NanoMed Europe

June 17-19, 2019 Braga, Portugal

Conference Booklet

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A warm welcome

The European Technology Platform on Nanomedicine will meet on June 17-19, 2019 for its fourteenth year in Braga, Portugal.

We are very happy to cooperate with the INL - International Iberian Nanotechnology Laboratory, to make this event a great success. To reinforce the scientific sessions, the Annual meeting of ETPN is organized jointly with Nanomedicine Europe (last edition in London 2017, Grenoble 2015). It will make NME19 a unique place in Europe to meet scientists, companies, agencies, EC officers with interest in nanomedicine development in research, clinic and industry.

The fantastic and unique environment in Braga will favor a friendly atmosphere and lively interactions among participants, to initiate new projects, new cooperation opportunities. We are glad welcoming you at NME19 in Braga, 17-19 June 2019!

Sincerely,
Patrick Boisseau
VP Healthcare at CEATech

A warm welcome

On behalf of the organizing team of INL and partners, I am very pleased to be able to welcome you to INL, to the beautiful city of Braga, to the beautiful Minho Region and to Portugal for the NanoMed Europe 2019 Conference.
The NanoMed 2019 Conference is the premier event for the European Nanomedicine research community. The conference is designed to increase participation, to foster interaction and networking, and to propitiate new dialogues. Nowadays, Science, Technology and Research is, to a great measure, a work in partnership, motivated towards the search for solutions to societal great challenges of present times. The European Nanomedicine research community has a large tradition and a large potential of collaborative work, which is inherent to the fact that enabling nanotechnologies are in the core of the activities.
We are convinced that the 2019 flagship conference fostered by EuroNanoMed3, the ERA-Net Cofund Action on Nanomedicine under Horizon 2020, will consolidate these EuroNanomed conferences as the major conference in the world to discuss about present and future nanotechnologies within the nanomedicine area and attract the main leading players in the area. Lastly, I wish that you will enjoy not only science and technology discussions and presentations, but also the beautiful North of Portugal. We encourage you to taste the multiple possibilities to explore Portugal, through culture, food and wine.

Finally, we would like to thank YOU ALL for attending the Conference. It is the quality of your presentations and the dynamism of your exchanges that will make the conference a real success. We sincerely wish you all a fruitful conference, a memorable visit to INL and to Braga and an enjoyable stay in Portugal.

Sincerely,
Prof, Dr ***Lars Montelius***
INL Director-General

About NME2019

The Conference

NanoMed Europe 2019 | NME19 is born from the merge of the 14th annual event of the ETPN & the European scientific conference ENM (after London 2017 & Grenoble 2015). Together, these two major events form a new and unique conference for the European Nanomedicine community, bringing together scientists, technology providers, entrepreneurs, industry and clinicians.

NME19 is co-organized this year by the ETPN and INL - International Iberian Nanotechnology Laboratory.

NME19 is a unique place reserved for you to:
:: Discover and share your best nanomedical innovations
:: Disseminate your most recent scientific discoveriee;
:: Share your H2020 projects results
:: Pitch your SME
:: Initiate or consolidate your new H2020 or EuroNanoMed project

A prize for the best short talk and best poster will be organized and therefore the participation of young researchers and PhD students is strongly encouraged.

On the networking side, a very effective matchmaking system will be open in advance to increase the impact of your participation to the event.

An exhibition hall within the lunch and break area will allow you to exhibit your company and/or European Projects. Various sponsoring opportunities are also proposed.

We look forward to welcoming you at NME19!
INL Director-General

Agenda

DAY 1

June, 17 | Monday

08h00 - 09h00 ***Registration***

09h00 - 11h00 **ETPN Association, General Assembly (Part 1)**
By the ETPN Bureau & Secretariat (**for ETPN Members only**)

11h00 - 11h30 ***Coffee-break***
Poster Exhibition & Industry Exhibition

11h30 - 13h30 **ETPN Working Groups Meetings**
By the ETPN WG Chairpeople (**for ETPN Members only**)

13h30 - 14h30 ***Networking Lunch***
Poster Exhibition & Industry Exhibition

14h30 - 16h30 **ETPN Association, General Assembly (Part 2)**
By the ETPN Bureau & Secretariat (**for ETPN Members only**)

16h30 - 17h00 ***Coffee-break***
Poster Exhibition & Industry Exhibition

17h00 - 17h10 **NanoMed Europe 2019**
Open to all NME19 Attendees

17h10 - 18h00 **Plenary Session**
NanoXray: From the Idea to Market. Behind the Scenes.
By, ***Laurent Lévy***, CEO and Founder of the ***Nanobiotix***

Laurent Lévy holds a doctorate in physical chemistry, specialized in nanomaterials, from the Pierre and Marie Curie University (Paris) and from the CEA (Commissariat à l'Énergie Atomique et aux Énergies Alternatives) and a DEA (advanced studies and diplomas) in physics of condensed matter from the UPVI-ESPCI (Paris).
He holds an extensive experience in sciences and techniques related to nanotechnologies, a field in which he worked for more than 10 years. His research at the frontier of

biotechnology and nanotechnologies has resulted in the development of a number of concrete applications such as NanoXray, which could open a new method for cancer treatment.
For many years, Laurent was a consultant in the development of application of nanotechnologies with large companies such as Sanofi (pharma), Guerbet (medical imaging), Rhodia (chemistry), as well as for biotechnology start-ups. Laurent is the author of 35 international scientific publications and communications, has applied for several patents and completed his training by a post-doctoral fellowship at the Institute for Lasers, Photonics and Biophotonics, SUNY (State University of New York), Buffalo, USA.

18h00 - 18h30 ***INL Tour***

18h30 - 21h00 ***Welcome Reception***

DAY 2

June, 18 | Tuesday

08h30 - 09h00 ***Registration***

09h00 - 09h30 Keynote Session
Nanomedicine Against Infectious Diseases
Jennifer Grossman,Senior Scientific Manager at NIH/NIAID

Jennifer Grossman, PhD is a Senior Scientific Program Manager in the Vaccine Translation Research Branch (VTRB) in the Division of AIDS of the National Institute of Allergy and Infectious Disease (NIAID), one of the US National Institutes of Health (NIH). She oversees the production of a large portfolio of investigational HIV vaccine products, including nanoparticles, peptide and protein immunogens (monomers, trimers, germline-targeting, lineage-based, epitope-based), viral vectors, DNA, RNA, and adjuvants. Jennifer provides nanotechnology subject matter expertise for R&D, manufacturing, analytics, formulation, Q/A and regulatory support for HIV vaccine products. Before joining the VTRB, Jennifer was at the National Cancer Institute (NCI), where she led alliance management for the Nanotechnology Characterization

Lab (NCL). There, she established and managed productive collaborations within NCI, FDA, NIST and a network of over 150 drug development labs in industry and academia.
Jennifer's areas of scientific expertise include analytical methods for assessing drug delivery systems and modeling of nanoparticle structures and interactions. She has experience in a variety of issues related to nanotech drug development and regulation. Prior to joining the NCL in 2006, Jennifer conducted research in Physical Chemistry at the University of Maryland, where she focused on modeling and measuring protein motion under the guidance of Dr. David Fushman, Professor of Biochemistry. She holds a Ph.D. in Physical Chemistry, an M.S. in Chemical Physics and a B.S. in Physics.

