Dear Delegates,

It is a great pleasure and extreme honor to welcome you to Cyprus on the occasion of the Second International Conference on Nanotheranostics - ICoN2015.

In the vast field of nanomedicine, “Theranostics” combine therapeutics and diagnostics, aiming to provide a comprehensive platform for diagnosis, therapy and monitoring of the patient, leading to customized approaches and personalized treatment. Emerging nanotechnology discoveries provide a unique opportunity to design and develop such combination agents, permitting the delivery of therapeutics and concurrently allowing the detection modality to be used not only before or after but also throughout the entire treatment regimen, defining new supra-disciplinary fields in major clinical specialties such as Radiology, Surgery, Neurology and Oncology, to mention few. A Nanotheranostics Conference is an important initiative, expected to provide the forum for idea exchange and create a potential high-impact nanomedicine paradigm. Inspired by the success of ICoN2013- the 1st International Conference of Nanotheranostics, ICoN 2015 aims to provide the optimal venue to expand nanotheranostics research in a multidisciplinary environment which will bring together all the key researchers in the field.

ICoN2015 has attracted the contribution of prominent entities in the Nanomedicine field and has succeeded in bringing together high-caliber researchers and a promising population of young scientists at the dawn of their career from all five continents. It combines thoughtfully topic-oriented plenary and keynote lectures with innovative research papers. Major thematic areas include (i) the roadmap of nanotheranostics development in a patient-oriented approach, (ii) emerging challenges for nanotheranostic applications, (iii) nanoscience technologies for theranostic approaches, (iv) an emphasis session on cancer nanotheranostics, (v) toxicology, regulatory issues and ethics. Specially designated sessions on (i) breaking through cellular and biological barriers and (b) facing challenges in thermotherapy are reflecting translational challenges under EC-funded schemes. Featuring two specially designated events, the “Meet with the Professor Session”, wholeheartedly devoted to the younger generation of Theranostics scientists and a Clinically-oriented seminar opening horizons towards clinical translation, ICoN2015 is expected to set the grounds for the establishment of a lasting inter-sectoral and trans-disciplinary network on novel diagnostics and therapeutics both locally and on an international dimension.

Cyprus, an island of nano-scale dimensions from a global prospective, but with tremendous potentials when it comes to creativity and effectiveness, is certainly the place for a Nanotheranostics Conference to be! Limassol, the “Princess of Mediterranean” has attracted and assimilated onto its Hellenic trunk, cultures from around the world, has hosted Saints and Crusadors and is now waiting to serve you its splendid flavor. Surrounded by pre-historic, Hellenistic, Roman and Byzantine monuments the city of wine is laying for you to discover.

I wholeheartedly wish that you make the most of ICoN2015 and take home a unique scientific and cultural experience.

Andreani D. Odysseos
General Chair, ICoN2015
PLENARY LECTURE:

IEEE Engineering in Medicine & Biology Distinguished Lecture
Drug Delivery and Ultrasound
Ghaleb Husseini, American University of Sharjah, United Arab Emirates

BIOGRAPHICAL SKETCH
Dr. Ghaleb A. Husseini graduated with a PhD in Chemical Engineering (Biomedical Engineering emphasis) from Brigham Young University in 2001 and joined the American University of Sharjah (AUS in the United Arab Emirates) as an Assistant Professor in the Chemical Engineering Department January 2004. He has been elected into the Distinguished Lecturer Program- IEEE-EMBS (Jan 2014-Dec 2015). He was promoted to Associate Professor and Professor in 2008 and 2013, respectively. Four years ago, Dr. Husseini took a sabbatical leave which enabled me to travel to Ecole Polytechnique Fédérale de Lausanne (EPFL, and work in Dr. Jeffrey Hubbell’s laboratory).

Dr. Husseini has published 76 journal articles (in addition to 1 book chapter and 1 patent) and 40 conference papers/abstracts. In addition, he was a Theme Editor for a special issue in Advanced Drug Delivery Reviews and is currently serving on the Editorial Board of the International Review of Applied Sciences and Engineering (IRASE).

ABSTRACT
Chemotherapy is the most extensively used treatment in the fight against malignant neoplasms. Unfortunately, chemotherapy use is plagued with numerous side effects. These side effects are caused primarily because of the non-specific nature of the treatment as the drug is capable of killing normal and cancerous cells alike. Several drug delivery systems have been investigated to reduce these side effects by encapsulating the chemotherapeutic agent in a nano-sized carrier until it reaches the tumor site. These carriers include: solid nanoparticles, micelles, liposomes and e-liposomes. Once the nanoparticle reaches the desired location, ultrasound is applied to release the chemotherapy drug directly to the cancer site, thus avoiding any interaction with the healthy cells in the body. This way the adverse side effects of chemotherapy are minimized.

This presentation will discuss two novel chemotherapy carriers (micelles and emulsion-Liposomes) used in conjunction with acoustic radiation to treat malignancies.
PLENARY LECTURE:

Health and Safety Challenges in Nanotheranostics: An Update from the Biomaterials Perspective

Rena Bizios, University of Texas at San Antonio, United States of America

BIOGRAPHICAL SKETCH

Rena Bizios is a Peter T. Flawn Professor in the Department of Biomedical Engineering at the University of Texas at San Antonio, San Antonio, TX. She earned her B.S. (Cum Laude) degree in Chemical Engineering from the University of Massachusetts (Amherst, MA), M.S. degree in Chemical Engineering from the California Institute of Technology (Pasadena, CA), and Ph.D. degree in Biomedical Engineering from the Massachusetts Institute of Technology (Cambridge, MA). She has pursued an academic career. Professor Bizios’ research interests include cellular and tissue engineering, tissue regeneration, biomaterials (including nanostructured ones) and biocompatibility. She has co-authored a textbook (entitled An Introduction to Tissue-Biomaterial Interactions), co-edited a book (Biological Interactions on Material Surfaces: Understanding and Controlling Protein, Cell and Tissue Responses), authored/co-authored 108 scientific publications and book chapters, and is co-inventor of several patents/disclosures. Professor Bizios is a member, and has been an active participant (including elected officer positions) in several professional scientific/engineering societies. She is a member of the editorial board of five scientific/engineering journals.

Professor Bizios’ contributions to education and her research accomplishments have been recognized by the: Rensselaer Alumni Association Teaching Award (1997); Clemson Award for Outstanding Contributions to the Literature by the Society for Biomaterials (1998); Distinguished Scientist Award by the Houston Society for Engineering in Medicine and Biology (2009); Women’s Initiatives Mentorship Excellence Award by The American Institute of Chemical Engineers (2010); Founders Award by the Society for Biomaterials (2014); Theo C. Pilkington Outstanding Educator Award by the Biomedical Engineering Division, American Society for Engineering Education (2014); Amber Award, The UTSA Ambassadors, The University of Texas at San Antonio (2014); and by her election as Charter Member of the Academy of Distinguished Researchers, The University of Texas at San Antonio (2015). Professor Bizios is Fellow of five professional scientific/engineering societies, specifically, the American Institute for Medical and Biological Engineering, International Union of the Societies for Biomaterials Sciences and Engineering, Society of Biomedical Engineering, American Institute of Chemical Engineers, and of the American Association for the Advancement of Science.

ABSTRACT

The advent of nanostructured materials has set off major new developments in the biomaterials field because these materials have properties similar to those of physiological tissues (most of them characterized by surface grain sizes in the nanometer range), exhibit unique chemical and physical properties, and have untapped potential for novel biomedical applications. In the case of prosthetic, implantable devises, for example, nanostructured biomaterials provide promising alternatives to conventional ones because the nanostructured formulations promote interactions of proteins that mediate subsequent mammalian cell functions which are select, specific, and different that those observed on conventional materials of the same chemistry.

The potential of nanostructured biomaterials for clinical applications, however, cannot be fulfilled before several current challenges are successfully addressed and resolved. In order to design and fabricate the next generation of nanostructured biomaterials for implant applications pertinent material structures and properties, material synthesis and preparation methodologies, must be developed and standardized. In addition, the underlying mechanisms of biomolecule and cell interactions with nanostructured material must be elucidated, understood, and integrated into biomedical applications. Most importantly, the biocompatibility and safety of nanoparticles (produced by either bio-resorbable or biodegradable implant materials) must be definitively established prior to clinical use of nanostructured materials. The aforementioned and related issues provide justification, motivation, and opportunities for current and future research endeavors in the field of nanostructured biomaterials for a wide scope of biomedical applications. Relevant health-related safety concerns have prompted various national/international academic, government, and research organizations to initiate efforts towards standardizing methodologies and analyses as well as coordinating data interpretation and assuring dissemination of pertinent scientific information. Such endeavors aim at establishing international standards to assure the biosafety of nanostructured materials which are used for clinical diagnostic purposes and therapeutic treatment applications.
Keynote Lectures:

**Design considerations for nanotherapeutics in oncology**
Triantafylllos Stylianopoulos, Cancer Biophysics Lab, University of Cyprus, Cyprus

**ABSTRACT**

Nanotherapeutics have improved the quality of life of cancer patients, primarily by reducing the adverse effects of chemotherapeutic agents, but improvements in overall survival are modest. This is in large part due to the fact that the Enhanced Permeability and Retention effect, which is the basis for the use of nanoparticles in cancer, can be also a barrier to the delivery of nanomedicines. A careful design of nanoparticle formulations can overcome barriers posed by the tumor microenvironment and result in better treatments. In this review, we first discuss strengths and limitations of clinically-approved nanoparticles. Then, we evaluate design parameters that can be modulated to optimize intratumoral delivery. The benefits of active tumor targeting and drug release rate on intratumoral delivery and treatment efficacy are also discussed. Finally, we suggest specific design strategies that should optimize delivery to most solid tumors and discuss under what conditions active targeting would be beneficial.

**Conception of Nucleic Acid-Based Nanodrugs**
Jan Mollenhauer, Lundbeckfonden Center of Excellence NanoCAN and Molecular Oncology, University of Southern Denmark, Odense, Denmark

**ABSTRACT**

In the recent years the versatility of nucleic acids for rational drug design dramatically expanded. siRNAs, for example, are suitable for targeting every desirable human gene, including those commonly considered as non-druggable. miRNA mimics and inhibitors can be utilized to supplement or inactivate cellular miRNAs, which represent key regulators of important cellular processes. Aptamers can be designed to target cellular surface structures for inhibition or therapeutic delivery. Nucleic acids, based on size and chemical nature have the advantage to escape common drug resistance mechanisms in cancer and thus may qualify for designing novel drugs that are able to eliminate the so-called cancer stem cells (CSCs). The CSCs are a stem-like tumor subpopulation with high tumorigenic, metastatic and drug resistance potential, so that they are thought to be responsible for cancer recurrence.

