy of nanomedicines in population of CSC we developed an original CSC model, in which we stably tagged CSC population. This CSC model we used to study the impact of targeted/non-targeted DDS on CSC presence and behavior in continuous cell culture.

27.

Development of gene-expressing liposomes as drug delivery systems

Alicia Soler Canton,[a] Christophe Danelon[b]

a. *Department of Bionanoscience, Kavli Institute of Nanoscience, Delft University of Technology, Lorentzweg 1, 2628 CJ, Delft, The Netherlands*

b. *Department of Bionanoscience, Kavli Institute of Nanoscience, Delft University of Technology, Lorentzweg 1, 2628 CJ, Delft, The Netherlands*

a.solercanton@tudelft.nl

Targeted drug delivery in human body can be mediated by diverse nanocarriers. Among the different types, liposomes – artificial phospholipid vesicles – have received the most attention during the last three decades for numerous reasons. Current liposomal systems deliver therapeutic compounds that have been directly packaged upon manufacturing. However, recent advances on the development of synthetic gene circuits prompt us to explore the biomedical potential of gene-expressing liposomes. In this work we aim to express small interfering RNA (siRNA) molecules of therapeutic value from a DNA template inside < 200 nm sized liposomes. Two main reasons drag us to work on the development of gene-expressing liposomes for the production of therapeutic siRNA. First, we hypothesize that the siRNA concentrations reached upon synthesis inside liposomes could be higher than those obtained through the encapsulation of molecules produced in bulk (current method). Second, we believe that future genetic-circuit-containing liposomes could manage a controlled expression and release of the therapeutic molecule. Early steps towards the realization of this new generation of programmable drug delivery systems include 1) the validation of the production of functional shRNA by in vitro transcription and 2) the development of a protocol for optimized transcription inside small unilamellar vesicles, which we have managed so far inside 800nm liposomes. Later steps on this project will involve the merging of these advances to transfect shRNA-expressing liposomes into cells and track specific gene silencing.

28.

Self-assembling nanoparticles based on calixarene-cyclodextrin heterodimers for targeted delivery and controlled release of docetaxel

Laura Gallego-Yerga,[a] Francesco Sansone,[b] Carmen Ortiz Mellet,[a] Alessandro Casnati[b] and José M. García Fernández.[c]

a. *Departamento Química Orgánica, Facultad de Química, Universidad de Sevilla, E-41012 Sevilla Spain;*
b. *Dipartimento di Chimica, Università degli Studi di Parma, I-43124 Parma, Italy;*
c. *Instituto de Investigaciones Químicas (IIQ), CSIC – Universidad de Sevilla, E-41092 Sevilla, Spain*

lgallego@us.es

The development of drug delivery systems able to improve bioavailability of insoluble or biodegradable drugs and avoid secondary effects through targeted transport is essencial for the success in administration of therapeutic agents. In this work we have succeeded at preparing molecularly defined calixarene-cyclodextrin heterodimers filled with the capacity to self-assemble in water or buffer media to form functional nanoparticles. These nanostructures have an inner core formed by hydrophobic calix[4]arene (CA_4)[1] units and an external hydrophilic shell exposing b-cyclodextrin (bCD) moieties[2,3]. The CA_4 scaffold is very well suited to promote tight packing of fatty chains installed at the narrower ring in its cone conformation, providing a lipid matrix where hydrophobic drugs can be entrapped, whereas the presence of hydrophilic bCD moieties at the nanosphere surface allows nanoparticle solubilization in water as well as ligand incorporation by inclusion complex formation. The potential of the new systems in nanomedicine is illustrated by their capacity to encapsulate and provide sustained release of the anticancer agent docetaxel (DTX) and undergo supramolecular surface post-modification with adamantane-armed glycoligands targeting the human macrophage mannose receptor (MMR). Since docetaxel can induce apoptosis in tumor cells which overexpress this receptor, this targeting is expected to improve cancer immunotherapies.

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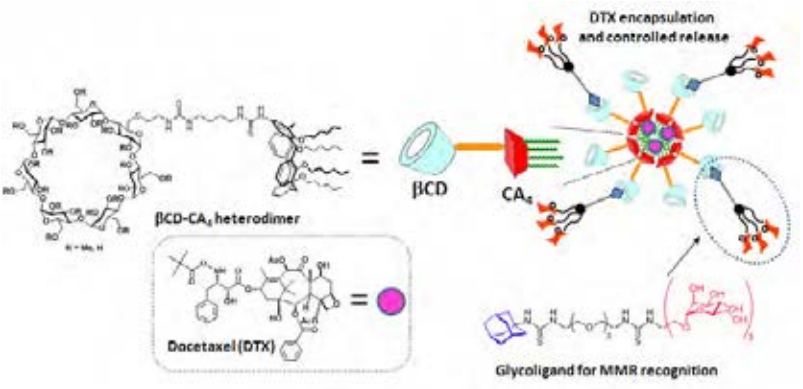

29.

Design of biocompatible magnetic nanoplatforms for potential platinum-based drug delivery applications

Gustavo B. da Silva,[a,b] Rocío Costo,[b] Marzia Marciello,[b] Maria D. Vargas,[a] Célia M. Ronconi,[a] M. Puerto Morales[b] and Carlos J. Serna[b]

a. *Instituto de Química, Universidade Federal Fluminense, Campus do Valonguinho, Centro, 24020-150 Niterói-RJ, Brazil*

b. *Department of Biomaterials and Bioinspired Materials, Instituto de Ciencia de Materiales de Madrid (ICMM)/CSIC, Sor Juana Inés de la Cruz 3, Cantoblanco, 28049 Madrid, Spain*

gustavobezerrads@gmail.com

Cisplatin (cis-[Pt(NH_3)$_2$$Cl_2$]) and its derivatives have been widely used in cancer treatment, however they have shown several side effects. To overcome these drawbacks, the use of magnetic nanoparticles as carriers and drug delivery systems could be a good strategy [1]. Iron oxide nanoparticles e. g. maghemite (g-Fe_2O_3) are biocompatible, superparamagnetic and can reach different regions of the body upon an external magnetic field. Furthermore, they are easily modified to which platinum(II) derivatives can be attached and protected against early deactivation, with consequent enhancement in their cytotoxicity.