09h30 - 10h00 ***Official Opening***
Lars Montelius, Director-General INL
Manuel Heitor, Portuguese Minister of Science and Technology
José Juan Sánchez, Spanish Deputy Director-General for the Internationalization for Science and Innovation
Ricardo Rio, Mayor of Braga
Patrick Boisseau, VP Healthcare at CEATech

Smart Therapeutic Nanosystems **| Chair: Maria de la Fuente**

10h00 - 10h30 ***Nanomedicine Strategies to Improve Outcomes in Metastatic Cancer***
Maria de la Fuente
Health Research Institute in Santiago de Compostela (IDIS)

María de la Fuente is the principal investigator of the Nano-Oncology Unit of the Health Research Institute of Santiago de Compostela (IDIS), and member of the cancer research area of the CIBER (CIBERONC). Dr. de la Fuente obtained her PhD degree in 2006 in the field of nanomedicine and nanotechnology, and performed several research stages at different national and international institutions. In 2013, she established the Nano-Oncology Unit. Dr. de la Fuente's research is devoted to developing innovative nanotools to provide solutions to confront one of the biggest current challenges in oncology, metastases.

She is the author of more than 45 scientific articles, books and book chapters, and has more than 100 contributions to national and international congresses. She has been directly involved in more than 30 research projects, being principal investigator in 12 of them. She is inventor of five patents, and is actively participating in tech transfer and valorization initiatives to move the developed technology forward to a

clinical setting (as NANOMEDTAB and the IGNICIA program of the Axencia Galega de Innovación).

10h30 - 10h45 ***Development of a Targeted Therapy for Triple Negative Breast Cancers by Using Docetaxel-loaded Polymeric Nanoparticles, iPeps and RGD Peptides***

Anabel Sorolla, Harry Perkins Institute of Medical Research
University of Western Australia

Abstract: Triple negative breast cancers are very aggressive malignancies which comprises 15-20% of all breast cancers. These tumours lack expression of estrogen receptor, progesterone receptor and human epidermal growth factor receptor 2 (HER2). Thus, patients cannot be treated with anti-hormonal and anti-HER2 compounds, remaining standard chemotherapy as the only treatment option. However, many TNBC acquire chemotherapy resistance, notably docetaxel, which has been associated with the overexpression of transcription factors, such as ENGRAILED1 (EN1). Transcription factors have largely been considered "undruggable" due to the lack of binding pockets for small molecules to bind. We solved this obstacle by designing interference peptides. Interference peptides are short versions of transcription factors that are able to bind and interfere with the binding with other binding partners and the DNA, ultimately abrogating oncogenic transcriptional programs.
Here, we have developed a tumor delivery system for docetaxel and interference peptides designed to specifically inhibit EN1 (EN1-iPeps) by using PAA-PGMA nanoparticles. To promote tumor specific targeting, we have functionalized these nanoparticles with EN1-iPeps engineered with RGD sequences, which were found in TGFβ and FMDV proteins and showed high affinity for αvβ3 and αvβ6 integrins. We found that these peptides reduce cell viability and induce apoptosis in triple negative breast cancer cells with negligible effects on normal cells (EN1-). Moreover, EN1-RGD-iPeps-mediated nanoparticle internalization into breast cancer cells was via integrins and intravenous injection of the functionalized nanoparticles increased their tumor accumulation. Furthermore, docetaxel nanoparticles targeted with EN1-RGD-iPeps significantly reduced triple negative breast cancer growth both in vitro and in vivo without showing changes in mice weight and toxicity in mice organs. Our results suggest that this targeted nanoformulation represents a new and safe therapeutic approach for chemoresistant triple negative breast cancers, paving the way for the so-needed targeted therapy for these cancers.

10h45 - 11h00 ***Advanced Delivery Systems for the Suppression of Cancer Stem Cells***

Marcos Garcia-Fuentes
CiMUS Research Center and Department of Pharmacology, Pharmacy and Pharmaceutical Technology
Universidade de Santiago de Compostela, Spain

Abstract: The presence of a subpopulation of malignant cancer stem cells (CSCs) is major cause of treatment failure in many of the most lethal cancers. CSCs can resist to cytotoxic drugs by the over expression of efflux pumps and by maintaining a quiescent state. They can also restore a tumour even from minimal amounts of surviving cells. The design of new cancer treatments that provide long-term survival need to consider how to address this key tumor cell subpopulation. Our team has focused on engineering new delivery solutions for biotechnological drugs that selectively suppress glioblastoma CSCs. For instance, some bone morphogenetic proteins (BMPs) European Technology Platform on Nanomedicine – ETPN Association 64-66 rue des archives 75003 Paris France www.etp-nanomedicine.eu have been described to induce glioblastoma CSC differentiation and suppression of their tumour initiating capacity. To prolong the presence of BMPs in the brain upon implantation, these proteins can be integrated in a polymer nanocomplex that is encapsulated in biodegradable polymer microspheres by an anhydrous method. This delivery technology provides more than 2 months of sustained BMP release in bioactive form. In vivo studies performed with human primary glioblastoma CSC tumors have shown that protein-loaded microspheres can activate BMP signaling in the tumours even 2 months after implantation. The group treated with the protein-loaded microspheres showed smaller tumours and a more benign tumour cell phenotype consistent with CSC differentiation. Currently, the same protein delivery technology is being explored for attracting CSCs to defined sites, where the cells could be managed with local interventions. The epidermal growth factor receptor dependent pathway is another promising target for glioblastoma CSCs suppression. The protein DYRK1A modulates the recycling of this receptor, and DYRK1A inhibition by RNA interference can eliminate glioblastoma CSCs. To deliver siRNA against DYRK1A (siDYR) to the intracellular compartment in glioblastomas, we generated a small polymer library starting from a polyphosphazene precursor with click handles. This polymer precursor can be easily modified by thiol-ene addition reactions. By performing serial screening tests, we identified a mixed composition with excellent activity/toxicity ratio and a remarkable capacity to penetrate and transfect 3D tumour spheres. The nanoparticles loaded with siDYR suppressed glioblastoma CSCs tumour initiating capacity in vitro and produced further reductions in tumour volume in vivo when combined with temozolamide.

Nanomedicine Translation to Market **| Moderator: Rui Sousa**

10h00 - 10h20 ***The HealthTech Translation Advisory Board (TAB)***
Rui Sousa
Tech Transfer Officer & Program Manager at TecMinho

The HealthTech Translation Advisory Board (TAB) aims to accelerate the development of emerging medical technologies in Europe by offering tailored support to the best innovative project. It is a unique and free-of-charge service to help the translation to the market of the best innovation coming from emerging medical technologies, including nanomedicine, robotics & photonics for healthcare, digital health, etc. It gives access to a group of world-class industry experts in IP, innovation funding, strategy, company development, industrial R&D. It helps to remove the specific roadblocks identified in HealthTech product development by providing its beneficiaries with continuous support It is open to all: entrepreneurs, SMEs, industry, academic labs, etc.