Our main strategy is to identify sites of vulnerability in cancer cells/CSCs with special focus on breast cancer. For this purpose, we set up a pipeline, starting from the systematic functional evaluation of mutations found by molecular profiling of breast cancer. The knowledge is then used to design systematic high-throughput screens for CSC-targeting nucleic acid-based drugs. A first pilot screen identified 27 miRNA inhibitors with selective killing activity for breast CSCs versus normal, tissue-matched stem cells. It will also be introduced into our initial approaches to characterize aptamers for delivery to cancer cells/CSCs and to design peptide-nucleic acid nanoparticles for delivery purposes.
SESSION I – EMPHASIS SESSION: CHALLENGES IN THERMOTHERAPY
in Collaboration with COST Action TD1402-RADIOMAG

Chairs: NT Thanh (UK), E. Tombácz (HU)

I-1. The hyperthermia effect in magnetosome suspension
Milan Timko1*, Matus Molcan1, Hubert Gojzewski23, Andrzej Skumiel4, Silvio Dutz5, Peter Kopcansky1, Ladislau Vekas6

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KEYWORDS
magnetosomes; hyperthermia; Specific Absorption Rate

ABSTRACT
Bacterial magnetosomes were isolated from Magnetotacticum Spirillum-AMB1 bacteria. Two samples were compared: magnetosomes normally prepared of a “standard” length (IM) and magnetosome of a short length (SM). Chains of magnetosomes are shortened by mechanical cleavage by means of centrifugation and sonication treatment. The centrifugation for 30 minutes at 18000 rpm of the rotation speed and sonication process at 20 kHz at power of 120 W for 3 h in a constant power mode. This procedure leaded to chains shortening. Second, to reduce the chains aggregation after cleavage, the sample was ultrasonically treated in the sweep power sonication mode at 80 kHz at power of 180 W for 5 h in the bath. The effect of the sonication was analyzed using transmission and electron microscopy, atomic force microscopy, and dynamic light scattering. Scanning imaging reveals three types of shortening effect in SM sample, namely, membrane collapse, membrane destruction, and magnetosomes cleavage along its length with a comparable contribution of these effects in the sample. Dynamic light scattering showed a reduction of hydrodynamic diameter in SM sample. Magnetic properties of magnetosome were analyzed in DC and AC magnetic field based on evaluation of quasistatic hysteresis loops (energy losses) and calorimetric hyperthermia measurements (specific absorption rate), respectively. SM sample magnetically behaves in a different manner, showing that energy loss and specific absorption rate are noticeable reduced, and thereby indicates variation in the relaxation process and heat distribution. This showed that SM type of magnetosomes can be auspicious material for application in hyperthermia, primarily in cancer treatment, as the short chain magnetosomes demonstrates good distribution and penetration properties providing an uniform heating in the tumor tissue.

ACKNOWLEDGMENTS
This work was supported within the COST Project RADIOMAG Action TD1402, VEGA 0043 and 0045 and Ministry of Education Agency for structural funds of EU in frame of projects Nos. 26110230097 and 26220220186.
I-2: Comparing PEGylated soft- and condensed clusters of magnetite nanocrystals for magnetic hyperthermia application

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KEYWORDS
magnetic hyperthermia; magnetic nanocrystals; magnetic iron oxides; magnetite; clustered colloids; superstructure

ABSTRACT
Magnetic hyperthermia has evolved into one of the key sectors of magnetic theranostics, with established clinical use,1 while continuous development of new particle systems push its limits even further.2 Although well studied, ferrofluids based on magnetic iron oxide nanoparticles attract considerable interest, since they are an excellent biocompatible hyperthermia substrate. Profound research in this field gave a better understanding on the importance of crystal shape3 and superstructure of magnetic colloids and nanocarriers,4 when employed for anti-cancer treatment in combination with magnetic hyperthermia. In this work we report on in vitro magnetic hyperthermia experiments studying magnetic PEGylated soft clusters and condensed clusters. The former pertain to colloidal iron oxide nanocrystallites where the polymer coating (polymethacrylic acid-graft-polyethylene glycol), acting as a bridge, connects the otherwise independent nanocrystallites. In the latter case, the nanocrystallites are organized in clusters of condensed motif and with their crystallographic planes oriented in parallel to each other.5 Both particle systems were fractionated to obtain the same hydrodynamic diameter, of about 80 nm (z-average). The static magnetic properties were recorded after tailoring the nanocrystallite diameter for both systems at the same value (~13 nm). By having the same diameter, their saturation magnetization was practically identical. Under these circumstances, we could probe the importance of the superstructure/organization of the nanocrystals in the two different colloidal architectures. Calorimetric measurements were thus performed under identical conditions in order to calculate the specific loss power of both particle systems for different concentrations. The results clearly show that the nanocrystallites in the softclustered system display dramatically higher SAR and ILP values than their condensed-clustered counterparts. However, their performance as T2 MRI contrast agents and their response to magnetic gradient fields, as deduced with magnetophoresis, displayed the opposite trend.

REFERENCES
I-3: Synthesis and characterization of a magnetic nanosystem for application in thermo-chemotherapy of cancer

HERVAULT, Aziliz; MAY, Lim; DUNN, Alexander; TANIKE, Toshiaki; MATSUMURA, Kazuaki; MOTT, Derrick; MAENOSONO, Shinya; THANH, Nguyen Thi Kim*

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KEYWORDS
magnetic hyperthermia; thermoresponsive polymer; magnetic nanoparticles; synthesis of magnetic nanoparticles; characterisation of magnetic nanoparticles

ABSTRACT
Magnetic nanoparticles (MNPs) have emerged as promising technology for their applications in biomedicine. MNPs offer the possibility to deliver drugs and heat at specific locations and to benefit from the synergistic effect of the combined thermo-chemotherapy. The development and characterization of the nanosystem are therefore important steps to ensure that the nanosystem has the desired properties to be used for both magnetic hyperthermia and drug delivery. For example, functionalisation of the MNPs with a suitable polymer layer can provide biocompatibility, colloidal stability, drug loading capability and stimuli-responsive behavior.

This work aims to develop a dual pH- and thermo-responsive magnetic nanosystem allowing for the triggered release of chemotherapeutic drugs as a consequence of hyperthermia and acidic tumor microenvironment. Iron oxide NPs were synthesized by a microwave-assisted co-precipitation method. The thermo-responsive polymer p(DEGMA-co-OEGMA-b-[TMSPMA-co-VBA]), synthesized using RAFT polymerization, was then grafted onto the MNPs surface via a silanisation reaction. The nanocomposites were characterized by XRD, SQUID, TEM, DLS and TGA. Finally, the heating performances of the nanohybrids were investigated.

The MNPs exhibit a superparamagnetic behavior with a saturation magnetization around 70 emu/g. The LCST of the polymer could be easily tuned by varying the initial monomers ratio to be in the hyperthermia temperature range. FTIR analyses confirmed the successful grafting of the polymer by the presence of characteristic carbonyl ester bonds at 1750 cm\(^{-1}\). By varying the MNPs to polymer ratio and the pH of the solution during the functionalization step, suspensions with long term (be specific how many days?) colloidal stability could be obtained. Their potential as magnetic hyperthermia agents was confirmed, demonstrating at the same time the importance of colloidal stability on the heating performances.

REFERENCES
I-4: Study of magnetic nanoparticles modified by polyethylene glycol

Vlasta Zavisova*, Martina Koneracka, Matina Kuboveckova, Iryna Antal, Alena Jurikova, Jozef Kovac, Peter Kopcansky
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KEYWORDS
Magnetic particles; Biocompatibility; Characterization; Polyethylene glycol

ABSTRACT
Control of particle size distribution and functionalization techniques are critical steps needed to effectively implement the use of magnetic nanoparticles in medical applications. Polyethylene glycol (PEG) modified surface resists protein adsorption and therefore the circulation of PEG-functionalized magnetic nanoparticles in blood stream is prolonged. Moreover PEG is non-immunogenic, non-toxic, non-antigenic, biocompatible and soluble in water and organic solvents. For this reason the PEG was chosen to modify magnetic nanoparticles surface in magnetic fluid (MF).

The freshly prepared magnetite nanoparticles (Fe3O4) were stabilized by sodium oleate and modified by polyethylene glycol with molecular weight 2 kDa (MFPEG). We have found that hydrodynamic diameter (measured by dynamic light scattering, DLS), zeta potential and isoelectric point depend on the amount of PEG used for magnetic nanoparticles functionalization. These properties were studied in the range PEG/Fe3O4 from 0.05 up to 20 (w/w). Ratio PEG/Fe3O4 up to 10 had no significant influence neither on magnetic particles hydrodynamic diameter nor their zeta potential. Increase of PEG content above value 10 caused increase of the hydrodynamic diameter and decrease of the zeta potential absolute value. Adsorbed amount of PEG on magnetic particles surface was determined by spectroscopic method. The SQUID measurements confirmed the superparamagnetic behaviour of magnetic particles at room temperature. Saturation magnetization of the MFPEGs was 0.9 Am2/kg and prepared MFPEGs contained 15 mg Fe3O4 per 1 ml. Morphology of the coated magnetic particles in MFPEGs studied by scanning electron microscopy (SEM) confirmed primarily smooth surface with mean diameter ca. 58 nm. In order to verify the coating formation on the surface of magnetic particles differential scanning calorimetry (DSC) for pure PEG, lyophilised MF (magnetic particles coated with sodium oleate), MFPEGs with different PEG to Fe3O4 ratios and physical mixture of PEG and MF, were carried out.

Prepared modified magnetic particles with PEG have high absolute values of the zeta potential (ca. -50 mV), suitable diameter and good colloidal stability. Biocompatibility of the prepared samples was proven by measuring of fluorescence intensity using Texas red–labelled bovine serum albumin (BSA) and is crucial for future hyperthermia treatment.

ACKNOWLEDGMENTS
This work was supported within the COST Project RADIOMAG Action TD1402, VEGA 0041, 0045, Slovak Research and Development Agency – contracts No. APVV-0742-10.
II-1: Protein-based hybrid magnetic nanoplatforms for theranostic applications

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KEYWORDS
protein assembly; protein capsules and nanoparticles; MRI; drug delivery; gene silencing; magnetic nanoparticles

ABSTRACT
The development of polymer-based hybrid nanoparticles has become central for the design of innovative and efficient theranostic nanoparticles. A key issue is the development of simplified approaches to design polymer nanocoatings at the surface of inorganic nanoparticles or to assemble polymer nanoparticles with improved properties compared with existing methods in terms of biodegradability, toxicity and processing. Furthermore, there are very few methods allowing the efficient synthesis of particles made of biomacromolecules especially proteins. Human proteins such as human serum albumin (HSA) are emerging as relevant building blocks to design non-toxic and efficient macromolecular nanocarriers. In coll. with the Univ. of Melbourne (Prof. F. Caruso), we pioneered an original approach using isobutyramid (IBAM) grafts to assemble non-covalently, protein-based hollow capsules and particles without the need of an additional cross-linking or other adjuvant. The process consists in a single adsorption step of proteins onto silica templates preally grafted with IBAM groups or derivatives (e.g., bromoisobutyramid, BrIBAM) followed by template removal[1]. The driving force for the adsorption is attributed to strong H-bonds between the IBAM interface and the polypeptide chains of the proteins. We applied this method towards the design of bioresponsive hollow capsules and particles made of a range of proteins, including enzymes, insulin and human serum albumin. [2] Furthermore, such carriers were shown to release chemotherapeutic drugs upon biological stimuli e.g. through protease degradation or reductive cytosolic mimetic conditions[3]. This approach was also demonstrated for the design of ca. 100 nm size multifunctional protein-based nanoparticles displaying simultaneously delivery of silencing RNA (siRNA) to cancer cells and magnetic resonance imaging (MRI) via the grafting of gadolinium complexes (Figure 1.A). The efficacy of such theranostic nanoparticles in gene silencing was demonstrated with a capacity of gene expression knockdown superior to 50% and a MRI contrast enhancement that would be suitable for visualization of targeted tumors (Figure 1B) [4].
In a recent work, we apply this protein nanoassembly method for the design of novel hybrid magnetic core-mesoporous silica nanoparticles loaded with antitumoral agents (doxorubicin, DOX), covered by a HSA shell to ensure biocompatibility, stealthiness and biodegradability. DOX is loaded by impregnation into the small pores of the silica shell and the original grafting of IBAM binders at the silica shell surface ensures a tight HSA coating and an efficient encapsulation of DOX. The efficient bio-responsive property of such HSA-coated core-shell NPs theranostic NPs in protease media mimicking intracellular lysosomes was shown through the efficient HSA shell biodegradation and subsequent DOX release. These new theranostic magnetic hybrid NPs are currently assessed in various biological studies (ca. cell viability/toxicity, cell uptake, intracellular behavior).