Aim of this work was the synthesis of superparamagnetic g-Fe_2O_3 nanoparticles and subsequent surface modification with a biocompatible polymer for the conjugation of cisplatin derivatives with potential anticancer properties. The g-Fe_2O_3 nanoparticles were synthesized

by co-precipitation method followed by an acid treatment [2]. Then they were coated with citric acid (CA) and amine dextran (ADex), through amide bonds. Investigation of the conditions (iron and dextran concentrations, reactive type and pH) to best attach the ADex polymer to the surface of g-Fe_2O_3 resulted in g-Fe_2O_3@CA@ADex containing ~70% of anchored polymer. The materials were characterized by TEM, DRX, IR, TG-DTA and ICP-OES measurements. Magnetic data confirmed a superparamagnetic core. The colloidal properties (hydrodynamic size - D_{Hyd}, potential z) were evaluated and the stability in the physiological environment were enhanced after functionalizations. Finally cisplatin related compounds (cis-[$Pt(NH_3)_2(NO_3)_2$] and cis-[$Pt(DMSO)_2Cl_2$]) were attached to the ADex polymer to yield cis-[$Pt(X)_2(ADex)_2$], X = NH_3 or Cl, (25-45%). These platinum-modified polymers were then anchored to the surface of g-Fe_2O_3@CA nanoparticles under the conditions employed for the synthesis of g-Fe_2O_3@CA@ADex. Studies of colloidal stability and drug release are in progress.

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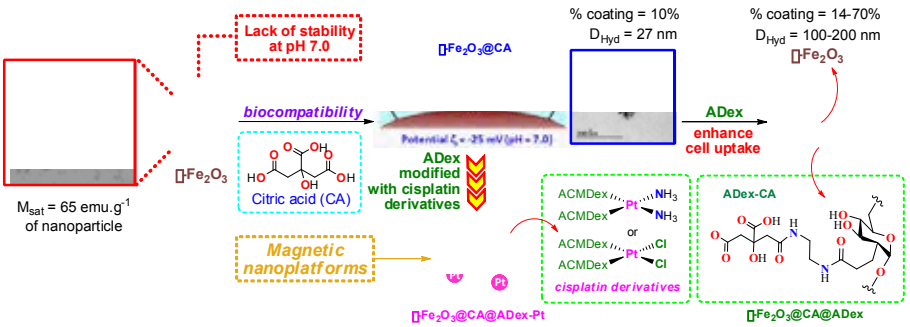

Figure 1. General route for design of magnetic nanoparticles of iron oxide (g-Fe_2O_3) and its modification with citric acid, amine dextran and cisplatin derivatives.

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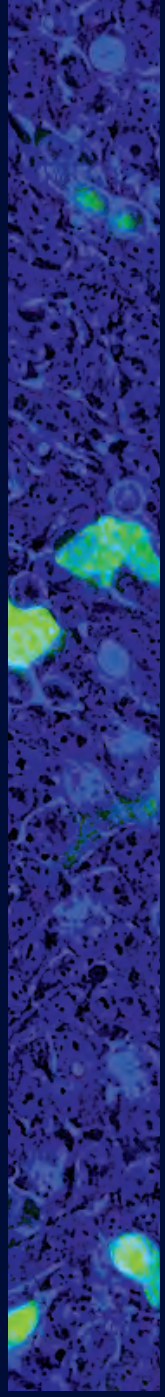

Interaction of multifunctional magnetic nanoparticles with biological membranes

María Josefa Rodríguez[a], Marzia Marciello[b], Antonio Aires[c], Sandra M. Ocampo[c], Aitziber L. Cortajarena[c], Puerto M. Morales[b], José L. Carrascosa[a]

[a] Department of Structure of Macromolecules, Centro Nacional de Biotecnología-CSIC (CNB-CSIC), Campus Cantoblanco, 28049 Madrid, Spain
[b] Instituto de Ciencia de Materiales de Madrid (ICMM)-CSIC, Campus Cantoblanco, 28049 Madrid, Spain
[c] Instituto Madrileño de Estudios Avanzados en Nanociencia (IMDEA Nanociencia). Cantoblanco, Madrid, Spain.

mjrguez@cnb.csic.es

Abstract

The possibility to selectively introduce magnetic nanoparticles (MNPs) inside cells for further manipulation is a key objective in nanomedicine. To achieve this purpose, MNPs need to overcome a first barrier represented by biological membranes, which also define the selectivity characteristics of the target cells. The biological functionalization of the MNPs will enhance the selectivity toward the desired cells, improving the efficiency of incorporation of therapeutic molecules and reducing the effects.

In this study, imaging of thin sections of cultured cells by transmission electron microscopy (TEM) have allowed us to evaluate at an ultrastructural level the interaction of MNPs with cellular membranes and the characteristics of uptaking and accumulation inside the cells of nanoparticles with different coatings and functionalizations. In particular, MNPs with two different coatings, a small molecule (dimercaptosuccinic acid, DMSA) and a biocompatible polymer (polyethylene glycol, PEG) have been studied. We have found that the different nature of the coating and the functionalization influence nanoparticle biodistribution in cellular membranes and entry characteristics.

Biodistribution of magnetic nanoparticles evaluated by AC magnetic susceptibility in a murine model

Eduardo Lorente-Sorolla,[a] Gustavo B. da Silva,[a,b] Sonia Perez-Yagüe,[c] Rocío Costo,[a] Sabino Veintemillas-Verdaguer,[a] Domingo F. Barber,[c] M. Puerto Morales,[a] Marina Talelli [c] and Lucía Gutiérrez [a]

[a] Department of Biomaterials and Bioinspired Materials, Instituto de Ciencia de Materiales de Madrid (ICMM)/CSIC, Sor Juana Inés de la Cruz 3, Cantoblanco, 28049 Madrid, Spain
[b] Instituto de Química, Universidade Federal Fluminense, Campus do Valonguinho, Centro, 24020-150, Niterói, RJ, Brasil
[c] Department of Immunology and Oncology, and NanoBiomedicine Initiative, Centro Nacional de Biotecnología (CNB)/CSIC, Darwin 3, Cantoblanco, 28049 Madrid, Spain

eduardo.lorentesor@estudiante.uam.es

Abstract

Iron oxide nanoparticles for biomedical applications preferentially accumulate in the liver and spleen, therefore reducing their possible uses in other organs, unless external magnetic fields or other functional molecules are used to target the particles to specific sites.