10h20 - 10h40 ***EUNCL Review After 4 Years: Successes and Lessons Learned***
Simon Baconnier
TNA manager at EUNCL

EUNCL, the European Nanomedicine Characterisation Laboratory, aims at fostering innovation in nanomedicine by providing access to state of the art full characterisation of nanomaterials intended for medical applications, prior to the regulatory application, developed by public labs, spin –offs and innovative SMEs. EUNCL provides a trans-disciplinary comprehensive testing infrastructure covering a comprehensive set of preclinical characterisation assays (physical, chemical, in-vitro and in-vivo biological testing) allowing researchers to fully comprehend the biodistribution, metabolism, pharmacokinetics, safety profiles and immunological effects of their medical nanoparticles. EUNCL is also fostering the use and deployment of standard operating procedures (SOPs), benchmark materials, and quality management for the preclinical characterisation of medical nanoparticles (nanoparticles used for medical applications) to promote inter-sectorial and inter-disciplinary communication among key drivers of innovation, especially between developers and regulatory agencies. In two years, EUNCL has processed 30 applications on nanoformulations for medical applications.

10h40 - 11h00 ***NanoPilot: Pilot Plant for the GMP Production of Polymer-based Nanopharmaceuticals***
Iraïda Loinaz
CIDETEC

A Pilot Plant for the Production of Polymer-based Nanopharmaceuticals in Compliance with GMP NanoPilot has received funding from the European Union Framework Programme for Research and Innovation Horizon 2020. The objective of this four-year long project is to set-up a pilot plant operating under Good Manufacturing Practice (GMP) for the production of polymer-based nanopharmaceuticals. This pilot plant will accelerate the development of nanomedicine, currently in its infancy within the pharmaceutical sector.Three different processes will be established for the production of three different nanopharmaceuticals. State of the art production processes including micro reactors and highly advanced characterization techniques will ensure the quality of the nanodrugs. Existing laboratories owned by the coordinator, will be adapted and certified within this project to enable the operability of the pilot plant.

11h00 - 11h30 ***Coffee-break***
Poster Exhibition & Industry Exhibition

Smart Therapeutic Nanosystems

11h30 - 11h45 ***Preclinical Evaluations of Tri-mannose Bearing Lipid Polymer Shell mRNA Nanoparticles that Combine Strong Antitumor T-cell Immunity with Improved Inflammatory Safety.***
Chantal Pinchon
Center for Molecular Biophysics CNRS, Orléans (France)

These last years, we are witnessing the emergence of new class of biopharmaceuticals based on messenger RNA (mRNA). It can encode any antigenic protein allowing development of preventive and therapeutic vaccines. It offers a strong safety compared to DNA because it cannot be integrated in host genome. The translation machinery being located in the cytosol, mRNA expression does not require nuclear import, which is of benefit for hard to transfect cells as dendritic cells (DC).
We developed an advanced hybrid histidylated lipid polymer shell mRNA nanoparticle (LPR) endowed a tri-antenna of α-D-mannopyranoside (triMN-LPR). We evaluated (i) their binding to human and mouse DCs able to recognize specifically mannose ligands, (ii) the nature of induced immune response after immunization and (iii) their therapeutic anti-cancer vaccine efficiency. After intradermal injection to mice, TriMN-LPR provided DCs recruitment and activation in draining lymph nodes. In therapeutic pre-clinical tumor models, triMN-LPR carrying mRNA encoding respective antigens significantly exert curative responses in mice vaccinated after initial tumor inoculation. Interestingly, LPR were also extremely effective in conferring antitumor T-cell immunity upon intravenous injection. When benchmarked against

ex vivo generated DCs electroporated with mRNA, LPR treatment was superior in controlling tumor growth. TriMN-LPR have the potential for a low inflammatory feature since its functionality did not rely on type I IFN, allowing the use of modified mRNA nucleosides for effective T-cell immunity by contrast to liposomal formulations. Altogether, our data provide evidence that triMN-LPR give rise to an efficient stimulatory immune response allowing for therapeutic anti-cancer vaccination in mice. LPR could be low inflammatory alternatives to the mRNA lipoplexes currently explored in early phase clinical trials.

11h45 - 12h00 ***Nanomedicines for the Therapeutic Manipulation of Tumour Associated Macrophages***

Fernando Torres Andón
Universidade de Santiago de Compostela

Tumor-associated macrophages (TAMs) play a key role in tumor progression, metastasis, and recurrence after treatment. In the tumor microenvironment of many solid tumors, such as lung cancer, they acquire an immunosuppressive phenotype, inhibiting the immune response to fight against cancer cells. Toll like receptor (TLR) ligands are well-known activators of immunostimulation, however their ability to reprogram TAMs from their M2-tumor-promoting phenotype towards an M1-antitumor mode has still not been effectively achieved in vivo. Thus, the aim of this work has been to engineer a series of polymeric nanostructures to improve the efficacy and pharmacokinetics of selected TLR ligands, Poly I:C, Imiquimod or Resiquimod, intended to reach their respective intracellular receptors, TLR-3 or TLR-7/8, inside TAMs. The TLR-loaded-nanostructures (TLRNS) presented low and narrow particle sizes (<200 nm and PDI <0.2), with surface charges ranging from highly positive to slightly negative values. In vitro models using primary human macrophages alone or with cancer cells were used to evaluate the ability of the TLRNS to manipulate the phenotype and polarization of macrophages into an M1-anti-tumor mode. These experiments allowed the selection of TLRNS with a favorable biocompatibility profile and ability to enhance the cytotoxic activity of treated-macrophages towards cancer cells. In parallel, we have developed a murine lung tumor model expressing a mutation of KRAS, in which colony assays and immunohistochemistry methodologies have been optimized for the evaluation of new nanoparticles designed to target TAM. The TLRNS are currently being investigated in vivo using these immunocompetent murine lung cancer models.

12h00 - 12h15 ***The Homeostatic Activities of Empty SV40 Nanoparticles***

Ariella Oppenheim
The Hebrew University of Jerusalem, Israel

We previously showed that treatment with empty capsid of SV40 (SV40 Nanoparticles - NPs) led to amelioration of toxic acute kidney injury through the induction of HSP70 and Akt-1. Survival increased from 12 to 60%. Even though Akt-1 is an oncogene, we have not seen over proliferation in any of the NP treated mice or in cell culture experiments. In further in-vitro studies we found that the NPs function in reversing the effect of both growth inhibitors and growth stimulators. In the present study we have shown that NPs improved survival in severely septic rats from 0 to 75%. RNAseq studies demonstrated that unlike AKI, here the NPs did not induce survival pathways. Instead they affected thousands of genes and many cellular functions. Six hours post the 2CLP operation the NP treatment has already eliminated 462 of a total of 2029 deleterious genes, and induced 1088 therapeutic genes. 24 pathways were modified in the NP treated rats, which function in 4 major areas: antipathogenic and immune response, resolution of inflammation, tissue regeneration and cell and system homeostasis. In contrast, only 4 genes were affected following NPs administration to healthy rats. We propose that Ca2+ signaling, a central cellular signaling mechanism plays a central role in the therapeutic process elicited by NPs. This is supported by Ca2+ oscillations that are seen in cells following NP infection (unpublished), which are presumably induced via activation of PLCγ, previously demonstrated to be required for the signaling networks elicited by the NPs. Moreover, the NPs attenuate cell death induced by etoposide in tissuecultured cells and the effect is inhibited by the calcium signaling inhibitor 2-APB. It appears that during virus-host coevolution these NPs gained the properties that enable them to keep the organism healthy, to ensure optimal viral gene expression and maximal propagation and spread.