REFERENCES
II-2: Vesicles with Colloidal Particle Shells (Submicron Colloidosomes) from Mesoporous Silica coated Iron Oxide Nanoparticles

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KEYWORDS
colloidosomes; microcapsules; nanoparticles; mesoporous silica; superparamagnetism

ABSTRACT
Vesicles with colloidal particle shells can be assembled via several synthesis routes which are either based on Pickering-emulsification,[1] amphiphilic polymer-tethered nanoparticle self-assembly,[2] or by nanoparticle adsorption on hard colloidal templates.[3] The resulting microcapsules can be used to cargo drug molecules; while depending on the synthesis method, either hydrophobic or hydrophilic molecules can be encapsulated. Until shortly, nanoparticle vesicles formed via Pickering-emulsification were too big to be potentially used in drug delivery applications, as small sizes are essential, for example, to make use of the enhanced permeation and retention effect. We recently introduced vesicles with decreased sizes (submicron colloidosomes) based on a surfactant-assisted mini-emulsion synthesis, thus positioning colloidosomes within closer reach for biomedical applications. The submicron colloidosomes with positive or negative zeta-potentials and varying pore sizes were formed from metal oxide nanoparticles.[4] Subsequently, we demonstrated a simple integration of multiple nanoparticles featuring distinct properties (fluorescence and superparamagnetism) in a single colloidosome shell.[5] Our current research focuses on the integration of mesoporous silica coated iron oxide nanoparticles (Fe3O4@mSiO2) within the submicron colloidosome shell. Mesoporous silica particles have recently proven to be an excellent candidate for the targeted treatment of cancer cells by encapsulation of cytostatic agents within the porous network of the silica particles.[6] The Fe3O4@mSiO2 colloidosome forming nanoparticles feature particle sizes below 30 nm and are formed by a seed-growth method (Figure 1a – 1. Particle synthesis and Figure 1b) where iron oxide nanoparticles are employed as heterogeneous nucleation sites.[7] The resulting nanoparticle vesicles (Figure 1b – 1. Vesicle formation and Figure 1c) potentially allow a dual active agent encapsulation, within the shell-forming nanoparticle mesopores and within the aqueous colloidosome core. To further tailor our structures for specific theranostic applications, the iron oxide seed may be replaced by other nanoparticles such as quantum dots or gold nanoparticles. These nanoparticle vesicles have great potential for the field of nanotheranostics. They present a versatile building block system that can be fine tuned and tailored for the specific challenges faced in modern nanomedicine, and potentially serve as a great complement to liposome-nanoparticle hybrids.[8]

REFERENCES


II-3: Long Term Stability of Photoluminescent Mesoporous Nano Silicon for Theranostics Applications

Ali Ghafarinazari1*, Erica Locatelli2, Mauro Comes Franchini2, Federico Boschi1, Claudia Laperchia3, Paolo Cortelletti4, Marina Scarpa4, Nicola Daldosso1

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KEYWORDS
Mesoporous Silicon; in vitro; bioimaging

ABSTRACT
In this study, we report about the effects of biopolymers (PEG and chitosan) functionalization on luminescent mesoporous silicon (pSi). Photoluminescence stability in biological conditions, such as PBS buffer, has been demonstrated for the first time up to 90 days, also maintaining good optical quantum efficiency (from 1.7% to 1.1%).

The discovery of photoluminescence (PL) from pSi and the lack of evidence for its toxicity has stimulated a huge research of effective methods to provide silicon nanostructures functionalization for biomedicine. We recently demonstrated that light emitting pSi particles can be uptaken by human dendritic cells without any toxicity or reduction in cell viability and can be tracked in cells by fluorescence imaging in real time. Moreover, no stimulation of the secretion of pro-inflammatory cytokines has been found [1]. The pSi particle size is in the range of 3-10 µm with high porosity: about 30 nm the average diameter.

Emission of pSi in the orange-red regime is well-known due to quantum confinement. But this band is sensitive to quenching due to surface oxidation. To avoid it, carboxyl functionalization leads to stabilize emission for years without significant variation in the optical properties. Although pSi microparticles present an optical stability in ethanol for years at room temperature, the possible applications have been severely limited because of the incompatibility with water solutions, which leads to degradation of the material and to the loss of its optical properties, thus limiting its effective utilization as delivery system [2].

The pSi is prepared by anodization of p-type Si wafer in aqueous HF:ethanol solution; pSi microparticles were obtained by sonication and then suspended in acetic acid under illumination thus resulting in carboxyl groups on the surface as shown by FTIR measurements (pSi-COOH sample). Furthermore, polymeric covering carried out by HCl:H2N-PEG-COOH to covalent conjugation of PEG on pSi-COOH in different ratio was done together with chitosan functionalization. On the other hand, a diblock copolymer made of polylactic-co-glycolic acid and polyethylene glycol (PLGA-b-PEG) was selected for entrapment (pSi-PEG-PLGA).

Based on the shrinkage of silicon crystals during chemical reaction with biopolymers, PL is blue-shifted but maintaining an optical quantum yield efficiency of about 1-2%, as determined by comparative method.

For the first time, experimental results showed that chemically coverage by using biopolymers maintain the PL stability in in vitro (i.e. water and PBS buffer) for at least 90 days. Moreover, we demonstrated by two photon absorption technique that the system can be efficiently excited at biological window (700-810 nm).

The present findings are very promising for several biomedical applications such as bio-imaging, photo-thermal therapy and drug delivery, i.e. Theranostics.

REFERENCES

ACKNOWLEDGMENTS
This work has been done within the Nanomedicine Initiative funded by Fondazione Cariverona (Verona).
SESSION III - NANOSCIENCE TECHNOLOGIES FOR THERANOSTICS APPLICATIONS-II

III-1: Biological active surface modification and functionalization of magnetic nanoparticles

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KEYWORDS
nanostructures; nanoparticles; surface functionalization; magnetic materials

ABSTRACT
Nanotechnology became in XX century very popular area of science. It was caused because of the discovery of an amazing properties of materials in nano size. Due to that, especially interesting became particles which are bioactive in any way. Magnetic nanoparticles, are a special case of it, because of possibility of its easy manipulation by external magnetic field [1]. In addition superparamagnetic properties are very desirable from the application point of view. These days, magnetic nanoparticles like magnetite, hematite or maghemite, are best known structures investigated in nano form. Therefore derivatives of this compounds, become natural following candidates to study. Ferrites doped with other 3d metals like Ni, Co, or Mn exhibit different magnetic properties in comparison to pure Fe-oxide [3]. Magnetic core shows at room temperature various magnetic state, dependents on the substituted elements. When structure and magnetic properties of those ferrite nanoparticles are well described, surface modification and then functionalization can be made. Nanoparticles bond with enzyme or other active compound are known as nano- or bionanocomposites. Such structures can be widely used in medicine, pharmacy or environment protection [2]. Prior to in vitro studies all characteristic of such system should be find out. In this presentation we would like to show studies on ferrite nanoparticles doped with Ni, Co, or Mn 3d metals and based on that biocomposite fabrication. Their surface functionalization with glutaraldehyde, as first attempts prior to attachment of enzymes such as albumin, glucose oxidase, lipase, and trypsin will be discussed. We have investigated also, if in every case, surface modification with linker (here glutaraldehyde) is needed. Structural analysis of the obtained bionanocomposites were done by TEM, X-Ray diffraction, FTIR spectroscopy. Magnetic properties of the particles were investigated by Mössbauer spectroscopy.

REFERENCES
III-2: A Magnetically Responsive Drug-Delivery System Based on Nanoparticle Clusters

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KEYWORDS
nanoparticle clusters; iron oxide nanoparticles; hollow silica nanostructures; magnetic drug delivery system

ABSTRACT
Among the various inorganic materials investigated as promising drug-delivery systems over the past 10 years, porous and hollow silica-type nanostructures have received particular interest. Unlike polymeric composite materials and liposome-type formulations, silica-based materials offer a superior chemical stability, the possibility of simultaneously integrating both hydrophilic and hydrophobic molecules, and, at the same time, increased space available for carrying the active substances inside hollow nanostructures. However, despite all the advantages offered by hollow silica particles, there remains the scientific challenge of developing highly magneto-responsive, hollow, silica nanostructures for magnetically guided drug delivery.

We have developed a flexible approach to the preparation of magnetic, hollow silica nanostructures loaded with fluorescent dye molecules as model drugs (Figure). First, commercially available iron oxide nanoparticle clusters coated with a thin, porous and uniform silica layer (iNANOvative™| BIO silica) were used as a high-quality source material (1). Next, the iron oxide nanoparticles, as the building blocks for the clusters, were partially dissolved in concentrated HCl (4 M) in order to produce the hollow compartment for subsequent drug loading and, at the same time, retain the hollow nanostructure’s magneto-responsiveness due to the remaining, undissolved, iron oxide nanoparticles. Finally, these magnetic hollow nanostructures were loaded with fluorescent dye molecules, representing a model drug. The nanostructures and drug-loading efficacy were characterized with transmission electron microscopy (TEM), vibrating-sample magnetometry (VSM), dynamic light scattering (DLS), zeta-meter measurements, BET, TGA, and fluorescent microscopy.

REFERENCES
III-3: Synthesis of biocompatible dendritic nanoparticles for Multimodal Imaging

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KEYWORDS
Nanoparticles; Dendrons; MRI contrast agent; Biodistribution; Multimodal Imaging

ABSTRACT
As one of the newest areas of science, nano-scale science and technology are seen by many as the key technology of the 21st century, which of course raises the question as to what role this technology will play in medicine. An important area of nano-scale science is the development of nano-structured carriers for medical applications. Various colloidal inorganic nanoparticles that exhibit unique inherent properties such as fluorescence properties (e.g. semiconductor quantum dots (QDs) up-/down-conversion nanoparticles), magnetic properties (e.g. metal oxide nanoparticles) and plasmonic properties (e.g. noble metallic nanoparticles) have been widely explored, particularly for biological and medical applications. For instance, various types of magnetic nanoparticles have a widespread range of applications such as in magnetic resonance imaging (MRI) as T1 and T2 contrast agent, magnetically guided drug/gene delivery, magnetic hyperthermia and magnetic biosensors; up-/down-conversion nanoparticles and QDs and their niche in biological and medical imaging; noble metallic nanoparticles can be used for photothermal therapy and biosensing. Designing nanoparticles for molecular diagnosis and targeted therapy is of the utmost importance. The wish list of such systems is long: they should selectively home in on the cells and organs of the body that are involved in the disease process, specifically targeting their potent healing effects on these cells and organs, while sparing cells not involved in the disease process. They should be completely non-toxic, biodegradable or capable of natural excretion, not be recognized or eliminated by the body’s own immune system before they have reached their target, and not induce any allergic reactions. Ideally, they are generic, i.e. they can be “programmed” to combat a wide variety of diseases by docking onto any target structures one chooses and being capable of carrying any medicines.