It is known that in thalassemia patients undergoing chronic blood transfusions, the excess iron from the blood cannot be properly bound to transferrin, leading to accumulation in the heart though the formation of iron-citrate complexes called NTBI (non-transferrin bound iron), as in the heart the metal-ion transporter ZIP14 is present, which mediates NTBI uptake [1]. The aim of this project was to mimic this NTBI function using citrate coated magnetic nanoparticles, in order to generate bio-inspired materials that may target the nanoparticles to the heart, therefore changing their biodistribution pattern.

We have performed a single administration of magnetic nanoparticles coated with citric acid and phosphonoacetic acid in mice and evaluated the presence of these nanoparticles in the heart, liver, lungs, spleen and kidney 24h after their administration. Nanoparticles with both coatings were clearly observed by AC magnetic susceptibility measurements in the liver, lungs and spleen tissues. The presence of nanoparticles in the heart was very small in comparison to the other tissues. Transmission electron microscopy in liver sections showed the presence of magnetic nanoparticles inside lysosomal structures. In addition, ferritin, the iron storage protein, was frequently observed localized near the lysosomes containing the magnetic nanoparticles in the liver tissues.

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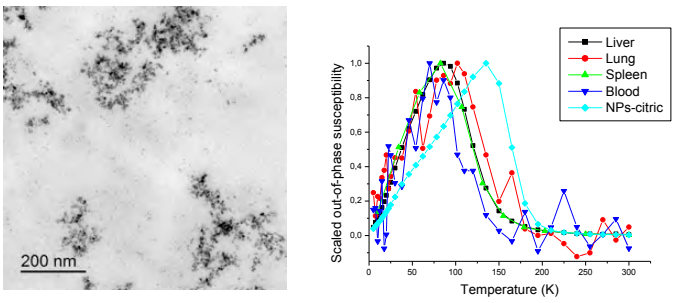

Figure 1. (*Left*) Transmission electron micrograph of a liver tissue section showing the presence of magnetic nanoparticles and ferritin. (*Right)* Temperature dependence of the out-of-phase susceptibility of different tissue samples from the same mouse showing a maximum corresponding to the presence of magnetic nanoparticles.

C. elegans: an *in vivo* model to evaluate nanoparticles?

Anna Roig, Laura Gonzalez-Moragas, Elisa Carenza, Siming Yu, Anna Laromaine

Nanoparticles and Nanocomposites Group, Institut de Ciència de Materials de Barcelona, Consejo Superior de Investigaciones Científicas (ICMAB-CSIC), Campus de la UAB, Bellaterra, Catalunya, E-08193 Spain.

roig@icmab.es

Abstract

Caenorhabditis elegans (*C. elegans*) is a 1-mm long free-living soil nematode widely used in biology as a model organism. The key attributes that make *C. elegans* a promising *in vivo* animal model to evaluate nanoparticles include simplicity, transparency, short life cycle, sequenced genome, small body size and ease of cultivation in the lab.

Here, we investigate the effect of surface functionalization (citrate and protein-coated) of superparamagnetic iron oxide nanoparticles in *C. elegans* combining techniques from materials science and biology confirming the worm as a suitable *in vivo* platform to advance in the understanding of nano-bio-interfaces (Figure 1). The survival and nanoparticle distribution of worms treated with the two nanoparticles was studied. By using magnetometry, quantitative uptake and magnetic properties of the nanoparticles inside the worms were evaluated. Interestingly, results indicate the protective effects both to the protein to the nanoparticles and to the worms especially at high concentrations. Thus, our results endorse the potential of *C. elegans* to assess nanomaterials at early stages of their synthetic formulations and to reduce the number of candidate materials to be screened in mammalian models.

Figure1. Visualization of a *C. elegans*: Prussian Blue stained worm where iron oxide nanoparticles appear blue.

Iron quantification inside cells incubated with SPION by Soft-Xray Absorption Spectro-Tomography (SXAST)

José Javier Conesa,[a] Francisco Javier Chichón,[a] Michele Chiappi,[a] Eva Pereiro,[b] and José L. Carrascosa.[a,c]

[a] Centro Nacional de Biotecnología (CNB-CSIC), Cantoblanco, 28049 Madrid, Spain.
[b] ALBA Synchrotron Light Source. MISTRAL Beamline - Experiments division. 08290 Cerdanyola del Vallès, Barcelona, Spain.
[c] Instituto Madrileño de Estudios Avanzados en Nanociencia (IMDEA Nanociencia), Cantoblanco, 28049 Madrid, Spain

jj.conesa@cnb.csic.es

Abstract

One of the mayor issues related to Superparamagnetic nanoparticles (SPION) internalization is the estimation of the amount of nanoparticles that are inside the cells. The answer to this question will determine the dose for drug-delivery or whetherhyperthermia treatments are feasible. To get an insight into this problem we used 15 nm DMSA-SPIONs interacting with MCF-7 breast cancer cells [1] as an experimental model, and Soft-X-ray spectral-microscopy to calculate the iron content -main component of the SPION- using the specific absorption of the element.

X-ray absorption is a widely used technique that exploits the singular interaction of the elements with X-rays [2]. For this study we developed a new approach called Soft X-ray Absorption Spectro-Tomography (SXAST) acquiring spectral tilted series of the same cell between at 700 and 709 eV. In this context, the iron abortion changes dramatically from 700 to 709 eV, while the rest of the cellular compound remains with the same contrast (Figure). We used this property to subtract the images corresponding to the tilted series acquired at 700 from the ones form the 709 eV tilted series. The result is a differential tilted series where the cellular background was eliminated. Then, tomographic reconstructions allowed us to obtain iron absorption coefficients, hence the iron mass, associated to three-dimensional cellular ultrastructure (Figure).