12h15 - 12h30 ***Sub-micrometric Capsules for Drug Delivery***

Beatriz Pelaz
CiQUS/ Universidade de Santiago de Compostela

One of the main limitations of nanomedicines is their very low specificity in vivo. Thus rendering efficient targeted delivery among the most wanted breakthroughs in the fields of nanotechnology and medicine. The development of "smart" materials capable to react to an external stimulus may become a solution to overcome the specificity problems associated with nanomedicines. The cargo-release triggered by external stimuli such as light, magnetic fields or ultrasounds, allow for controlled drug release from nanocarriers with spatiotemporal resolution. Here we present the synthesis of a sonosensitive, sub-micrometric layer-by-layer (LbL) capsule system for ultrasoundcontrolled delivery of macromolecules in vivo. We adapted methods typically used for the fabrication of LbL microcapsules (3-5 microns in diameter) and achieved sub-micrometric macromolecule-loaded nanocapsules (ca. 600 nm in diameter). As a proof of concept, a thrombolytic drug, the thrombolytic drug recombinant tissue plasminogen activator (rtPA), widely employed for the treatment of acute ischemic stroke and other thromboembolic pathologies, is used as encapsulated active compound. This drug is highly unstable, which requires continuous perfusion

and the administration of high doses to efficiently "dissolve" clots, increasing the risk of hemorrhagic transformation. The activity of the encapsulated drug and its ultrasound-induced delivery are presented. The drug encapsulation presents some advantages, such as the drug protection from biological inactivators while its activity is preserved. Finally, the in vivo behavior of the system is studied in a mice model.

12h30 - 12h45 ***Antiangiogenic Biodegradable Nanoparticles Enhancing Anticancer Activity of Chemotherapeutics***

Fabiana Quaglia, Department of Pharmacy,
University of Napoli Federico II, Italy

The formation of new blood vessels is fundamental for the supply of nutrients and oxygen to cancer cells and greatly contributes to tumor progression, invasion and metastasis. "Angiogenic switch" in tumors is strictly related to the secretion of pro-angiogenic factors such as Vascular Endothelial Growth Factor (VEGF). Over-expression of FLT1 (VEGFR1), a subtype receptor of VEGF receptor family, has been correlated with severe disease progression and poor prognosis, as well as metastasis and cancer recurrence in humans. Here we combine the concept of limiting angiogenesis to the delivery of a lipophilic chemotherapeutic through multifunctional NPs with the final aim to ameliorate antitumor efficacy. To this purpose biodegradable NPs bearing an antiangiogenic anti-FLT1 hexapeptide on the surface and delivering the model chemotherapeutic Docetaxel (DTX) were developed and their impact on biological behavior evaluated. Amphiphilic diblock poly(ethyleneglycol)-poly(ε-caprolactone) (PEG-PCL) copolymers conjugated with an anti-FLT1 peptide (GNQWFI-NH2) at PEG-OH end where synthesized and characterized. This copolymer was mixed in appropriate ratios with unmodified PEG-PCL to give core-shell NPs entrapping DTX by nanoprecipitation. Formulation parameters were selected to give NPs with a size around 100 nm, entrapping DTX with high efficiency, and sustaining its release along time in simulated biological conditions. anti-FLT1 was partly confined to the surface of NPs and located amid PEG chains. A soft protein corona was formed around NPs in human serum. Anti-FLT1 NPs were internalized in HUVEC through VEGFR1 receptor and found more antiangiogenic than free peptide at equivalent peptide concentrations. Moreover, the loading of DTX potentiated the inhibition of formation of properly conformed tubes in vitro. Concerning cytotoxicity, DTX delivered by NPs showed a clear dose-dependent cell mortality in both HUVEC and MDA-MB-231, reaching 20% of cell survival after 72 h of treatment. Finally, we tested the formulations in MDA-MB-231 cells xenografted on chicken embryo chorioallantoic membranes (CAM). In this model, antiangiogenic properties and tumor weight decrease of anti-FLT1 NPs were found to be higher than those found for free DTX. Taken together, these results point at anti-FLT1 NPs as a novel platform to potentiate anticancer activity of chemotherapeutics devoid of antiangiogenic properties in highly resistant triple negative breast cancer.

12h45 - 13h00 ***Inhalable Nano-antibiotics to Treat Lung Antimicrobial-resistant Bacterial Infections***

Iraida Loinaz
CIDETEC

Due to the past misuse and over-prescription of antibiotics, diseases caused by antimicrobial resistant (AMR) bacteria are emerging with an impact expected to be higher than cancer within 30 years in term of patients death. Thus, this alarming situation requires the development of new strategies against bacterial infections. In the case of pulmonary infection, it has been demonstrated that direct inhalation of the antibiotic improved its performances. However, AMR bacteria are creating biofilm protection that makes even more complicated for antibiotics to reach the bacteria and treat the infection. In this context, the development of new drugs based on nanotechnology could allow more effective and safe antibiotic delivery bringing a potential alternative to the current weapons to fight infectious diseases, especially regarding pulmonary infections.In the current work, a novel therapy based on nanoantibiotics (NA) which comprises biocompatible polymeric nanocarriers (single-chain polymer nanoparticles, SCPN) and new antibiotics was investigated. Specifically designed SCPNs were able to carry large amount of antibiotics via electrostatic interaction. Interestingly, the resulting NAs showed lower cytotoxicity towards lung cell lines compared to the antibiotic in free form. However, the use of SCPN to carry the antibiotic did not affect the minimum inhibitory concentration (MIC) and time kill kinetics (TKK), which are the most important characteristics to fight infections, compared to the antibiotic in free form . In addition, biodistribution of the SCPN and NA using singular nuclear imaging equipment (PET/SPECT) via intra-tracheal administration followed by inhalation to uninfected rats showed a very good distribution of the nanomaterials in all lung lobes. Finally, therapeutic efficacy of NA was studied in a model of ESBL-positive Klebsiella pneumoniae pneumonia/ septicaemia in rats showing a highly significant survival of rats (4 days) at the maximum tolerated dose of NA after intratracheal administration.