We thus propose a concept combining a dendritic coating with phosphonate anchors. Indeed, phosphonates ensure a strong anchoring at the NPs surface while preserving their magnetic properties, and dendritic shells, in addition to their small and easily controllable size (as a function of their generation), are promising building blocks simultaneously solving the problems of biocompatibility, large in vivo stability and specificity. Iron oxide NPs synthesized by co-precipitation and thermal decomposition were coated with functional oligoethyleneglycol or poly(amide)amine (PAMAM) dendrons to improve colloidal stability, graft fluorophores and investigate cell interactions. Different grafting strategies were optimized as a function of the NPs synthesis and dendron nature. The size distribution, colloidal stability in isosmolar media, nature of surface complex, biodistribution and contrast enhancement properties evaluated through in vitro and in vivo MRI experiments were compared as a function of the nature of both dendrons and nanoparticles. All functionalized nanoparticles (whatever the synthesis method) display good colloidal stability in water but, in isosmolar media, best results were observed with functional dendronized NPs bearing carboxylates at their periphery. The contrast enhancement properties of all dendronized nanoparticles were found higher than those of commercial products (polymer-decorated) and the best values were recorded for the nanoparticles synthesized by coprecipitation due to their higher saturation magnetization. Moreover, no evident adverse effect was observed in rat after injection, even at high concentrations and a long time after injection. The biodistribution of such nanohybrids was also studied by optical imaging thanks to Alexa labelling at the dendron periphery. In this case, a fast hepatobiliary, together with a low urinary, elimination was observed. Luckily, no RES uptake could be highlighted. Such study confirmed the interest of the dendritic approach to develop new, smart and multimodal contrast agents.
III-4: A comparative study of functionalized single-core and multi-core magnetic particle systems for biomedical applications

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KEYWORDS
magnetic particles; MRI contrast enhancement; magnetic hyperthermia

ABSTRACT
Magnetic particle systems have gained high interest in the field of biomedicine because they provide advanced therapeutic and diagnostic capabilities with dual-mode manipulation controlled by a magnetic field and through an appropriate design of surface properties [1]. The high magnetic moment of the functionalized magnetic particles is among the most important requirements for successful applications in biomedicine. We report a comparative study of different core-shell type magnetic particles systems with tunable size, morphology, magnetic and surface properties designed for biomedical applications. Different synthesis methods have been used to prepare single-core or multi-core magnetic particle with organic coating shells to provide chemical and colloidal stability in biological media. High magnetization single-core magnetite nanoparticles coated with glycerol phosphate or ascorbic acid phosphate have been prepared by co-precipitation method. Due to the presence of the hydroxyl groups on the surface these nanoparticles were used either to attach further biomolecules (biotin, glucose) or to initiate ring opening polymerization of lactones [2]. The multi-core magnetic particle systems have been obtained by oil-in-water miniemulsion technique using highly stable ferrofluid [3] or using solvothermal procedure. Densely packed clusters of magnetite nanoparticles form the core coated with polyethylene glycol or Pluronic 68 or polyacrylic acid. The physical-chemical properties of the magnetic nanocarriers have been investigated by transmission electron microscopy (TEM), X-ray photoelectron spectroscopy (XPS) and magnetization measurements. TEM investigations show the close packing of magnetite nanoparticles into well defined multi-core spherical particles with sizes in the range 50-200 nm, while for single-core nanostructures the magnetic core (mean diameter 10 – 13 nm) is covered with a thin organic shell. The magnetic particle systems show superparamagnetic behaviour at room temperature and relatively high saturation magnetization values (50-70 emu/g). Two standard in vitro assays, blood sedimentation (i.e., erythrocyte sedimentation rate) and peripheral blood smear tests were used to assess the hemocompatibility of the synthetized samples. Their MRI contrast enhancement and magnetic hyperthermia efficiencies were tested in a clinical MRI apparatus at 1.5 T and a magneTherm™ instrument in AC field at different frequencies, respectively. The preliminary experiments revealed a promising theranostic potential of the studied magnetic nanocarriers.

REFERENCES
SESSION IV - EMERGING CHALLENGES FOR NANOTHERANOSTICS APPLICATIONS-I

Chairs: G. Constantinide (CY), L. Vekas (RO)

IV-1: Facile autoclave polyol synthesis of superparamagnetic iron oxide nanoparticles as potential MRI contrast agents for tissue engineering

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KEYWORDS
MRI; Nanoparticle synthesis; Tissue engineering

ABSTRACT
Monodisperse iron oxide nanoparticles (IONPs) were obtained through a simple polyol synthesis in high pressure and high temperature conditions. The size and morphology of the nanoparticles can be tuned by varying the solvents, the amount of iron acetylacetonate Fe(acac)3 and the reaction times. Compared with other common synthetic methods such as thermal decomposition or co-precipitation, this new process yields monodisperse nanoparticles in a simple, reproducible and cost effective manner without the need for an inert atmosphere. The surface of the IONPs could be tailored with various ligands post synthesis which provided functionality and stability in water, PBS buffer, high electrolyte concentrations or cell culture medium. Phantom experiments on the contrast agent (clinical 3 T MRI scanner) revealed an enhanced ratio r2/r1 when compared to commercially available NPs. The biocompatibility with biological were performed with primary human mesenchymal stem cells (hMSCs) confirmed the suitability of IONPs for tissue engineering.
IV-2: Magnetoactive electrospun (nano)fibers in biomedical applications

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KEYWORDS
electrospinning; magnetoactive fibers; iron oxide nanoparticles; drug delivery; magnetic hyperthermia applications.

ABSTRACT
Electrospinning is a simple, and highly versatile fiber fabrication technique used for the production of (nano)fibrous materials. It can be employed for the preparation of pristine polymer fibers as well as polymer-based fibrous nanocomposites via the combination of polymers with inorganic nanofillers. Among others, magnetic nanoparticles, are highly attractive due to their potential applications in the biomedical arena including magnetically-triggered drug delivery, tissue engineering, magnetic bioseparation, biosensing, hyperthermia cancer treatment and MRI contract enhancement.

In this presentation different literature-reported fabrication routes for generating magnetoactive electrospun polymer-based (nano)fibers will be briefly discussed [1] whereas specific emphasis will be given on the fabrication and characterization of PEO/PLLA/Fe₃O₄ magnetoactive electrospun membranes that were recently developed in our group and their potential applicability in drug delivery and magnetic hyperthermia processes [2].

ACKNOWLEDGEMENTS
This work is supported by the University of Cyprus and the Center for Fundamental and Advanced Technical Research of the Romanian Academy at Timisoara.

REFERENCES
IV-3: Hemocompatibility of Albumin microspheres as Drug delivery system, 
In vitro study

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KEYWORDS
Hemocompatibility; Albumin; Microspheres

ABSTRACT
The main objective of the present work is to evaluate the Hemocompatibility of albumin microspheres. Albumin microspheres were prepared by coacervation method. The characteristics of Albumin microspheres such as particle size, particle morphology, and drug loading were evaluated. That coacervation method is well suited to produce albumin microspheres and the preparative variables of the procedure can be fine tuned depending on the clinical application.
IV-4: Electrospun PEO/PPLA fibrous membranes for sustained tyrosine kinase inhibitors delivery in situ

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KEYWORDS
Electrospinning; drug release studies; in vitro application; anti-EGFR targeted therapies; solid tumors

ABSTRACT

Background
Electrospinning has gained huge attention due to its capability to prepare nanofibers with tailored characteristics, rendering drug-loaded membranes suitable for biomedical applications. Such membranes enable us to treat locally minimally residual disease along surgical margins in very invasive and malignant tumors where biological barriers render them inaccessible to conventional therapies and targeting is not possible.

Objectives
Our aim, herein, has been to (i) achieve the fabrication of functional Tyrosine-Kinase Inhibitor (TKI)-loaded electrospun nanofibrous membranes, and (ii) provide evidence that they may be further developed for biomedical applications.

Methods
Before the assessment of TKI delivery and anti-cancer efficacy of these membranes in cell culture, drug release studies were conducted, by UV-visible spectroscopy, in an environment mimicking tissue culture conditions to evaluate the kinetics and mechanism of drug release and the quantity of the released drug was evaluated. Studies on potential cytotoxic effects followed, to evaluate their impact on human health under extended use. Towards this objective, cytotoxicity studies were carried out to estimate membranes potential cytotoxic effects by testing the short and long time exposure of cells to unloaded scaffolds. Colon cancer and glioblastoma cell lines with differential expression of the Epidermal Growth Factor Receptor (EGFR), a well characterized membrane tyrosine kinase, were used in this study. The in vitro cytotoxicity induced by unloaded PEO/PLLA scaffolds was determined using two different assays: the short-term assay (MTT) and the long-term (up to 3 weeks) clonogenic assay to estimate more accurately the potential cytotoxicity of degradation products. Subsequently, the antiproliferative and pro-apoptotic efficacy of the nanomembrane released inhibitor was assessed against that of i) equivalent concentration of free TKI and ii) exposure (short/long term) to PEO/PLLA scaffolds.

Results
Data revealed that following incubation in the presence of unloaded PEO/PLLA membranes for 5 days and MTT colorimetric analysis, cell viability was substantially unaffected. Additionally, the results of clonogenic assay performed under the same conditions remarkably disclosed no significant effect on cloning efficiency. PEO/PLLA TKI-loaded scaffolds demonstrated comparable antiproliferative efficacies when tested against equivalent concentrations of free TKI. Furthermore, it was shown that cancer cell death was induced only by the released TKI and not from scaffolds degradation products. This demonstrates that exposure to drug-loaded fibrous PEO/PLLA membranes can cause decrease of cell viability as a function of released drug and time of exposure.

Conclusion
In conclusion, drug-loaded PEO/PLLA nanofibrous membranes can provide similar therapeutic effects to the free-drug therapeutic levels, while no toxicological problems were induced from the polymer degradation byproducts. Hence, they consist a promising drug delivery system in cancer therapy, increasing the therapeutic benefit and minimizing the side effects.

ACKNOWLEDGEMENTS
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SESSION V– EMPHASIS SESSION ON CANCER THERANOSTICS-I

Chair: C. Pitris (CY)

V-1: Quatro targeted Stimuli Nanocontainers: In-vitro and in vivo study

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KEYWORDS
Multistimuli; nanocarriers; theranostic

ABSTRACT
A versatile drug that responds to external stimuli is synthesized via the emulsion polymerization process. This facile two-step process is carried out by using Poly (Methyl Methacrylate) as a soft template and a series of monomers with desired properties as coating materials. The highlight of this work is the ability of carriers to target the cancer cells through their physicochemical characteristics in which the carrier is sensitive. An important key step of this work is the formation of an inner cavity inside the microspheres during shell fabrication (2nd step). The monomers that are used in the coating procedure are monomers with thermo-, pH- and redox sensitivity. The surface of the multi-stimuli nanocontainers is functionalized with magnetite nanoparticles in order to attach sensitivity in external alternating magnetic field (AMF). By using AMF in various strengths and frequencies, the temperature of the final multi-stimuli microcontainers (Q-Spheres) increases in a control manner resulting in hyperthermia phenomenon. The NCs are further modified by specific agents such folic acid and leuprolide for specific targeting. Loading and release studies are carried out by using the anthracycline anticancer drug Doxorubicin and their cytotoxicity is evaluated by using the MTT assay. In vivo studies were performed in order to evaluate the biodistribution, pharmacokinetics and toxicity per se by intravenously injection.
V-2: Photodynamic Quenched Cathepsin Activity Based Probes for Cancer Detection and Macrophage Targeted Therapy

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KEYWORDS
Cathepsins; Photodynamic Therapy; Activity-Based Probes; Macrophages; Non-Invasive Imaging