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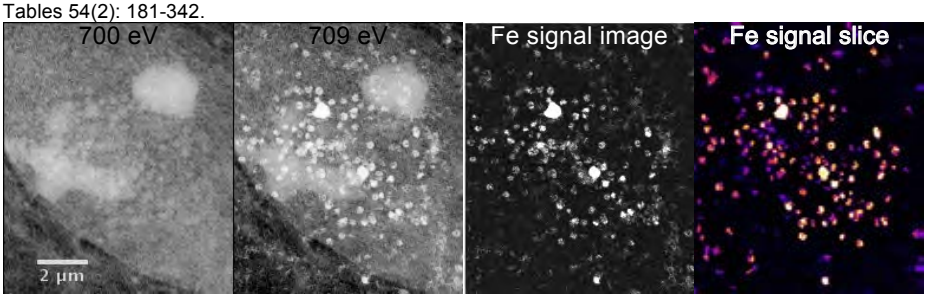

Figure. Soft-X ray Absorption Spectro-Tomography (SXAST) workflow. First two images represent 0º projection images of a dehydrated MCF7 cell incubated with OD15-DMSA SPION using Fe pre edge and edge energies (700 and 709 eV respectively). Alignment of projections at each different energy yield ratio images that must be aligned and then submitted to tomographic reconstruction (fourth image, central slice of the quantitative volume). Modified EFTEM TOMOJ software was used to create differential maps and reconstruction.

Following the transformation of magnetic nanoparticles over time in macrophages by AC magnetic susceptibility

Vanesa del Dedo,[a] Marina Talelli,[b] José M. Rojas,[b] Gustavo B. da Silva,[a,c] Rocío Costo,[a] Sabino Veintemillas-Verdaguer,[a] Domingo F. Barber,[b] M. Puerto Morales,[a] Marzia Marciello [a] and Lucía Gutiérrez [a]

[a] Department of Biomaterials and Bioinspired Materials, Instituto de Ciencia de Materiales de Madrid (ICMM)/CSIC, Sor Juana Inés de la Cruz 3, Cantoblanco, 28049 Madrid, Spain
[b] Department of Immunology and Oncology, and NanoBiomedicine Initiative, Centro Nacional de Biotecnología (CNB)/CSIC, Darwin 3, Cantoblanco, 28049 Madrid, Spain
[c] Instituto de Química, Universidade Federal Fluminense, Campus do Valonguinho, Centro, 24020-150, Niterói, RJ, Brasil

vanesa.dedo@estudiante.uam.es

Abstract

Little is known about the degradation processes that magnetic nanoparticles (MNPs) undergo after their administration, which can give insight on their potential side effects when used for biomedical applications. A promising approach to follow the MNPs transformations in tissues is the use of AC magnetic susceptibility measurements [1,2]. However, once the nanoparticles start to transform in the body, the analysis of the magnetic susceptibility data for quantification purposes is not straightforward, as a reduction in the particle size cannot be easily distinguished from a disaggregation process that can also reduce inter-particle interactions.

To gain insights into the transformations of magnetic nanoparticles in biological systems, and therefore be able to better understand the changes of their magnetic properties, we followed their degradation in a macrophage cell line (THP1) for 5 days. Transmission Electron Microscopy (TEM) and AC magnetic susceptibility measurements have been performed at different time points after treatment of the cells with the nanoparticles. Results indicated that a partial degradation of the nanoparticles occurs during this time period, with a shift of the particle size distribution towards smaller particles. Further reduction of the particle sizes may require longer incubation times, however, this will require optimization of culture conditions.

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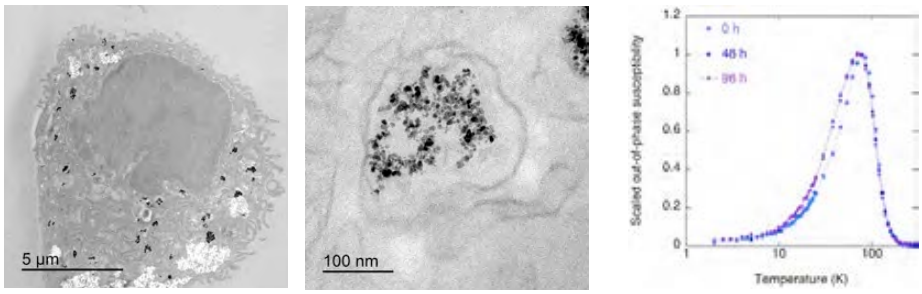

Figure 1. (*Left and center*) Transmission electron micrographs at two different magnifications of a THP1 cell line incubated with magnetic nanoparticles. (*Right*) Temperature dependence of the out-of-phase susceptibility of cells incubated with the MNPs at different times after the administration.

pH-Dependent Switch for Anticancer Metallodrugs

Francisco Martínez, Ana M. Pizarro

IMDEA Nanociencia, *Ciudad Universitaria de Cantoblanco, 28049, Madrid*

Francisco.Martinez@imdea.org

Abstract

The successful development of platinum-containing anticancer drugs that began with cisplatin (*cis*-diamminedichloridoplatinum(II)) has triggered the investigation of other metal-based agents with cytotoxic activity for drug design. Ruthenium, osmium, iridium and rhodium organometallic complexes have been in the last few years one of the most promising findings due to their inherent anticancer activity and to the possibility of a different cytotoxic profile than platinum-based drugs.

In general, ruthenium(II) arene-complexes of type $[(\eta^6\text{-arene})Ru^{II}(XY)Z]^{n+}$ (Z = monodentate ligand; XY = chelating ligand such as ethylenediamine, en) are cytotoxic to cancer cells including cisplatin-resistant cell lines. Recently, new ruthenium(II) arene compounds with a hemilabile amino-derivatised arene ligand have been published,[1,2] where the amino group offers two reversible functionalities: (i) binding to the Ru^{II} center to form a tether-ring-closed (inactive) complex, or (ii) dissociation of the amine from the Ru^{II} center (as a dangling arm) to afford an open-tether complex with a pendant free amino group. In the open form the vacant site on the ruthenium is occupied by either chloride, a solvent molecule or a biomolecule (active complex). Most importantly, in aqueous solution, the tether-ring dynamics are pH dependent, giving the potential to finely tune the activation process to biological conditions, for example, the acidic microenvironment of the tumor.[3]

In the present work, we show a series of ruthenium(II) "tethered" arene complexes bearing different hemilabile amino-derivatised arene ligands. They have been characterized by NMR and elemental analysis.

The understanding of the conditions and kinetics that control the opening of this tether would be crucial for rationally designing drugs with controlled biological target recognition, enhanced cytotoxic activity and minimized off-target effects. The aquation and anation phenomena for this type of compounds are under investigation to finely tailor the structures where the opening-closing pK_a is most suitable for switching on in tumours, sparing healthy cells and therefore optimising selectivity and efficacy.