13h00 - 13h15 ***Anti-EGFR Targeted Drug Loaded Polyavidin Nanoparticles Bypass Drug Resistance in a Triple Negative Breast Cancer Model and are Superior to Their Anti-EGFR Antibody drug conjugate***

Margherita Morpurgo, University of Padova,
Dept. of Pharmaceutical and Pharmacological Sciences

While Antibody-Drug-Conjugates (ADC) represent the first successful example of targeted drug delivery systems for personalized cancer therapy[1], more complex nano-based architectures are also actively investigated, but their advantage over ADCs has not been demonstrated yet. Using the Avidin-Nucleic-Acid-Nano-ASsemblies (ANANAS), a class of poly-avidins multifuctionalizable with stoichiometric

control, we compared quantitatively anti-EGFR antibody(cetuximab)-targeted NPs to the corresponding ADC. We showed that ANANAS-tethering of cetuximab promotes a more efficient EGFR-dependent vesiclemediated internalization, leading to a potent drug delivery system capable to overcome resistance to therapy in a triple negative breast cancer model. Cetuximab-guided ANANAS carrying doxorubicin are more cytotoxic in vitro and much more potent in vivo than the corresponding ADC, leading to 43% tumour reduction at very low drug dosage (0.56 mg/kg).The results (Fig.1) [4], for the first time, provide a quantitative evidence that advantage of antibodyguided nanoparticles with respect to the ADC may go beyond the increase in drug-to-antibody ratio.

13h15 - 13h30 ***Carbon Nanotubes as Intelligent Systems for Cancer Therapeutic Nano-Delivery***

Monica L. Fanarraga
Universidad de Cantabria-Idival

Carbon nanotubes (CNTs) are nanomaterials with a high interest in the industry that recently have drawn special attention in the field of nanobiotechnology. As other carbon allotropes, CNTs display an extraordinary capacity to capture biomolecules from the environment, acquiring different biological identities. But their unidimensional nature endows CNTs with unique properties in vivo. These nanofilaments can cross many different types of membranes, penetrating inside cells or tissues. More interestingly, their unique morphology and surface properties prompts their biomimetic interaction with the intracellular biological nanofilaments, namely microtubules, actin and DNA, triggering interesting effects at the cellular level that deserve special attention when developing nano-vectors for anticancer therapies. In the treatment of human cancer, multiple-drug resistance is a major problem. To circumvent this issue, clinicians combine several drugs. However, this strategy could backfire resulting in more toxic or ineffective treatments. We propose the use of multi-walled nanotubes (MWCNTs), in the design of active-by-design nanocarriers, attempting to enhance the effect of broadly used chemotherapies. MWCNTs display intrinsic properties against cancer interfering with microtubule dynamics[1] and triggering anti-proliferative,[2] anti-migratory[3] and cytotoxic[4] effects in vitro that result in tumor growth inhibition in vivo.[5,6] Remarkably, these effects are maintained in tumors resistant to traditional microtubule-binding chemotherapies such as Taxol®.[5] Our results demonstrate how the total effect of the drug 5-FU is remarkably improved (50% more effective) when delivered intratumorally coupled to MWCNTs both in vitro and in vivo in solid tumoral models.[7] Our results demonstrate how using MWCNTs as anti-cancer drug delivery platforms is a promising approach to boost the efficacy of traditional chemotherapies, while considerably reducing the chances of resistance in cancer cells. All these intrinsic properties of CNTs have a huge potential if exploited in the development of smart materials in nanomedicine against.

Clinical Applications **| Moderator: Alexandre Ceccaldi**

11h30 - 12h00 ***RUBYnanomed: Non-invasive Monitoring of Cancer***
Lorena Diéguez
RUBYnanomed | Medical Devices Group Leader at INL

RUBYnanomed has developed a precise cancer snapshot tool, the RUBYchip®, a microfluidic device for isolating all types of circulating tumour cells (CTCs) from unprocessed whole blood. In this way, RUBYnanomed offers a non-invasive and real-time snapshot of cancer progression to oncologists. In a first preliminary clinical test, we demonstrated the higher efficiency and sensitivity of our cartridge against the reference CellSearch® equipment in a small pool of 9 metastatic colorectal cancer patients. We were able to isolate a higher number of CTCs from unprocessed whole blood samples, and showed the potential to provide improved correlation with clinical prognostic information (Scientific Reports, 2019).Currently, RUBYnanomed is running pre-clinical trials at 8 hospitals for 5 different types of cancer in different countries in Europe.

12h00 - 12h30 ***Curadigm, a Platform Shifting Therapeutic Delivery to Elevate Treatment Outcomes for Every patient***
Matthieu Germain
Curadigm

Curadigm's platform technology is based on engineered, biocompatible nanoparticles called nanoprimer that transiently occupy the liver pathways responsible for therapeutic clearance and toxicity. This has the potential to lead to enhanced systemic bioavailability and improved accumulation in the target tissues. Curadigm's unique approach is designed to augment the beneficial aspects of therapeutics while mitigating toxicities, with the goal of increasing patient quality of life, improving outcomes, and decreasing costs. The technology is widely applicable and can be adapted to work with a variety of therapeutic classes including nanomedicines, nucleic acid therapeutics, small molecules, and gene editing technologies.

12h30 - 13h00 ***Taking a Nanoparticle Medical Product To the International Market***
Eric Mayes
EndoMag

Given their ability to be detected and influenced from outside the body, magnetic nanoparticles are a popular theme in the field of nanomedicine. Endomag has developed a platform of devices that use magnetism for guidance in cancer surgery.

Endomag's first marketed product is called "Magtrace", which is a dispersion of iron oxide nanoparticles coated in polysaccharides. Following injection, Magtrace follows the lymphatic drainage pathways from a tumour to the lymph nodes that could be implicated in the spread of cancer. Dr Mayes will discuss the challenges involved in translating a nano-based medical product from concept to international commercialisation, particularly highlighting the nano-specific regulatory challenges.

13h00 - 13h30 **Panel discussion** ***"Mission Cancer In Horizon Europe: What Nanomedicine can Bring?"***
Moderator: Alexandre Ceccaldi

13h30 - 14h30 ***Networking Lunch***
Poster Exhibition & Industry Exhibition

Nanostructured Theranostic Systems **| Chair: Graeme Stasiuk**

14h30 - 15h00 ***Development of PDT Based Theranostics***
Graeme Stasiuk
Non-Clinical Lecturer in Molecular Imaging at University of Hull

Dr Graeme Stasiuk was appointed as Non-Clinical Lecturer in Molecular Imaging at the University of Hull, in the School of Life Science in June 2014. He has seven years of research experience, post PhD, into the design, synthesis and development of molecular imaging agents for all modalities.
Following a year at the French Alternative Energies and Atomic Energy Commission (CEA) in Grenoble, developing multimodal nanoparticle MR/Fluorescent contrast agents, he joined Professor Nick Long's group at Imperial College London in 2011 as Post-Doctoral Research Assistant, this work was focused on multimodal and targeted imaging.