ABSTRACT
Elevated cathepsins levels and activities are found in several types of human cancer, making them valuable biomarkers for detection and targeting therapeutics. We designed small molecule quenched activity-based probes (qABPs) that fluoresce upon activity-dependent covalent modification, yielding cell killing by Photodynamic Therapy (PDT). These novel molecules are highly selective theranostic probes that enable both detection and treatment of cancer with minimal side effects. Our qABPs carry a photosensitizer (PS), which is activated by light, resulting in oxidative stress and subsequent cell ablation, and a quencher that when removed by active cathepsins allow the PS to fluoresce and demonstrate PD properties. Our most powerful and stable PS-qABP, YBN14, consists of a selective cathepsin recognition sequence, a QC-1 quencher and a new bacteriochlorin derivative as a PS. YBN14 allowed rapid and selective non-invasive in vivo imaging of subcutaneous tumors and induced specific tumor macrophage apoptosis by light treatment, resulting in a substantial tumor shrinkage in an aggressive breast cancer mouse model. These results demonstrate for the first time that the PS-qABPs technology offers a functional theranostic tool, which can be applied to numerous tumor types and other inflammation-associated diseases.
IV-1: Magnetic Nanocarriers for Specific Targeting and Noninvasive MR Imaging of IL4Ra Asthma Biomarker in the Lung

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KEYWORDS
Superparamagnetic iron oxide nanoparticles; Lung imaging; Magnetic Resonance Imaging; Specific targeting; Asthma biomarkers

ABSTRACT
Simultaneous inhibition of IL-4 and IL-13 via the common receptor chain IL4Ra represents a promising therapeutic approach to bring the additional relief required for asthma patients. Enhancing the ability of these blocking antibodies to be administered directly to the inflamed lung tissue will both improve treatment efficacy and decrease systemic side effects. In this study, we aimed to target IL4Ra asthma biomarker in the lung by developing an antibody-conjugated iron oxide based nanocarriers that would enhance the delivery of the antibodies specifically to the site of lung inflammation while allowing simultaneous noninvasive monitoring of inflammatory responses and tracking of the developed nanocarriers using MRI. Special attention was devoted to evaluate the biocompatibility of the polyethylene glycol (PEG) functionalized SPION-IL4Ra and the length effect of the PEG chain.

Biocompatibility assays which were performed to evaluate cell viability, mitochondrial membrane potential, reactive oxygen species generation and oxidative DNA damage after incubation with airway smooth muscle (ASM) cells confirmed the safety of the developed nanocarriers for pre-clinical investigations. For all the investigated formulations, nanocarriers were found to be greatly stable at neutral pH. However, the stability noticeably decreased with the PEG length in acidic environment and thus preferentially releasing the loaded antibodies.

Immunofluorescence and fluorimetry assays confirmed the binding of the nanocarriers to IL4Ra asthma biomarker. Challenging of ASM cells with either ovalbumin or IL-4 and to a high extent when combined was found to increase the expression of IL4Ra in ASM cells revealing the importance of this biomarker in asthma management and the feasibility of blocking with anti-IL4Ra antibodies conjugated magnetic nanocarriers.

Pulmonary MRI performed using ultra-short echo time (UTE) sequence allowed simultaneous noninvasive monitoring of inflammatory responses induced by ovalbumin challenge and tracking of the developed nanocarriers, which were found to co-localize with the inflammatory sites in the lung. Void signal dots related to anti-IL4Ra conjugated SPION were found to co-localize preferentially with the inflammatory regions induced by ovalbumin challenge. Histological data confirmed that, contrary to non-conjugated iron oxide nanoparticles, anti-IL4Ra conjugated nanocarriers distribution within lung tissue mostly co-localized with areas rich with IL-4Ra. This was also confirmed using flow cytometry revealing that most of the lung Th-2 CD4 and CD8 cells (i.e. > 70%) were targeted by the IL4Ra loaded nanocarriers, contrary to non-loaded nanoparticles. This data clearly confirm the successful targeting of the nanocarriers to IL-4Ra+ inflammatory cells.

The anti-IL4Ra conjugated nanocarriers developed here have been confirmed to be efficient in targeting key inflammatory cells during chronic lung inflammation following intrapulmonary administration and is expected to better control lung inflammation with a much lower side effect to alternative ordinary approaches including direct intrapulmonary administration of whole IL-4Ra antibodies.
IV-2: Breaking through the brain tumor barrier

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KEYWORDS
Blood Brain Barrier; Blood Brain Tumor Barrier; Glioblastoma Multiforme; Transcytosis; Nanomedicine

ABSTRACT

Blood Brain Barrier (BBB) is a neurovascular tissue unit composed by endothelial cells, pericytes, astrocytes, oligodendrocytes and neurons. Glioblastoma Multiforme (GBM) is a fast-growing, highly morbid type of brain malignancy and the most common adult primary intracranial neoplasm, arising from glia cells. GBM is characterized by microvascular proliferation and a highly abnormal dysfunctional vasculature. The GMB blood brain tumor barrier (BBTB) differs significantly from normal BBB morphologically, functionally and molecularly. The molecular and cellular transport mechanisms that govern the BBB and BBTB integrity and permeability during GBM tumorigenesis though remain elusive, limiting the development and generation of effective therapeutic agents that can cross and precisely target GBM cancer-generating cells. Multi-parametric characteristics such as lipophilicity, charge, low planar area and molecular weight of both macromolecules and nanoparticulate systems define the delivery potential across the BBB.

Innovative nanoparticulate systems show a promising therapeutic approach for overcoming this major hindrance. In order to study these pharmaceutical targeting methods, a series of prototyype in vitro and in vivo models have been generated recapitulating the development of GBM in human cerebral cortex. Standard histopathologic analysis and early angiogenesis marking with the PECAM-1, CD31 monoclonal antibody displayed that vascularization is highly patterned in the proliferative periphery of the tumor than in its necrotic core and normal brain region. Platelet Derived Growth Factor Receptor-beta (PDGFRbeta) positive pericyte progenitors and Claudin-5 positive endothelial tight junctional cells are highly expressed during BBTB development, compared with healthy BBB. Additionally, we identified that Major Facilitator Super Family Domain Containing 2a (MFSD2a), a well-known CNS-endothelial-cell vesicular transcytosis regulator that is expressed during BBB development in embryogenesis, is also highly expressed in the newly vascularized area of tumor models. It is therefore suggested that receptor-mediated transcytosis may play a crucial role in the differential uptake and transport of nanopaticulate systems across either BBB or BBTB.

Elucidating the cellular structures and molecular mechanisms underlying the delivery of nanoparticles through either healthy BBB or BBTB, both intact and disrupted, is expected to provide new insights in the physic-chemical characteristics required for effective targeting of both barrier structures and malignant cells throughout GBM tumorigenesis.

ACKNOWLEDGEMENTS

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SESSION VII - EMERGING CHALLENGES FOR NANOTHERANOSTICS APPLICATIONS-II

Chairs: T. Stylianopoulos (CY), J. Mollenhauer (DK)

VII-1: Relevance of physical, chemical and colloidal characterization in the potential theranostics application of SPIONs

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KEYWORDS
magnetic nanoparticles; physical chemical characterization; colloidal stability; theranostic application

ABSTRACT
Superparamagnetic iron oxide nanoparticles (SPIONs) seem to have great potential for diagnostic and therapeutic (theranostic) use due to their unique magnetic properties and inherent biocompatibility. The latter refers to the iron metabolism within a living organism, however, the interaction of SPIONs with any biological entities (e.g., proteins and cells in blood, cell membranes in tissue) acts through the solid-liquid interfacial layer and leads to the formation of protein coronas, particle wrapping, intracellular uptake and biocatalytic processes [1]. So the expected, mostly biocompatible outcome of SPIONs’ products unequivocally depends on their coating. In vivo experiments have showed the size, zeta potential (in relation with the sign and magnitude of surface charge) and dispersibility in biorelevant aqueous media (hydrophilic/hydrophobic feature) are the main parameters influencing nanoparticle biocompatibility [1]. Therefore, characterization of both the magnetic core and the biocompatible/bioactive coating of the fabricated SPIONs’ product is crucial in their biomedical use. All of their magnetic, physical chemical and colloidal parameters (single or multicore magnetic feature, saturation magnetization, size, shape and chemistry of SPION crystals, hydrodynamic size and charge state at the given composition of equilibrium aqueous phase, dissolved iron and organic matter content) and propensities (hydrophilicity, pH-dependent charging and salt tolerance), which might be needed in biomedical use have to be characterized by correct laboratory methods in details before expensive in vitro and in vivo testing. We have optimized the methods of physicochemical and colloidal characterization of our carboxylated SPION products; and some biomedical tests (hemocompatibility, cell proliferation, MRI contrast and hyperthermia efficiency) have been also applied [2,3]. The magnetic core was the same in each product and the carboxylated shells were also similar regarding their hydrophobicity and surface charge; and so a very similar behavior should be expected according to the understanding biophysicochemical interactions at the nanobio interface [1]. Nevertheless, we observed occasionally significant differences in both colloidal and biophysicochemical interactions. Though none of the products was cytotoxic, the intracellular uptake of carboxylated SPIONS was unexpectedly different [2, 3]. The protein corona on carboxylated SPIONs formed in the interaction with human plasma also somewhat altered. In the lecture, we attempt to explain the relevance of physical, chemical and colloidal characterization of SPIONs in their potential theranostic application through several novel examples for our carboxylated SPIONs.

REFERENCES

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VII-2: Solid stress accumulation inhibits the delivery of drugs

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KEYWORDS
hypoxia; vessel collapse; tumor perfusion; nanomedicine; chemotherapy

ABSTRACT

Introduction
Accumulation of mechanical stresses within structural components of the tumor microenvironment compresses intratumoral blood vessels reducing drastically oxygen supply and drug delivery, leading to hypoxia, necrosis and compromised treatment [Griffon-Etienne, 1999]. In the present study, we developed a continuum biphasic computational model for tumor growth and drug delivery that highlights the interdependence between oxygen supply, cancer cell proliferation, accumulation of solid stress, compression of blood vessels and reduction in vascular density and drug delivery. To investigate the extent to which solid stress inhibits drug delivery, we modeled the intratumoral transport of two systems: (i) chemotherapy alone, and (ii) a nanoparticle delivery system where the chemotherapy was contained in a nanocarrier and was released in a controlled fashion.

Methods
To describe kinematics we used the multiplicative decomposition of the deformation gradient tensor $F = F_e F_g$ [Rodriguez, 1994], which includes tumor growth $F_g$, set to be homogeneous and isotropic [Stylianopoulos, 2013], and the elastic interactions between host tissue and tumor $F_e$. The total stress was obtained as the sum of the fluid and solid phase stresses [Mow, 1980]. Oxygen and drug concentrations were determined using convection, diffusion and reaction equations.

Results
The model predicted the magnitude of the compressive solid stress to be higher at the center compared to the periphery of the tumor resulting in heterogeneous stress distribution. When solid stress exceeds a critical value for vessel collapse, tumor vascular density reduces inside the tumor to values less than the normal tissue baseline and the tumor interior becomes hypo-vascular and hypoxic. For both drug delivery cases the concentration of the internalized drug is lower in the center of the tumor compared to the periphery. In the normal tissue the concentration of the drug for the case of chemotherapy alone is lower than in the tumor interior owing to the assumption of higher vessel permeability inside the tumor. In the case of nanotherapy, there is leakage of drug from the tumor to the surrounding normal tissue owing to the fluid pressure difference between the two tissues; however, the concentration of the drug becomes negligible away from the tumor because of selective extravasation of the nanocarrier through the hyper-permeable tumor vessels.