Finally, the extraordinary versatility of the metallodrug structure is amenable for engrafting onto nanoparticle carriers. Nanoparticles as drug delivery systems have received much attention due to their elegant capabilities to target and accumulate in tumour tissues as well as drop deadly cargoes in a more efficient, controlled, on-target manner.[4] For example, the bidentate chelating ligand in the metal complexes can be modified to carry groups that can react with suitable functionalities on the surface of the selected nanoparticles.

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Superparamagnetic properties of hydrothermally prepared $CoFe_2O_4$ as a function of size (6–10 nm) and coating (oleic/citric acid or TiO_2)

Daniel Nižnanský,[a] Anton Repko,[a] Jana Vejpravová,[b] and Taťána Vacková [c]

[a] Charles University, Faculty of Science, Department of Inorganic Chemistry, Hlavova 2030/8, 128 43 Prague 2, Czech Republic
[b] Institute of Physics AS CR, v.v.i., Department of Magnetic Nanosystems, Na Slovance 2, 182 21 Prague 8, Czech Republic
[c] Institute of Macromolecular Chemistry, Academy of Sciences of the Czech Republic, 162 06 Prague 6, Czech Republic

email: niznansk@natur.cuni.cz

Abstract

Magnetic properties of cobalt ferrite nanoparticles prepared by hydrothermal hydrolysis of Co-Fe oleate in the presence of pentanol/octanol/toluene and water at 180 or 220 °C were prepared. The particle size (6.2, 9.1 or 10.5 nm) was controlled by the organic solvent and temperature. The inter-particle distance was then changed by a surface modification with citric acid or titanium dioxide.

The as-prepared hydrophobic nanoparticles (coated by oleic acid) had an inter-particle distance of 2.5 nm. Their blocking temperature (estimated as a maximum of the zero-field-cooled magnetization) was 179 K, 283 K and 331 K. Replacement of the oleic acid by citric acid imparted hydrophilicity and the inter-particle distance decreased to almost zero, while the blocking temperature increased by approximately 10 K, as a consequence of stronger dipolar inter-particle interactions.

Another modification was achieved by coating with titanium dioxide, supported by nitrilotris(methylphosphonic acid). The increased inter-particle distance implied lowering of the blocking temperature by ca. 20 K. The $CoFe_2O_4$@TiO_2 nanoparticles were sufficiently stable in water, methanol and ethanol.

Magnetic structure was also investigated by Mössbauer spectroscopy. The effect of dipolar interactions on relaxation phenomena of the nanoparticles of different size and coating was finally studied by alternating-current (AC) susceptibility measurements.

Targeting cancer cells with photoactive silica nanoparticles

Wioleta Borzęcka [a, b, c], Tito Trindade [b], Tomás Torres [c] and João Tomé [a, d]

[a] QOPNA and [b] CICECO, Department of Chemistry, University of Aveiro, 3810-193 Aveiro, Portugal
[c] Department of Organic Chemistry, Autonoma University of Madrid, 28049 Madrid, Spain
[d] Department of Organic and Macromolecular Chemistry, Ghent University, B-9000 Gent, Belgium

wioleta.borzecka@gmail.com

Abstract

Photodynamic therapy (PDT) is an emergent cancer medical treatment that combines a nontoxic drug, a particular type of light and oxygen. This drug, called photosensitizer (PS) or photosensitizing agent, when in contact with molecular oxygen and exposed to light can produce reactive oxygen species (ROS) that are strongly cytotoxic to cancer cells. An ideal PS should be a single pure compound with high absorption peak between 600 and 800 nm (red to deep red), should have no dark toxicity, and relatively rapid clearance from normal tissues [1].

Like other clinical protocols, PDT also has some limitations. To avoid some of these disadvantages, different PS nanoformulations have been tried. This target strategy can improve PDT treatments by increasing the aqueous solubility of the hydrophobic photosensitizers, their blood circulation, and their selective accumulation in tumor tissue, thanks to the enhanced permeability and retention effect [2]. In this field, silica nanoparticles (SNPs) have recently emerged as promising vehicles for PDT owing to their: high biocompatibility, controllable size formation, unique physicochemical properties, the possibility for tumor targeting through facile surface modification [3].

Some of our recent work on smart porphyrin-SNP materials, presenting their synthetic strategies and obtained photo-chemical and -physical results will be highlighted.

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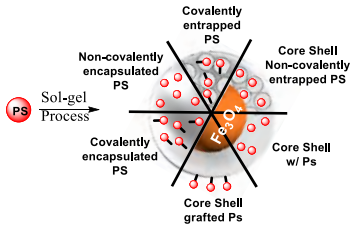

Figure 1. Differente methodes for the synthesis of nanoparticles [4].

Acknowledgements: Thanks are due to the Universities of Aveiro and Autonoma of Madrid, FCT, European Union, QREN, FEDER and COMPETE for funding the QOPNA research unit (PEst-C/QUI/UI0062/2013; FCOMP-01-0124-FEDER-037296), CICECO Laboratory (Pest-C/CTM/LA0011/2013, FCOMP-01-0124-FEDER-037271). Thanks are also due to the Seventh Framework Programme (FP7-People-2012-ITN) for funding the SO2S project (grant agreement number: 316975).

Combinational sensitization of tumor cells with two photosensitizers synergistically enhances their photodynamic inactivation *in vitro*

Andrea Tabero,[a] Ana Lazaro-Carrillo,[a,b] Pilar Acedo,[c] Macarena Calero,[a,b] Juan Carlos Stockert,[a] Magdalena Cañete,[a] Angeles Villanueva [a,b]

[a] Departamento de Biología. Universidad Autónoma de Madrid. Spain
[b] Madrid Institute for Advanced Studies in Nanoscience (IMDEA). Spain
[c] Karolinska Institute. Stockholm. Sweden.

andrea.tabero@gmail.com

Abstract

Liposomal lipid-based nanocarriers are widely used in drug delivery, especially in the treatment of cancer disease. Photodynamic therapy (PDT) is a minimally invasive and clinically approved procedure for cancer treatment. It can be defined as the administration of a drug known as photosensitizer (PS), which preferentially accumulates in tumor cells and that does not induce a cytotoxic effect by itself. However, after being irradiated with light of appropriate wavelength, the PS is able to trigger photochemical and photobiological reactions that lead to the generation of reactive oxygen species (ROS, mainly singlet oxygen), which cause irreparable damage, cell death and tumor regression.