15h00 - 15h15 ***Approaches for Combined Therapeutics and Theranostics Using Hybrid Sensitive Nanoparticles***
Pablo Taboada
Universidade de Santiago de Compostela

Recent advances in nanotechnology and biotechnology have contributed to the

development of sensitive multifunctional nanoparticles able to produce different and complementary therapeutic outcomes and/or to be simultaneously used as imaging and therapeutic tools, opening a new filed of research coined as Theranostics. Theranostics as an integrated imaging and treatment strategy for individual patients encompasses personalized medicine, pharmacogenomics, and molecular imaging, in order to develop an efficient new targeted therapy and optimize drug selection via a better molecular understanding of wide world extended diseases such as cancer, cardiovascular or infectious diseases. Furthermore, Theranostics aims to monitor the response to the treatment, to increase drug efficacies and safety through an exquisite control of drug administration profiles and release rates, and to reduce the necessary treatment of patients, resulting in significant cost savings for the overall healthcare system. In the present talk, several examples of sensitive hybrid theranostic nanoparticles and nanodevices based on the combination of organic and inorganic materials and their potential biological outcomes, especially in cancer treatment, will be discussed. An special emphasis will be done in those nanoplatforms in which their physical and chemical properties can be modulated either by internal and/or external stimuli in order to optimise their potential responses.

15h15 - 15h30 ***DelNAM: Fighting the Crisis of Bacterial Resistance to Antibiotics***

Rita S. Santos
LEPABE, Department of Chemical Engineering,
Faculty of Engineering of the University of Porto

Antimicrobial resistance to antibiotics threatens the global public health; it is predicted to cause 10 million deaths in 2050, if no solution is found. Nucleic acid mimics (NAMs) such as Locked Nucleic Acid (LNA) and 2'-OMethylRNA (2'OMe) have the potential to become such solution. NAMs can act as novel antibacterial drugs by hybridizing and, thus, inhibiting the expression of essential bacterial genes. However, the envelope that surrounds the bacterial cytosol presents a stringent barrier to the internalization of the NAMs. Therefore, delivery strategies are needed to intracellularly carry the NAMs into bacteria. Although nanocarriers have been widely explored for nucleic acids delivery into animal cells, their use in bacteria is still on its infancy. DelNAM is a European project aiming to strengthen the research area of NAMs delivery into bacteria. In order to accomplish this task, DelNAM gathers expertise on the synthesis of NAMs (University of Southern Denmark), nanomedicine (Ghent University) and NAMs hybridization in bacteria (University of Porto). Carriers such as cell-penetrating peptides and fluid liposomes seem attractive to overcome the complex bacterial envelope. Indeed, Post-PEGylated DOTAP-DOPE liposomes were already shown to be able to intracellularly deliver LNA/2'OMe NAMs into the gram-negative bacterium Helicobacter pylori – the most prevalent chronic infection. DelNAM will further explore this concept in other infections, studying different liposomal and peptide formulations, as well as NAMs and therapeutic targets. As such, DelNAM is expected to fulfill the potential of NAMs to become an alternative to antibiotics.

15h30 - 15h45 ***Glucose-derived Carbon Nanoparticles are Promising Safe and Efficient Agents for the NIR-induced Phototherpy of Tumours***

Ida Kokalari
Department of Chemistry, University of Torino, Italy

Carbon nanoparticles (CNP) are attractive candidates as they could make possible the combination of multiple functions in a single nanostructure. However, their development is hampered by the reduced number of synthetic strategies that allow the production of CNP in good yield and in a reproducible way. In the present study, a green synthetic approach based on the hydrothermal carbonization (HTC) of glucose and non toxic solvents was used. CNP appear to be monodispersed and almost perfectly spherical in shape. The surface was characterized by negatively charged groups which confer high colloidal dispersion stability and could provide suitable linkage for a wide range of functionalization, interesting in drug delivery applications. Furthermore, CNP exhibited photothermal and photodynamic properties in cell free systems when irradiated with NIR light. CNP were firstly tested for uptake, cytotoxicity, and ability to interfere with the cellular redox homeostasis on macrophages (RAW 264.7) and alveolar epithelial tumor cells (A549). Transmission electron microscopy (TEM) revealed that nanoparticles internalization in both cell lines. However,CNP exhibited a safe profile for doses up to 80 μg/ml. In addition, CNP were found able to scavenge hydroxyl radical (EPR spectroscopy) displaying a possible antioxidant activity, which was confirmed in cells. In fact, CNP induced a reduction of LPS-activated ROS generation in macrophages. Oppositely, upon NIR irradiation (laser beam wavelength 945 nm), CNP were able to generate heatand singlet oxygen (1O2), promoting cell death of tumor cells thus acting as a promising photothermal/photodynamic agent for cancer treatment. In conclusion, the experimental evidences suggest that CNP may represent a promising agent for the NIR-photo-therapy of tumors.

15h45 - 16h00 ***Functionalized Magnetic Nanoparticles: a Versatile Platform for Cancer Diagnosis and Treatment***

Nuria Lafuente-Gómez
Institute for Advanced Studies in Nanoscience (IMDEA Nanociencia)

Nanotechnology has received tremendous attention due to its biomedical applications. Particularly, magnetic nanoparticles (MNP) offer a versatile platform based on the combination of the magnetic properties of the core with the versatility of an organic coating able to impart new and specific responsibilities [1]. Moreover, they can also be seen in magnetic resonance imaging and combined with hyperthermia treatment if an alternating magnetic field is applied. In this regard, we propose a system based

on MNP synthesized by co-precipitation method composed by a core of maghemite and coated with dextran-based molecules to covalently attached molecules such as fluorophores, drugs, oligonucleotides or antibodies. In cases in which we want to release the molecule inside tumoral cells, we use disulfide bonds which are prone to rapid cleavage in tumor tissues ([GSH] 0.5-10 mM) and not in healthy ones (0.5-10 μM. By doing so, we have a smart theranostic tool. On the one hand, by modifying MNP with the Cy5 fluorophore, we confirmed that MNP targeted the tumoral environment in mouse lung cancer model, probably due to the EPR effect. On the other hand, MNP functionalized with gemcitabine were tested in pancreatic cancer cell lines, and their cytotoxic effect was remarkable. The results show the enormous potential of our proposed nanosystem for both diagnosis and treatment of cancer.

16h00 - 16h15 ***Dendrimer Nanotechnology for Cancer Therapy***

Ling Peng
Centre Interdisciplinaire de Nanoscience de Marseille,
Aix-Marseille University, CNRS, Marseille, France

Cancer is one of the leading causes of death in the world, and remains a difficult disease to treat because of poor prognosis, rapid tumor metastasis and drug resistance. Therefore, there is high demand for innovative therapeutic modalities that facilitate early and precise diagnosis, deliver safe and effective treatment, and overcome drug resistance. Nanotechnology-based cancer therapy is widely expected to bring advances in this direction by exploiting the distinct tumor microenvironment. Dendrimers are ideal materials for constructing nanomedicine by virtue of their uniquely well-defined structure and multivalent cooperativity. We have recently established modular, responsive and adaptive dendrimer nanosystems which are able to carry either hydrophobic anticancer drugs 1 or hydrophilic nucleic acid therapeutics 2 for effective anticancer activity to combat drug resistance. Also, the dendrimer nanosystems are able to deliver imaging agents for better imaging quality by harnessing the multivalent feature of dendrimer and the EPR effect of the tumor microenvironment. 3 These dendrimer nanosystems are expected to offer new perspectives on nanotechnology-based biomedical applications for treating various cancers.