Discussion
Heterogeneous accumulation of stresses results in heterogeneous distribution of vascular density, with higher values at the periphery and lower at the center of the tumor. The hypo-vascular interior of the tumor results in inefficient delivery of drugs affecting treatment efficacy. Our results highlight the importance of alleviation of solid stresses to decompress blood vessels and improve drug delivery.

REFERENCES
SESSION VIII – EMPHASIS SESSION ON CANCER THERANOSTICS-II

Chair: C. Pitsillides (CY)

VIII-1: Carbon Nanotubes for siRNA delivery in Glioblastoma cells

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KEYWORDS
Glioblastoma; EGFR; Carbon nanotubes; siRNA

ABSTRACT
The treatment of patients affected by Glioblastoma Multiforme (GBM) has arisen as one of the biggest challenges in oncology. Even with therapeutic strategies targeting specific cancer-associated molecular pathways such as the Epidermal Growth Factor Receptor (EGFR) pathway, no major improvements in patient survival have been achieved so far. EGFR is involved in cell growth and division, and its overexpression is a striking feature of many GBM tumors. Alongside small drug molecules such as the tyrosine kinase inhibitors, RNA interference (RNAi) has emerged as a new strategy that could silence or knockdown the EGFR gene expression via small interfering RNAs (siRNA). Despite some promising advances, this technology is however hindered by the challenge of identifying a delivery system that could combine both safety and efficacy. Among the different nanomaterial based delivery systems, carbon nanotubes (CNTs) have been demonstrated to offer specific features in relation to their structural and biological properties. The capability of different types of functionalised CNTs to bind and intracellularly transport siRNA sequences was studied. We have established a method to improve the efficacy of complexation between siRNA through the protonation of surface amino-functionalised CNTs. We confirmed the complexation between the nucleic acid and the nanotubes by agarose gel electrophoresis. Following administration to cells, we observed that the CNT/siRNA complex localised in the cytoplasm using flow cytometry. Our strategy allowed further exploration of the hypothesis that the CNTs/anti EGFR siRNA vectors can be further explored as a potential therapy for the treatment of glioblastoma.
VIII-2: Gadolinium as a novel nano-probe to enhance image-guided targeted x-ray therapy

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KEYWORDS
Radiation therapy; Gadolinium; Magnetic resonance imaging

ABSTRACT
Heavy metal nanoparticles have attracted widespread interest for their potential use as radiosensitising agents that can enhance the therapeutic effect of both targeted x-ray and heavy-ion beams in radiotherapy applications [Butterworth et al 2012, Porcel et al 2014]. Nanoparticle enhanced radiotherapy is highly suited to targeted therapies for difficult to treat cancers such as resistant glioblastoma and paediatric cancers. The ability of high-Z atoms to generate more secondary electrons than normal tissue following irradiation leads to a higher absorbed dose in the treated region through a chain of physical interactions (including Auger electron cascades) that can produce large numbers of ionisations in the surrounding molecules. Where the nanoparticles can be located in the cell nucleus, complex damage may be caused in the DNA primarily mediated by radical oxygen species caused by ionisation of water molecules. However, high-Z nanoparticles are often observed to locate outside the cell nucleus [Stefancikova et al 2014] yet the cells still exhibit radiosensitisation responses, implying that the radioenhancement in these cases may be mediated indirectly by cell death signalling pathways.

While much attention to date has been paid to gold nanoparticle radiosensitisation, recent studies have begun to focus on gadolinium because of its additional advantage as a contrast agent for magnetic resonance imaging (MRI), allowing the exciting possibility of diagnostic imaging and enhanced radiotherapy treatment delivered by the same nanoparticle [Sancey et al. 2014]. Indeed, there is the possibility of developing new gadolinium-based nanotheranostic paradigms for emerging MR image-guided radiotherapies, including with the integrated MRI-linac technology now available.

We have conducted Monte Carlo radiation transport simulations to model the relevant physical processes involved in irradiation of high-Z nanoparticles, including gold and gadolinium, with x-rays. Our simulation results reveal that atomic de-excitation by both Auger electron emission and fluorescence (mainly K-alpha) photon emission both contribute to radiosensitisation [Byrne et al 2015]. Importantly, we find that while the Auger cascade process produces damaging low-energy electrons on nano-scales, de-excitation by fluorescence results in the production of secondary electrons on much larger scales (typically centimetres). This result also demonstrates the importance of in silico studies, since these insights cannot be readily gleaned from experiments alone.

We also present preliminary results on a novel gadolinium Gd(III) complex that exhibits excellent preferential uptake into the mitochondria of tumour cells and radiosensitivity to synchrotron x-ray irradiation. NMR relaxivity measurements and imaging confirm its enhanced MRI contrast capability. We aim to carry out the first study of this nano-complex for image-guided radiotherapy using an integrated MRI-linac system.

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VIII.3: Quantification of spatiotemporal distribution of the nanoparticles from fluorescence microscopy images of tissue sections

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KEYWORDS
Nanoparticles; Fluorescence; Spatial Distribution; Temporal Distribution; Image Processing

ABSTRACT
The temporal and spatial distribution and availability of both positively and negatively charged nanoparticles were quantified in normal and glioblastoma mouse brains to determine the distribution characteristics of such nanoparticles.

To evaluate the temporal distribution in normal tissue, nanoparticles were injected intravenously, the animals were sacrificed at different times post injection, and frozen sections of the brain were collected. The sections were subsequently imaged using an epi-fluorescence. The nanoparticle accumulations and tissue area were segmented using automatic, unsupervised, thresholding. The nanoparticle fluorescence intensity per tissue area, which is analogous to nanoparticle concentration, was subsequently estimated. The results for both negatively and positively charged nanoparticles, for time points ranging from 1 to 48 hours were extracted. Given these results, it was evident that the nanoparticles, independent of charge, reached their maximum concentration by 10 hrs and are maintained at that concentration for at least 14 hrs.

In order to evaluate the spatial distribution of the nanoparticles in glioblastomas, a mouse orthotopic tumor model was used where human glioblastoma cells were implanted in a mouse brain. At 10 hrs post injection, the animals were sacrificed and frozen sections of the brains where imaged with an epi-fluorescence microscope. The area occupied by tumor cells was automatically segmented and at the same time the tumor boarders were identified and the tumor area was divided into regions separated by 25 μm progressively deeper into the tumor. The areas of nanoparticle accumulation were also automatically identified using a similar approach. With these two types of information, the nanoparticle fluorescence intensity per tissue area was calculated both outside the tumor and inside as a function of distance from the tumor surface. This study indicated that the negatively charged nanoparticles preferentially accumulate at the boarders of the glioblastoma tumor more so than positively charged nanoparticles.

In order to explain the spatial patterns estimated above, frozen sections of the glioblastoma tumor were stained with CD31 (to identify the blood vessels) and DAPI (to identify the tumor cells). Several blood vessel characteristics (diameter, area, density (vessel area per volume) and wall thickness) were estimated per tissue area as a function distance from the surface of the tumor. The results imply that the vessels are bigger with thicker vessel walls closer to the surface as opposed to the deeper layers of the tumor. Comparing the nanoparticle concentration with the vascular density (i.e. the vessel cross-sectional area per tissue volume) as a function of the distance from the tumor surface, a clear correlation is evident. This outcome is consistent with the numerical modelling results.

The therapeutic implications suggested by the quantitative spatial analysis of the nanoparticle distribution, i.e. higher concentration in the periphery of the tumor, are quite significant for the effectiveness of glioblastoma management. The property of the proposed nanoparticles to concentrate in the periphery of a tumor offer the possibility of destroying the tumor cells at the periphery to minimize the residual malignancy after surgery.
SESSION IX - THE ROADMAP TO NANOTHERANOSTICS DEVELOPMENT: A PATIENT-ORIENTED APPROACH

PRECLINICAL AND CLINICAL RESEARCH IN (NANO)THERANOSTICS

Chair: A. Odysseos (CY)

A. Odysseos (CY), Introduction – Defining the clinical challenges

Costas Pitsillides (CY), *Preclinical research leading to novel therapeutic strategies*

Constantinos Pitris (CY), *Intercoupled technologies enabling the clinical applicability of (nano)theranostics*

Andreani Odysseos (CY), *(Nano)theranostics at the clinical fore - The Translational Pathway*

Round Table Discussion: How Close are We to Meeting the Clinical Challenges?
Rena Bizios (Moderator); J. Mollenhauer; C. Pitsillides; C. Pitris; A. Odysseos
IX-1 Facilitating the development of novel therapeutic strategies via in vivo optical imaging techniques

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KEYWORDS
In vivo imaging; optical imaging; cancer therapeutics

ABSTRACT
The BioLISYS Laboratory at CUT is developing novel fluorescence-based techniques for in vivo imaging of small animals with applications in cardiovascular and cancer therapeutics. These include the development of an in vivo flow cytometer in order to monitor fluorescently labeled cells in circulation as well as a whole body reflectance imaging system for detection of fluorescence and bioluminescence signal from cells and tissues in murine models of disease. The in vivo flow cytometer has been designed as a minimally invasive optical tool for the real time detection/quantification of fluorescent cells in circulation of living animals without the need to sequentially extract blood samples or sacrifice animals. Thus the system allows for the continuous monitoring of a cell population of interest over long time periods in order to assess dynamic changes in circulation. The optical reflectance imaging system combines fluorescence and bioluminescence imaging capabilities with a large field of view in order to enable imaging over a wide area of the animal. The noninvasive, quantitative method enables longitudinal studies of physiological changes in disease and allows for continuous monitoring in the same mouse over an extended time period, in order to evaluate biodistribution and therapeutic response of experimental therapeutic agents.

The imaging systems have been employed in the in vivo analysis of cardiovascular implants and novel biomaterials in order to evaluate the inflammatory response of vascular tissue to stent implantation and stent biocorrosion via the in vivo monitoring of the degree of inflammation, macrophage infiltration and cytokine expression in tissue surrounding stents deployed in mice abdominal aortas. In cancer therapeutics, the in vivo imaging systems have been used to develop a novel therapeutic system for targeted miRNA delivery to tumors, via microparticles that are derived from mesenchymal stem cells. Fluorescently labeled miRNA-loaded microparticles injected into the tail vein of tumor bearing mouse were monitored in circulation via the in vivo flow cytometer while their biodistribution and targeting specificity was detected in tumor sites via the fluorescence-based whole body reflectance imaging system. Furthermore, tumor progression and therapeutic response to miRNA therapy delivered via local and systemic administration of the MSC-derived microparticles was monitored in real time via the imaging of fluorescence and bioluminescence expressing tumors by whole body reflectance imaging.
IX-2 Intercoupled technologies enabling the clinical applicability of (nano)theranostics

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KEYWORDS
Enabling technologies; imaging modalities; quantitative microscopy

ABSTRACT

The success of theranostic applications, at any spatial scale, requires appropriate technologies and methodologies for monitoring the results and assessing the effectiveness of each intervention. Over the past decades, various technological advancements have reached developmental maturity to the point where they can now serve as building blocks of complex and innovative theranostic solutions. These technologies provide a wide range of options for every and each individual component of a theranostic solution including probe structure, probe therapeutic and diagnostic nature, as well as imaging modalities and mode of application. The choice of each property of the theranostic system cannot be decided out of the context of the envisioned clinical application but also the interactions between components must be appropriate and harmonized. This presentation will attempt to introduce several key components that can, or could in the near future, play an important role in the development of novel theranostic applications mainly from the view point of the diagnostic options offered rather than the therapeutic capabilities available which primarily depend on the clinical target. Topics include availability and applicability of probe structures (peptide, antibody, vesicle, nanoparticle), beacon types and detection modalities (nuclear, magnetic, optical), and example applications. MRI, nuclear, and optical molecular diagnostics and their applications will be introduced in the context of cancer diagnosis, management, and therapy guidance. The presentation will conclude with local experience in optical nanotheranostics involving different types of nanoparticles and optical in vivo imaging, with endoscopy, as well as ex vivo quantitative microscopy, in colon cancer and glioblastoma mouse models.
IX-3 - (Nano)theranostics at the Clinical Fore - The Translational Pathway

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KEYWORDS
Clinical nanomedicine; image-guided procedures; translational research

ABSTRACT

The development of nanomedicine this past decade has spurred a flurry of developments in novel diagnostic and therapeutic approaches which show great promise as a potential medicine paradigm for addressing current treatment roadblocks and persistent clinical needs, which are not successfully addressed with current methodologies. Emerging technologies in biomedical imaging combined with cutting edge nano-manufacturing modalities and state-of-the art molecular medicine platforms have enabled the evolution of a plethora of multimodal and multifunctional nanoactuators as drug delivery and imaging platforms. Concurrently, the inhomogeneity and adaptability that some diseases, such as cancer or inflammatory disorders, exhibit, in conjunction with the seemingly infinite variability of the patients’ genomes and responses, make the search for cures appear like an impossible task. Fully realizing these challenges, the medical community is increasingly turning towards personalized approaches to treatment. The exponential growth of research and discovery in the area opens pathways never before imagined. One of these new approaches is “nanotheranostics” which refers to a new paradigm of combined diagnosis and therapy, with a significant emphasis on personalization of care.