Our work is focused on a new photodynamic treatment based on the combination of two PSs: the hydrophobic Zinc(II)-phthalocyanine (ZnPc) incorporated into nanoliposomes and the hydrophilic cationic porphyrin *meso*-tetrakis(4-N-methylpyridyl)porphine (TMPyP). These PS have different subcellular location (Golgi apparatus for ZnPc and lysosomes for TMPyP) and therefore ROS are generated simultaneously in two different subcellular targets, which induces a synergistic effect using very low doses of PDT in human adenocarcinoma HeLa cell line. Cytotoxic MTT assay showed that the combined treatment has a powerful cell inactivation effect (cell death > 95%), while photodynamic treatments with each PS alone did not significantly affect cell survival. Using different morphological and biochemical analysis we have demonstrated that combined treatment triggers apoptotic cell death via mitochondrial-related pathway (1).

On the other hand, caspase-3 is an important effector protease that cleaves many proteins in the apoptotic pathway. For this reason, we also analyzed the response to this treatment in human breast carcinoma cells (MCF-7), which do not express caspase-3. Our results revealed that this new strategy of photodynamic treatment induces over 95% MCF-7 cell death 24 h after treatment by a caspase 3-independent apoptotic pathway.

In summary, combined PDT with ZnPc and TMPyP increases the ability to inactivate human tumor cells through apoptosis, even if they have activated a mechanism of evasion of this type of cell death, such as the caspase-3 deficiency. Our results provide a novel and valuable information that will contribute to the development of better PDT approaches for the treatment of various cancers.

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This work was supported by the Spanish Ministry of Economy and Competitiveness (CTQ2013-48767-C3-3-R).

Synthesis Strategies of Single-Core Magnetic Nanoparticles

Helena Gavilán,[a] Rocío Costo,[a] Sabino Veitemillas- Verdaguer,[a] Lucía Gutiérrez,[a] and M. Puerto Morales[a]

Dept. Biomaterials and Bioinspired Materials, Instituto de Ciencia de Materiales de Madrid ICMM/CSIC, Sor Juana Inés de la Cruz 3, Cantoblanco, 28049 Madrid, Spain

helena_gr@icmm.csic.es

Among other functional nanomaterials, magnetic iron oxide nanoparticles (MNPs) are especially promising as contrast enhancement agents for magnetic resonance imaging (MRI), drug carriers and for cancer treatment by hyperthermia. For all these biomedical applications, MNPs require among others, good magnetic properties, biocompatibility and stability. The former is deeply influenced by the crystal structure of the iron oxide nanoparticles and the magnetic interaction between the particles. It is essential therefore to control such characteristics.

Different synthesis strategies have been developed considering the fact that magnetic nanoparticles tend to form aggregates in the absence of specific coatings. There are mainly two kinds of systems that have been defined: multi-core and single-core particles.[1] The former is composed of several magnetic cores per particle and although it is generally easy to synthesize such particles, it is difficult to control the number of cores per particle. The latter contains just one core per particle and nowadays there are just few coating methods that can be used to prevent aggregation by minimizing the inter-particle interactions.

Various synthesis strategies can be used to obtain these systems, being thermal decomposition in aqueous or organic solutions the most common one.[2] Although thermal decomposition in organic media prevent the agglomeration of the particles as they are formed, it requires also complicated operations and sometimes expensive/toxic reagents. Nevertheless these particles possess high crystallinity and monodispersity and are the ideal material for standardization purposes.

As part of the NanoMag-project, we have prepared both single-core and multi-core magnetic particles. The iron oxide cores have been synthesized by thermal decomposition of iron oleate complex, and subsequently coated either individually with silica via microemulsion, or forming clusters with 2,3-dimercaptosuccinic acid (DMSA) by ligand exchange, being both colloids stable in water (essential pre-requisite for a biomedical application). In addition, we present a three-step aqueous approach to obtain Fe_3O_4 single-core particles based on the synthesis of antiferromagnetic nanoparticles, its coating and subsequent reduction to magnetite. This method has the advantage of the low inter-particle interactions of as-synthesized nanoparticles and the different morphologies that these materials exhibit.

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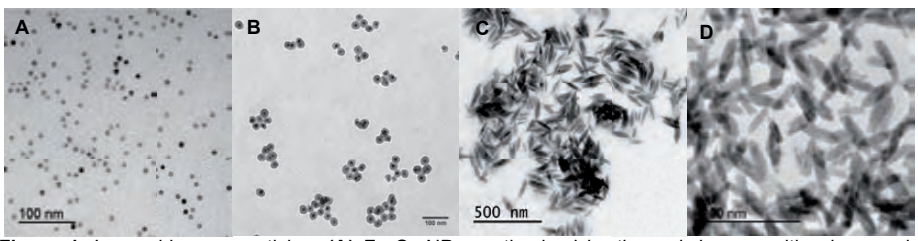

Figure 1. Iron oxide nanoparticles: **(A)** Fe_3O_4 NPs synthesized by thermal decomposition in organic media. **(B)** Fe_3O_4 NPs coated with silica by microemulsion process. **(C)** α-FeOOH NPs synthesized by thermal decomposition in aqueous media. **(D)** β-FeOOH NPs synthesized by thermal decomposition in aqueous media.

Silica encapsulation of magnetic nanoparticles

Leonor de la Cueva,[a] Paloma Rodríguez,[a] Rebeca Amaro,[a] David Cabrera,[a] María Acebrón,[a] Beatriz H. Juárez,[a,b] Francisco J. Terán,[a] and Gorka Salas[a]

[a] IMDEA Nanociencia
[b] Departamento de Química-Física Aplicada, Universidad Autónoma de Madrid

e-mail leonor.delacueva@imdea.org

Abstract

Iron oxide nanoparticles (IONPs) based on magnetite and maghemite are promising tools for biomedical applications in treatment and diagnosis of tumours.[1] For treatment they can be employed as selective drug carriers or as nano-heaters. The latter application is known as magnetic hyperthermia and is based on the ability of these materials to dissipate heat under the influence of an alternating (AC) magnetic field.[2] However, there exist some problems limiting the use of IONPs as hyperthermia mediators in *in vivo* tissues. It has been observed that once into the cells the heat generation ability is seriously harmed mainly due to aggregation caused by the intracellular environment and processing.[3] This aggregation leads to magnetic dipolar interactions between particles that may worsen their heat dissipation power. One plausible approach to avoid this problem is to encapsulate the individual IONPs in a stable and rigid material to prevent those destructive interactions. Alternatively, a controlled aggregation of IONPs in an ordered fashion may enhance their magneto-thermal capacity, instead of damaging it.