16h15 - 16h30 ***A Three-Colour Fluorescent Supramolecular Nanoassembly of Phototherapeutics Activatable by Two-Photon Excitation with Near Infrared Light***

Salvatore Sortino
University of Catania

Fluorescent photoactive nanoassemblies able to release multiple therapeutic species under control of light can open intriguing prospects for novel imaging-guided multimodal therapeutic treatments. This allows indeed the visualization of the phototherapeutic agent in cells through fluorescence techniques and can provide a highly localized "burst" precisely at the desired sites.1 Singlet oxygen (1O2) and nitric oxide (NO) represent fascinating multitarget therapeutic agents avoiding Multiple Drug Resistance problems encountered with several conventional drugs often target-specific. Furthermore, due to their short half-life and lack of charge, both 1O2 and NO diffuse in the cellular environment over short distances without inflicting systemic side effects common to general anticancer drugs. Therefore, the combination of 1O2 with NO appears to be very suited to tackle cancer diseases through light-triggered approaches.We have designed here a novel nanoconstruct in which a water soluble epichlorohydrin branched β-cyclodextrin polymer tagged with the green emitter fluorescein-isothiocyanate co-encapsulates a zinc phthalocyanine, as a red emitter 1O2 photosensitizer, and a tailored nitroaniline molecular hybrid, as NO photoreleaser bearing a blue fluorescent reporter. The host nanocarrier and the guest components can be operated in parallel under light inputs, imposing to the whole supramolecular nanoconstruct "five in one" photofunctionalities. In fact, it exhibits distinct green, red and photoactivable blue fluorescence and, at the same time, simultaneously photoreleases the cytotoxic 1O2 and NO. The nanoassembly internalizes with its cargo in melanoma cancer cells where the host and guests components can be visualized due to their distinct fluorescence colours upon simultaneous two-photon excitation in the phototherapeutic window at 740 nm. Enhanced cell mortality most likely due to the combinatory effect of the simultaneously generated cytotoxic 1O2 and NO is also observed.

Disruptive Medicine **| Moderator: Alexandre Ceccaldi**

14h30 - 14h55 ***The Continuum of Care: Why Emerging Medical Technologies Revolutionize Healthcare***

Kathleen Spring
Project manager at Bioanalytik Münster

The healthcare sector today seems to be at the verge of a new era. Driven by key enabling technologies such as nanotechnology, growing biomedical knowledge as well as the need to balance costs in healthcare, today's healthcare system will undergo an extensive transition in the coming years. New medical technologies and products shape this transition towards a continuum of care. The continuum of care is a holistic concept for healthcare which aims to change the current system with the help of emerging technologies towards more personalised and patient-oriented healthcare.

14h55 - 15h20 ***Simulation and Virtual Reality Enabling Better Safer Healthcare***

Paul Galvin
Tyndall Institute

Avoidable medical errors are the third biggest cause of death in US, resulting in an estimated 400,000 deaths per year. Some of the errors can be prevented by ensuring proficiency of all clinical personnel involved in a medical procedure. By designing and implementing simulation based training programmes, clinical staff can practice and become proficient, ensuring they implement all of the microtasks of a procedure according to established best practice. This process is well established in the aviation industry, but to date has not been widely used in medicine. This presentation will demonstrate how state of the art and emerging technologies are now enabling clinicians to perform simulations appropriate to the procedure, patient anatomy and context, even including simulations for infrequent complications during a procedure. Future medical devices will need to be designed not only for manufacture and test, but should also be compatible with an intuitive simulation based training programme to ensure clinical proficiency in the use of the device for all anticipated contexts.

15h20 - 15h45 ***PRIMA a Photovoltaic Sub-retinal Microchip Retinal Implant and a Brain-machine Interface for Dry AMD***

Priscilla Pagnacco
Pixium Vision

Pixium Vision is a bioelectronic company specialized in neuromodulation. The company's mission is to create a world of bionic vision for those who have lost their sight, enabling them to regain partial visual perception and greater autonomy. Pixium Vision develops bionic vision systems like PRIMA, a sub-retinal miniature photovoltaic wireless implant system, designed for patients who have lost their sight due to outer retinal degeneration, initially for atrophic dry age-related macular degeneration (dry AMD). The device is currently evaluated in two feasibility studies, one in Unites States and one in France for those the company recently published preliminary positive results at 6 months.

15h45 - 16h10 ***The Brain Computer Interface (BCI) Projet by CLINATEC***

Simon Baconnier
CEA

The BCI (Brain Computer Interface) by CLINATEC aims to develop compensating systems that will help people with a severe motor control disability recover mobility. The BCI project aims to demonstrate that it is possible to drive an exoskeleton thanks to an implant that records cortical signals, opening up the prospect of a better future

for people with motor function disabilities. The project is based on the fact that when we imagine making a movement, we trigger the same electrical activity in the motor cortex of the brain as when we actually perform that activity. The project therefore aims to record these electrical signals, known as ElectroCorticoGrams, and decode them to drive complex objects, for example, to move the limbs of an exoskeleton.

16h10 - 16h30 ***Q&A***

16h30 - 17h00 ***Coffee-break***
Poster Exhibition & Industry Exhibition

Organ-on-a-chip and Biomimetic Systems **| Chair: Elena Martínez**

17h00 - 17h30 ***Engineering Biomimetic 3D Models of Intestinal Epithelium***
Elena Martínez
Institute for Bioengineering of Catalonia

After finishing my BSc in Physics, I started my scientific career completing a PhD in Materials Science in 2001 (University of Barcelona, Spain), focused on the study of the mechanical and tribological properties of thin films by nanoindentation technique.
Afterwards, I was engaged as a postdoc associate at the Laboratoire de la Matière Complexe, led by Prof. Francis Levy, at the École Polytechnique Fédéral de Lausanne (EPFL, Switzerland). There, I worked with nanostructured materials based on chromium nitride (CrN). In 2003, I received a "Ramon y Cajal" grant to lead a five year research project on the micro and nanostructuring technologies of polymer materials at the Barcelona Science Park (www.pcb.ub.es). It meant building the facilities and expertise in the newer micro and nanofabrication techniques for biomedical applications such as soft lithography, microfluidics, microcontact printing, nanoimprint lithography, focused ion beam nanolithography and dip-pen nanolithography. In 2008, I joint as Senior Researcher the Nanobioengineering group of IBEC led by prof. J. Samitier. There, I have been developing micro and nanopatterning techniques for cell culture applications as an independent research line.
In 2013, I was formally able to constitute my own independent research group, "Biomimetic systems for cell engineering", by being appointed as a "Junior Group Leader" at the Institute for Bioengineering of Catalonia (IBEC). Our research focuses on the technological development of cell culture systems that mimic in vivo complex

signals (topographical, biochemical and mechanical) for applications in basic cell research, disease modeling and regenerative medicine. Such new technological setups are being developed by applying micro and nanofabrication techniques, including microfluidics, to soft polymer materials (extracellular matrix-like materials) in combination with tissue engineering approaches (mini-bioreactors with electrical and mechanical stimuli) to account for the 3D architecture, ligand distribution at the nanoscale, spatiotemporal biochemical gradients and mechanical properties of the living tissues. The aim of such systems is to provide reliable in vitro models of tissue-like constructs that help to fill the gap between conventional 2D cell cultures and animal experiments.