Nanotheranostics are designed to offer, both to the medical and pharmaceutical communities, a robust platform for personalized, minimally invasive, in vivo drug delivery with on-demand release and therapy, while enabling real-time treatment monitoring. Novel strategies are expected to emerge enabling integration of diagnosis and therapy across many major specialties and sub-disciplines of clinical practice. These approaches require highly interdisciplinary research integration which is the outcome of the synergism between molecular imaging, therapy, and nanomedicine. Potential benefits of nanotheranostics include, but are not limited to, (i) in vivo assessment of drug bio-distribution and accumulation at the target site, (ii) visualization of the drug release from a given nanocarrier and (iii) real-time monitoring of the therapeutic outcome. An important allusion to the potential of this concept is exemplified by the role of imaging in interventional procedures that at present, appears to be one of the applications that is much closer to clinical translation. The ultimate goal of any researcher in this field remains the realization of concept to clinical program. Herein, the author outlines the underlying problems and actions that need to be taken so that current challenges can be solved.
1. Photodynamic Quenched Cathepsin Activity Based Probes for Cancer Detection and Macrophage Targeted Therapy

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KEYWORDS
Cathepsins; Photodynamic Therapy; Activity-Based Probes; Macrophages; Non-Invasive Imaging

ABSTRACT
Elevated cathepsins levels and activities are found in several types of human cancer, making them valuable biomarkers for detection and targeting therapeutics. We designed small molecule quenched activity-based probes (qABPs) that fluoresce upon activity-dependent covalent modification, yielding cell killing by Photodynamic Therapy (PDT). These novel molecules are highly selective theranostic probes that enable both detection and treatment of cancer with minimal side effects. Our qABPs carry a photosensitizer (PS), which is activated by light, resulting in oxidative stress and subsequent cell ablation, and a quencher that when removed by active cathepsins allow the PS to fluoresce and demonstrate PD properties. Our most powerful and stable PS-qABP, YBN14, consists of a selective cathepsin recognition sequence, a QC-1 quencher and a new bacteriochlorin derivative as a PS. YBN14 allowed rapid and selective non-invasive in vivo imaging of subcutaneous tumors and induced specific tumor macrophage apoptosis by light treatment, resulting in a substantial tumor shrinkage in an aggressive breast cancer mouse model. These results demonstrate for the first time that the PS-qABPs technology offers a functional theranostic tool, which can be applied to numerous tumor types and other inflammation-associated diseases.
2. Synthesis of magnetic cobalt ferrite nanoparticles with controlled morphology, monodispersity and composition: the influence of solvent, surfactant, reductant and synthetic condition

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KEYWORDS
magnetic nanoparticles; synthesis; cobalt ferrite

ABSTRACT
In our present work, magnetic cobalt ferrite (CoFe₂O₄) nanoparticles have been successfully synthesized by thermal decomposition of Fe (III) and Co (II) acetylacetonate compounds in organic solvent in the presence of oleic acid (OA)/ oleylamine (OLA) as surfactants and 1,2-hexadecanediol (HDD) or octadecanol (OCD-ol) as accelerating agent. As a result, CoFe₂O₄ nanoparticles of different shapes were tightly controlled in size (range of 4-30 nm) and monodispersity (standard deviation only at ca. 5 %). Experimental parameters, such as reaction time, temperature, surfactant concentration, solvent, precursor ratio, accelerating agent, in particular, the role of HDD, OCD-ol, OA/OLA have been intensively investigated in detail to discover the best condition for the synthesis of the above magnetic nanoparticles. The obtained nanoparticles have been successfully applied for producing oriented carbon nanotubes (CNTs), and they have potential to be used in biomedical applications.
3. Multi-stimuli nanocarriers decorated with N-Phthalimides co-drugs with non-steroidal anti-inflammatory agents, for targeted anticancer therapy

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KEYWORDS
phthalimides; angiogenesis; cancer; targeting; VEGFR; COX-2; nanocarriers

ABSTRACT
According to literature there is a strong relation between cancer progression and inflammation and this phenomenon referred as vicious cycle. Inflammation is now considered as a risk factor of cancer and cyclooxygenase-2 (COX-2) enzymes which are inducible enzymes responsible for the synthesis of pro-inflammatory prostaglandins, are highly expressed in many cancers. Numerous studies in vitro, in vivo and epidemiological studies demonstrate that non-steroidal anti-inflammatory drugs (NSAIDs), particularly those that inhibit COX-2 have an important role in the treatment of various cancers. The anticancer activity exhibited by NSAIDs antiangiogenic activity of COX-2 inhibitors may constitute another mechanism to prevent tumor.[1]

Thalidomide ((2-(2, 6-dioxo-3-piperidyl)-isoindoline-1,3-dione) is a derivative of glutamic acid. Although thalidomide was withdrawn from the market because of teratogenicity, in recent years it has been discovered that the drug has various biological activities, such as the regulation of tumor necrosis factor-a (TNF-a) production and antinflammatory, antiangiogenic action and inhibits the activity of cyclooxygenase (COX) Based on above literature we designed synthesized and characterized co-drugs of thalidomide with non-steroidal anti-inflammatory drugs in order to evaluate their anticancer activity and use them for active targeting of nanocontainers in tumor areas.

The organic molecules’ cytotoxic activity was evaluated in multiple cancer cell lines (MCF-7, HeLa, U-87MG, PC-3) and healthy cells (3T3) by MTT assay in order to investigate the anticancer activity of synthesized phthalimides and their cytotoxicity in healthy cells. The analogue with the lower IC50 in all the cell lines was coupled with NSAID diclofenac and naproxen and the co-drugs that arised, were investigated for their anticancer activity in multiple cancer lines.

The purpose of the project was to decorate the surface of multiresponsive organic nanocontainers with a final co-drug analogue. These nanocarriers presented no toxicity at in vitro and in vivo studies. Actually, drug carriers that have been synthesized previously in published work were modified by compound and chemotherapeutic agent daunorubicin was encapsulated. Aiming at investigating their in vitro cancer targeting ability, confocal microscopy studies have been performed by functionalization of nanocarriers with FITC.

REFERENCES
4. Silica-Coated Core-Shell Nanoparticles define a Novel Theranostic System for Targeted Drug Delivery to Nuclear-Translocated phospho-EGFR

Panayiota Stylianou¹, Khodor Issa¹, Sofia Iliopoulou¹, Ana-Belen Davila², Jean-Michel Siaugue², Triantaphyllos Stylianopoulos³, Constantinos Pitris³, Andreani D. Odysseos¹,³*/

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KEYWORDS
EGFR; glioblastoma cells; Nanoparticles

ABSTRACT
Resistance to cancer therapies targeting tyrosine kinase receptors is epitomized by intrinsic and acquired resistance resistance to the Epidermal Growth Factor Receptor –axis (EGFR), due to genetic and epigenetic variations in critical downstream effectors and parallel pathways. Nuclear translocation and constitutive activation of phosphorylated EGFR (npEGFR) defines an emerging resistance biomarker in highly morbid malignancies such as lung cancer (LC), triple-negative breast cancer (TNBC), malignant gliomas (GM) and certain subtypes of colon cancer (CRC). Theranostic nanoparticles with anti-EGFR therapeutic attributes define a promising approach to image and inactivate npEGFR. We are introducing a novel system using rationally designed nanoparticles in vitro and in vivo for the management of malignant gliomas and potentially other malignancies expressing the npEGFR.

Citric acid functionalized, silica-coated maghemite nanoparticles encapsulated with organic fluorophores were PEGylated and end-functionalized with NH2 or COOH were successfully grafted with rationally designed irreversible tyrosine kinase inhibitors (TKI). Vibrating sample magnetometry and relaxometry confirmed appreciable magnetic properties and suitability for MR Imaging. Saturation of magnetization was reversible enabling applications for magnetically guided therapies while specific lost power values were suggestive of superior hyperthermia properties regardless of coating characteristics. Single photon spectrofluorometry under tissue culture conditions disclosed robust fluorescent attributes and disclosed clinically favorable drug release kinetics. Short and long-term nanosafety has been documented in standard clonogenicity assays.

Temporo-spatial trafficking and intracellular distribution in live glioblastoma cells (U87MG GFP) incubated with the NPs was assessed under confocal and fluorescence microscopy. There has been strong evidence of zeta-potential dependent, energy-driven, endosome/vesicle-mediated nuclear delivery with the NH2-end-functionalized NPs demonstrating higher perinuclear uptake and COOH-end-functionalized NPs showed higher nuclear predilection associated with homogenous biodistribution in the cytoplasm.

In vivo biodistribution and pharmacokinetics studies enabled by quantitation of fluorescence have confirmed the impact of zeta potential on tissue uptake and intratumoral distribution. Charged bimodal silica core-shell NPs with anti-EGFR attributes offer a promising solution for the management of resistance attributed to npEGFR. Their tunable fluorescent and magnetic properties enable concurrent tracking and validation of biomarker expression in real time, in vivo.

ACKNOWLEDGEMENTS
We acknowledge the EC FP7 for MC IAPP Grant “NANORESISTANCE”
5. Metal-based Bimodal Theranostic Systems Decipher Nuclear EGFR Activity in Colorectal Cancer

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KEYWORDS
Lanthanides; EGFR; Colon cancer cell lines

ABSTRACT
Nucleus-translocated Epidermal Growth Factor Receptor (npEGFR) defines an emerging marker of resistance to targeted cancer therapies. We are introducing a novel system of anti-EGFR targeted molecular imaging agents for the management of malignancies overexpressing the npEGFR with prototype metal based functionalized compounds engaging cutting edge synthetic and coordination approaches. Despite efforts to achieve bimodal imaging using a combination of d block transition metal (optical) and f-block lanthanide metal (magnetic) in a unimolecular organic framework, possessing anti-EGFR therapeutic potential, with optimized in vivo stability, targeting efficacy and desirable pharmacokinetics for clinical translation remains a major challenge.