In this work we show the encapsulation of individual IONPs in silica (IONP@SiO_2 core@shell nanostructures) with controlled thickness, along with magnetic measurements comparing IONP@SiO_2 with the initial non-encapsulated nanoparticles. Preliminary results on the encapsulation of nano-assemblies of IONPs will be also presented.

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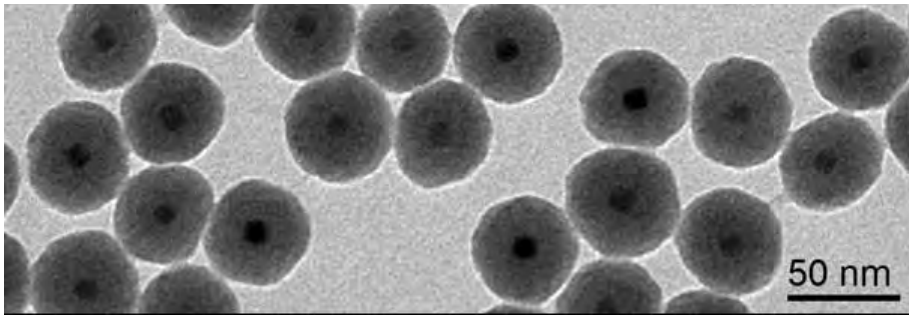

Figure 1. Individual IONPs encapsulated with a silica shell.

Synthesis of hybrid magneto-plasmonic nanostructures based on Au nanorods and iron oxides nanoparticles

Jesús G. Ovejero[a,b], Eva Mazario[b], Alexandra Muñoz[b], Patricia Crespo[a] and Pilar Herrasti[b]

[a]*Instituto de Magnetismo Aplicado Salvador Velayos (UCM) P.O. Box 155, 28230 Las Rozas, Madrid, Spain*
[b]*Departamento de Química Física Aplicada, Universidad Autónoma de Madrid, 28049, Cantoblanco, Madrid, Spain*

Abstract

Magnetic and plasmonic nanoparticles have brought a large number of biomedical solutions in the fields of detection, diagnosis and treatment. Both kinds of nanoparticles present their own limitations, but a question arises: Can we get unexpected synergetic properties in hybrid systems?

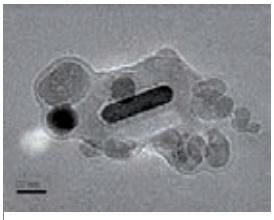

Figure 1. TEM image of Au@SiO NR coupled with Fe_3O_4

Surface plasmon resonance of Au nanoparticles is commonly used in biological sensing (SERS), medical imaging techniques (PAI, OCT, X-Ray, etc.) and in certain therapies (Photo-hyperthermia, thermally enhanced drug delivery, etc.). Moreover, different geometries of Au nanoparticles have been developed with the aim of shifting the plasmon resonance to the near infrared biological absorption window. Silica coated nanorods (Au@SiO NR) stand out from the rest of geometries due to their chemical stability and optimum optical properties [1],[2].

O the other hand, magnetic nanoparticles (MNP) have been used as alternative approach in fields such (MRI, MPI, magnetic hyperthermia, etc.). Thus, the combination of magnetic and plasmonic properties in a single nanoparticle offers, at first approximation, an interesting platform for multimodal imaging or a combined magneto-optic therapies. In addition, magnetomotive effect has been successfully used to improve the resolution of plasmon-based imaging techniques [3,4].

This work presents novel hybrid magneto-plasmonic nanoparticles based on the coupling of Au@SiO NR with different types of ferrite-based magnetic nanoparticles (MNP). Both, the Au@SiO NR and the MNP are synthesized separately and coupled afterwards. The followed process allows the tailoring of the magnetic and the plasmonic properties independently. Finally, the magneto-plasmonic nanoprobes have been functionalized with folic acid in order to improve their biocompatibility and internalization in cancer cells which overexpress folate receptors.

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This work has been supported by MAT2012-37109-C02-01 project of the Spanish Ministery of Economy and Competitivity

Functionalized magnetic nanoparticles for cancer therapy

Antonio Aires,[a,b] Sandra M. Ocampo,[a] David Cabrera,[a] Leonor de la Cueva,[a] Yael Fernandez,[a] Gorka Salas [a,b], Francisco J. Terán [a,b], and Aitziber L. Cortajarena.[a,b]

[a]Instituto Madrileño de Estudios Avanzados en Nanociencia (IMDEA Nanociencia).

[b] CNB-CSIC-IMDEA Nanociencia Associated Unit, Cantoblanco, Madrid, Spain.

email address of the Presenting Author: antonio.aires@imdea.org

Abstract

In recent years, magnetic nanoparticles (MNP) have been widely investigated for their potential in biomedical applications acting as contrast agents for magnetic resonance imaging (MRI),[1] nanocarriers for selective targeting and drug/gene-delivery,[2] and as magnetic heating inductors for thermal therapeutic approaches.[3] For successful *in vivo* application, MNP have to satisfy several requirements such as biocompatibility, invisibility to the immune system to avoid fast liver clearance, high colloidal stability and long blood circulation time.

To achieve a long-term colloidal stability and long blood circulation time of MNP, different polymers coatings including poly(ethylene glycol) (PEG), dextran, chitosan, poly(ethylenimine) (PEI), and dimercaptosuccinic acid (DMSA), have been employed over the last years. Among the commonly-used polymer materials, proteins or polypeptides are considered to be among the most promising materials as protective layers of MNP due to their biocompatibility and hydrophilicity.[4]

In this study, we have developed MNP functionalized with an anti-cancer drug, in particular gemcitabine (GEM) to target pancreatic cancer. In order to achieve formulations with improved properties for *in vivo* use in drug delivery and hyperthermia treatment we developed a formulation in which the MNP are previously coated with BSA to provide enhanced colloidal stability in biological environments and long blood circulation time to the final formulation. The effect of surface functionalization on the colloidal stability in different media, magnetic properties, internalization behavior and *in vitro* cytotoxic effect in pancreatic cancer cells were investigated for their potential *in vivo* application in controlled drug release and/or magnetic hyperthermia.