A series of hetero-bimetallic complexes containing (i) luminescent ruthenium polypyridyl complex having a carboxylic arm and (ii) functionalized cyclen (1,4,7,10-tetraazacyclododecane) based lanthanide chelators containing anilinoquinazolines pharmacophore and ethylenic linker to bridge ruthenium polypyridyl complex, have been synthesized and spectroscopically characterized. Lanthanides exhibit complementary properties over organic fluorophores, including resistance to photobleaching, long luminescence lifetimes, minimal or no reabsorption and sharp emission bands. The tunable fluorescence is aimed to enable the concurrent detection of two different target organs, with the same agent and to also allow metastasis imaging by MRI and therapeutic attack with the same diagnostic session. Sensing and therapeutic attributes are explored against those of citric acid functionalized, silica-coated, magnetite, rhodamine nanoparticles (rNPs) end-functionalised with NH2 or COOH and grafted with the same TKI. Most promising TKI was examined by state-of-the-art Surface Plasmon Resonance (SPR) for assessing drug-target interactions and emerging technologies for in vivo protein-protein and theranostic compound-protein interaction.

Vibrating sample magnetometry and relaxometry confirmed appreciable magnetic properties and suitability for MR Imaging while reversible saturation of magnetization enabled applications for magnetically guided therapies. Live colon cancer cell lines (CRC, SW480 and SW620) with differential expression of EGFR were assessed for inducible spatial expression of pEGFR and temporo-spatial trafficking of NPs or Ru-Gd complexes under confocal and fluorescence microscopy. Zeta-potential dependence of nuclear delivery was documented. Proapoptotic efficacies of (i) hetero-bimetallic complexes and (ii) irreversible TKI were assessed against Cis-Platinum, a widely applied metal-based chemotherapeutic and Gefitinib, a clinically approved TKI, respectively.

Nuclear-targeted bimodal systems define a promising approach for npEGFR driven malignancies. Live system biodistribution and pharmacokinetics disclose TKI-Ru-Gd complexes as promising theranostic solutions for better bioavailability and reduced long-term toxicity.

ACKNOWLEDGEMENTS
RPF for YTEIA/BIOΣ/0311/BIE/04 and EC FP7 for MC IAPP Grant “NANORESISTANCE”.

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6. Detection and therapeutic targeting of translocated phospho-EGFR in Triple Negative Breast Cancer with Silica –Coated Ferromagnetic Nanoparticles

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ABSTRACT

Triple negative breast cancers (TNBC) are among the most aggressive and deadly breast cancer subtypes. TNBC is characterized by biological aggressiveness and poor prognosis mostly due to the lack of any validated therapeutic target. The Epidermal Growth Factor Receptor (EGFR) has been known to be overexpressed in TNBC, however, clinical trials with anti-EGFR therapies have not been promising. Phosphorylated EGFR constitutively expressed and translocated to the mitochondria and nucleus, can enhance resistance to anti-EGFR therapies and is correlated with poor overall survival in breast cancer. Targeting the translocated EGFR can be a promising approach towards the management of resistance of TNBC.

We introduce citric acid functionalized silica-coated magnetite nanoparticles, rhodamine-doped, NH2- or COOH – end-functionalized, covalently grafted with irreversible anti-EGFR tyrosine kinase inhibitors, as potential carriers of anti-EGFR attributes to the mitochondria and nucleus of MDA-MB 231 TNBC cells.

Using both confocal microscopy (live and fixed) and transmission electron microscopy we managed to obtain data showing the internalization and localization of non-grafted NPs inside the cells. NP cytotoxicity was evaluated and the results showed that the non-grafted NPs tested did not inhibit cell proliferation and did not affect colony formation even after long-term exposure for 15 days, thus demonstrating long-term nanosafety.

The alteration of the nanoparticle surface in biological media with the formation of the protein corona changes their chemical-physical properties. The NPs protein corona was preliminary characterized by SDS-PAGE. The results revealed that more proteins bind to the NH2 - NPs comparing to the COOH-NPs. With this initial approach we confirmed that the composition and amount of proteins that form the hard corona depend on the surface charge and functional groups of NPs. Confocal and TEM imaging verified that the NH2 functionalized NPs were always enclosed in organelle structures throughout their trafficking to the nucleus. On the contrary the COOH-NPs seemed to be distributed in the cytosol. These findings give further credence to the notion that the protein corona not only plays a significant role in NP internalization but also determines their intracellular biodistribution. Tyrosine-Kinase Inhibitor-grafted NPs were not only uptaken by the nuclear membrane but also maintained their anti-EGFR efficacy as demonstrated by phosphor-EGFR quantification.

These findings disclose TKI-grafted silica –coated ferromagnetic NPs as a promising nano-theranostic solution for Triple Negative Breast Cancer.

ACKNOWLEDGEMENTS

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7. Growth-induced stress in tumors: implications for cancer progression and treatment
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ABSTRACT
Introduction
Growth-induced stresses are generated in solid tumors due to the uncontrolled growth of cancer cells within the confined space of the normal tissue [Stylianopoulos, 2012]. These stresses can contribute to tumor progression and pathological cellular behaviour. Furthermore, they might compress blood vessels [Padera, 2004], reducing tumor perfusion and the systemic administration of drugs. In this work, we developed a strategy to quantify growth-induced stresses in murine tumors and studied the effect of stress on tumor growth and perfusion.

Methods
Growth-induced, residual, stress can be retained as stress after a tumor is excised, even though external confining forces have been removed. The existence of this stress is realized when one makes a cut to the excised tissue along its main axis, the stress relaxes and the tissue deforms in a measurable way. To calculate the stress from the measured deformation, we developed a computational model assuming that tumors mechanical behaviour is compressible and neo-Hookean and applying an existing theory for tissue growth [Rodriguez, 1994]. We employed five tumor models (n=12, Table 1) grown in severe combined immunodeficient (SCID) mice and measured the tumor growth rate given by the doubling time. Then we used our methodology [Stylianopoulos, 2012] to quantify growth-induced stress. Subsequently, tumor sections were obtained and stained with CD31 antibody for endothelial cells to indentify vascular structures and with lectin to measure tumor perfusion. Finally, therapeutic depletion of tumor stroma was performed with administration of Saridegib, 40 mg/kg for 8 d.

Results
Growth-induced stress was confirmed in all tumor types considered in this study. The stress was compressive at the center of the tumor and switched to tensile at the tumor periphery. Table 1 presents the results of the doubling time and the estimated by our model radial stress at the center of the tumor.

<table>
<thead>
<tr>
<th>Cancer cell line</th>
<th>Doubling time (days)</th>
<th>Radial stress (mmHg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>U87</td>
<td>5.18</td>
<td>38.0 - 60.1</td>
</tr>
<tr>
<td>B16F10</td>
<td>0.94</td>
<td>2.8 - 4.7</td>
</tr>
<tr>
<td>E0771</td>
<td>2.21</td>
<td>4.9 - 8.2</td>
</tr>
<tr>
<td>MCaIV</td>
<td>2.50</td>
<td>4.5 - 7.4</td>
</tr>
<tr>
<td>4T1</td>
<td>2.25</td>
<td>5.3 - 8.8</td>
</tr>
</tbody>
</table>

From CD31 and lectin staining we found the mean vessel diameter of tumors to be 10 μm and only the 32% of the vessels was perfused. Depletion of tumor stroma with Saridegib alleviated stress levels and resulted in a 10% increase in vessel diameter and a 47% increase in the fraction of perfused vessels.

Discussion
Our results suggest that depending on tumor type, growth-induced stress in tumors varies from 2.8 to 60.1 mmHg. Interestingly, we found that tumors with the highest stress level (U87) had the slowest growth rate (i.e., the highest doubling time). In addition, we found that stress alleviation can be achieved by depleting tumor stroma and it can cause the decompression of blood vessels and an increase in perfusion, which is essential for effective delivery of drugs. Our research demonstrates the potential of a new therapeutic strategy - the anti-stress therapy - to improve perfusion and drug delivery in solid tumors.

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8. The effect of MIONs spatial arrangement onto the Hyperthermic efficiency, of magnetic nanocrystal clusters

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ABSTRACT

Magnetic hyperthermia appears as a promising strategy for cancer treatment due to the sensitivity of cancerous cells to increased temperatures (~ 42-45 °C) compared to healthy cells. Towards the quest for development of systems with increased tissue heating properties, clustering of magnetic iron oxide nanoparticles (MIONs) has emerged as an effective approach for the enhancement of magnetic nanoparticles hyperthermia efficiency [1]. In the present work we examine the in vitro hyperthermia behavior of magnetic Colloidal Nanocrystal Clusters (CNCs), consisting from MIONs of the same particle diameter and saturation magnetization. The magnetic nanocrystallites are organized in such a fashion that two distinctly different configurations of condensed and soft clusters have emerged [2]. In the first case scenario, MNPs are organized in a densely packed motif were magnetic nanocrystals are adopting the same crystallographic orientation, forming a condensed cluster system. In the second scenario, the MIONs are connected with their neighboring particles through their polymer coating, forming a soft clustered system. Calorimetric measurements at identical conditions (after tailoring their colloidal characteristics, i.e. hydrodynamic diameter) proves that the spatial arrangement of MIONs in multi-core systems can have a major impact onto their hyperthermic efficiency, with soft-cluster motif consistently outperforming the condensed superstructure. Nevertheless, a detailed study at different concentrations and tissue mimicking conditions demonstrates that soft-cluster configuration is much more susceptible to interparticle interactions [3] and immobilization effects.

REFERENCES

9. Theranostic nano-platforms based on PEGylated Condensed Colloidal Nanocrystal Clusters: Hyperthermia properties and evaluation of in vivo tumor targeting with Multispectral Optoacoustic Tomography

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ABSTRACT
Condensed Colloidal Nanocrystal Clusters (co-CNCs) based on Magnetic Iron Oxide Nanoparticles (MIONs) have recently attracted considerable research interest in the field of cancer theranostics [1]. Their unique superstructure with densely packed MIONs inside particles’ volume imparts enhanced magnetic properties, compared to similar soft-clustered systems [2], while their easily functionalized surface and inherent biocompatibility favors their use in biomedical applications. In the present work, magnetic nanoassemblies of PEGylated co-CNCs (coded as PEG-MagAlg) were evaluated for their potential use as hyperthermia mediators and tumor targeting agents in xenografted 4T1 breast tumor bearing mice using advanced in vivo imaging techniques.

Magnetic nanocarriers’ (MNCs) ability to generate heat when exposed to Alternating Magnetic Fields (AMF) for their use as hyperthermia agents was studied in detail. Calorimetric measurements at different concentrations and tissue mimicking conditions, manifested their particularly stable performance which was unaffected from interparticle interaction effects, demonstrating an ILP of 2.8 nHm²/kgFe. Optoacoustic studies using Multispectral Optoacoustic Tomography (MSOT), (which is emerging as a potent modality for molecular visualisation of nanomedicines [3]) and relying solely on particles’ magnetic core absorbance, unveiled the superb in vivo stealth properties of PEGylated MNCs. Their prolonged life-time in blood circulation enables them to utilize EPR effect and gradually accumulate to the tumor site of a xenograft mouse model. Furthermore, distribution profile of MNCs at tumor site can dramatically change under the influence of external low gradient magnetic fields, proving that effective PEGylation of MagAlg’s co-CNCs combined with their enhanced magnetic manipulation favors their use as magnetically driven vehicles for in vivo tumor targeting.

The overall unique characteristics of MagAlg-PEGs constitute it an ideal candidate nano-platform for the development of nanotheranostics, capable of combining tumor targeting ability through physical routes (EPR and magnetic targeting) and hyperthermia properties exhibiting high ILP values under the use of AMF.

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