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Detection and Inhibition of Mutated GNAQ Gene Using Spherical Nucleic Acids

Ana Latorre[a], Christian Posch[b], Alfonso Latorre[a], Michelle B. Crosby[b], Anna Celli[b], Igor Vujic[b], Martina Sanlorenzo[b], Gary A. Green[b], Jingly Weier[b], Mitchell Zekhtser[b], Jeffrey Ma[b], Gabriela Monico[b], Devron H. Char[b], Denis Jusufbegovic[b], Klemens Rappersberger[b], Susana Ortiz-Urda[b]. Álvaro Somoza[a]

[a] Instituto Madrileño de Estudios Avanzados en Nanociencia (IMDEA Nanociencia), & CNB-CSIC-IMDEA Nanociencia Associated Unit "Unidad de Nanobiotecnología"

[b] University of California San Francisco, Mount Zion Cancer Research Center, 2340 Sutter Street, San Francisco, USA

anabelen.latorre@imdea.org

Abstract

Uveal Melanoma (UM) is one of most common tumors of intraocular malignancies. In 90% of the cases, UM is generated due to a single point mutation of GNAQ gene. Currently, the diagnosis of this disease is based on morphological changes of medium-large sized lesions, which are prone to be disseminated to other organs. What is more, the treatment of this metastatic tumor is for now ineffective [1]. Therefore, the development of systems allowing an early detection and treatments of UMs could improve greatly the survival of the patients.

Spherical nucleic acid conjugates (densely oligonucleotide functionalized AuNPs), are nanostructures that present remarkable properties for biomedical applications such as high colloidal stability and biocompatibility. In addition, they show an excellent cellular uptake and due to the negative charged shell, the oligonucleotides are largely protected from nuclease degradation. These properties make them ideal systems to develop biosensors and delivery systems of drugs and oligonucleotides [2].

In this study, we report a single point mutation gene sensor using AuNPs modified with fluorescent Molecular Beacons (*Figure 1a*). Molecular Beacons were designed to target specifically the mutated GNAQ gene, which can be visualized due to an increase in the fluorescent signal. In addition, we have developed a new release system of siRNAs from AuNPs (*Figure 1b*), which have shown excellent efficiency to downregulate the mutated GNAQ gene and decrease the cellular viability.

Results showed in this work, evidenced that spherical nucleic acid are excellent new tools to detect the presence of mutated GNAQ gene and modulate its expression [3].

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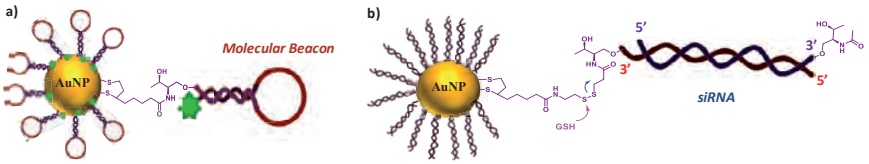

Figure 1: **a)** Schematic structures of AuNPs modified with fluorescent Molecular Beacon. **b)** New approach of delivery system of siRNA targeting GNAQ gene.

Acknowledgments: IMDEA Nanociencia and Asociación Española Contra el Cancer (AECC) are acknowledged for financial support.

venue

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28049 Madrid
Spain

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From Airport:

- Underground: Line C8 (to Nuevos Ministerios) y then Line C4A y C4B Local (Atocha, Nuevos Ministerios, ó Chamartín). Cantoblanco Universidad. Station
- Bus: Intercity services: Lines 827/828 (T4); Urban services: Line 204 (T4) and Line 200 (T1, T2, T3) to Avenida de América.

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From Airport:

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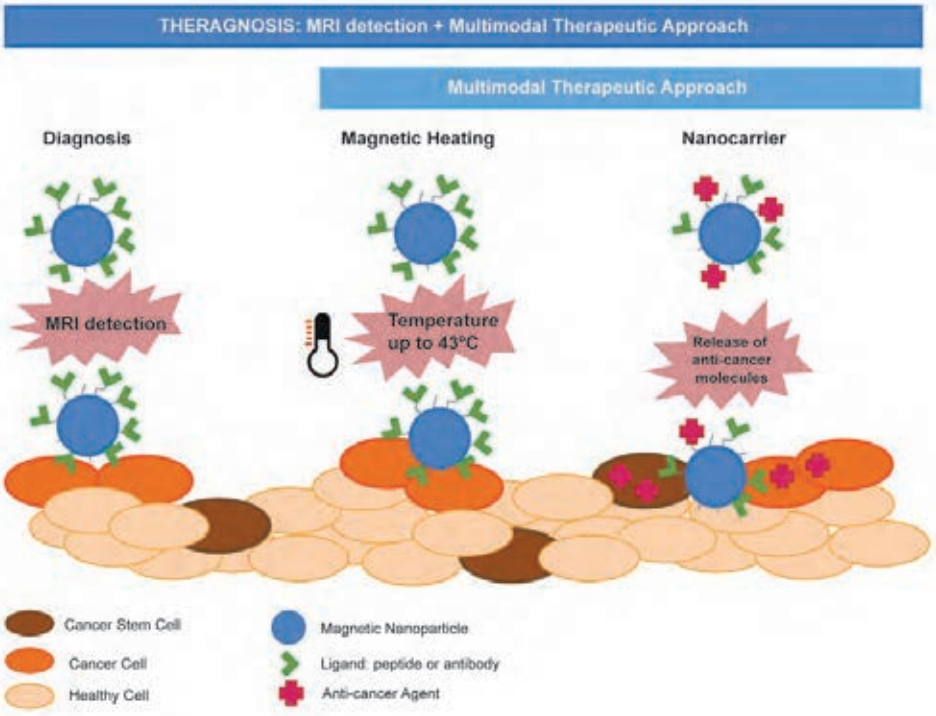

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The research is partly funded by the European Community's Seventh FrameworkProgramme under grant agreement no. 262943.

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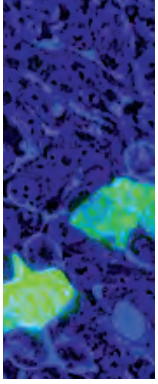

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