17h30 - 17h45 ***From Human Induced Pluripotent Stem Cell to Barrier-building Endothelial cells - Using a Realistic Model of the Blood-brain Barrier for the Identification of Essential Nanoparticle Characteristics***

Helen Nneka Onyema, Fraunhofer Institute for Microengineering and Microsystems IMM

Curing glioblastomas is still challenging as potential drugs (e.g. Doxorubicin) cannot cross the blood-brain barrier (BBB) for the treatment of this aggressive cancer. The cells commonly used in models of the BBB are often not of human origin or lack of the essential barrier function the way it can be found within the brain. Therefore, most models only give a first tendency but are not suited for directly translating the generated results to the clinic. Hence, barrier-building endothelial cells were differentiated from human induced pluripotent stem cells (iPS) which leads to a more realistic model as it is from human origin, expresses essential endothelial cell proteins (e.g. Claudin-5, CD31, Occludin) and builds a tight barrier. Due to their ability to cross the barrier and release their content in the brain nanoparticles (NP) have been considered as potential drug delivery systems. The well-characterized NP were synthesized and formulated by a combination of miniemulsion and solvent evaporation and were composed of Polylactid and stabilized by Tween 80. For the attribution of the successful internalization of the NP to their essential characteristics, not only their protein corona via mass spectrometry but also their size, aggregation behavior and surface charge are analyzed. Guaranteeing a safe application of the biodegradable NPs as drug delivery systems in the future, the toxic potential of all NPs is identified. The major aim is to establish a realistic 3D model of the BBB consisting of human glioblastoma as the target cells and the barrier-building endothelial cells differentiated from human iPS. Additionally, the system is equipped with a peristaltic pump simulating the blood flow within blood vessels which has a great influence not only on the barrier function but also on the efficiency of the NP crossing the barrier.

17h45 - 18h00 ***Fabrication of Microfluidic Chips and Preclinical Devices by Laser Technologies***

María Aymerich, Photonics4Life Research Group,
Departamento de Física Aplicada, Facultade de Física,
Universidade de Santiago de Compostela, Spain

Photonics4Life research group has a strong background in laser structuring of materials by laser technologies. In particular, fluidic devices can be manufactured with dimensions ranged from microns to millimetres by this technology. In this work, technologies employed, capabilities and final devices made by the group will be presented. By using different techniques, customizable devices from microfluidic chips to organ-on-a-chip models of big blood vessels can be obtained. Different laser equipments are available in the research group with different pulse durations and wavelengths, in order to optimize the fabrication process regarding the substrate. Laser technologies outstand among others due to advantages like accuracy, versatility, speed, non-contact nature or no requirement of special facilities such as cleanrooms. All of this features allow the fabrication of ad hoc devices with a competitive prototyping and fabrication cost since no mold, clean rooms, neither mass production are necessary. Devices can be manufactured in plenty of materials, outstanding glass when robustness and chemical resistance is desirable, PDMS when biocompatibility and long term cell culture is searched or flexible materials when the application requires so. Some of the validated applications of theses devices are organ-on-a-chip model of blood vessel for the study of vascular pathologies or circulating tumour cell deposition and the study of fluid dynamics in bloodstream.

18h00 - 18h15 ***A Microfluidic System to Sort Blood Cells and Assess their Deformability in Healthy blood and in Diabetes Mellitus Type II***

Vera Faustino, MEtRICs, Mechanical Engineering Department
University of Minho, Guimarães, Portugal

Microfluidic devices have been widely used as a valuable research tool for diagnostic applications, in particular since they have been related to the successful detection of different diseases and conditions by assessing the mechanical properties of red blood cells (RBCs). Passive microfluidic devices have long been a topic of interest for such applications, due mainly to its associated low cost and easy fabrication, when compared with active microdevices, since passive microdevices do not need any electronic, mechanical or acoustic components. In the present work a passive microfluidic device with several stages of cross-flow filtration barriers and a sequence of three hyperbolic contractions in each outlet was used, to separate and sort, simultaneously, red blood cells (RBCs) and white blood cells (WBCs) and measure the blood cells deformability. Diluted human blood from healthy donors

and from patients diagnosed with diabetes mellitus type II were flowed through the microfluidic device.
The experimental set-up has a high-speed camera combined with an inverted microscope where the image sequences were obtained at a frame rate of 2000 frames/s with a constant flow rate controlled by a syringe pump. The images sequences recorded during the flow visualizations were analyzed and processed by using image processing tools from the ImageJ software in order to measure the cells deformability. The cells separation efficiency was quantified by using an automatic hematology analyzer system and it was obtained a mean of 40% of RBCs separation from plasma. The deformability analysis has shown that the RBCs deformability is correlated with the presence of pathological blood. Detecting deformability changes in the cells and being able to separate those cells may be a key factor in assuring the success of detection of some blood diseases with diagnostic devices. Hence, the proposed microfluidic device has potential to perform in one single step a partial passive separation of RBCs based on their deformability. Evidences that the device is also capable of separating WBCs by size, were observed.

Pitch-me-up! Session #1: Manufacturing & Characterisation

17h00 - 17h15 ***Modular Manufacturing Platform for Reproducible Continuous Synthesis of Versatile Nanomaterials for Nanomedical Applications***
Regina Bleul
Fraunhofer IMM

17h15 - 17h30 ***Pilot line Development for the Scale-up of Nanopharmaceuticals***
Nikolas N. Daskalakis
National Formulation Centre, Centre for Process Innovation

17h30 - 17h45 ***IC-Tagging Methodology: Cost-effective Production of Proteins for Biomedicine & Vaccines***
José Manuel Martinez Costas
CiQUS - University of Santiago de Compostela

17h45 - 18h00 ***Measuring Particle Size Distribution of Nanoparticle Enabled Medicinal Products, the Joint View of EUNCL and NCI-NCL. A Step-by-step Approach Combining Orthogonal Measurements with Increasing Complexity***
Fanny Caputo
CEA-Leti

18h00 - 18h15 ***Multi-Detector Field-Flow Fractionation: A Powerful Analytical Tool in the Field of Nanomedicine***
Florian Meier
Postnova Analytics GmbH

18h15 - 18h30 ***TRPS: High Precision Characterization for Nano and Micro Particles***
Camille Roesch
IZON Science

19h00 - 19h30 ***Tour: Bom Jesus***

19h30 - 22h30 ***Gala Dinner***

DAY 3

June, 19 | Wednesday

Keynote Session

09h00 - 09h30 ***Multifunctional Polymeric Nanocapsules for the Targeted Delivery on Oncological Drugs- Learning from Euronanomed***
Maria José Alonso, Pharmacy and Pharmaceutical Technology, University of Santiago de Compostela, CIMUS Research Institute

María José Alonso is Professor of Biopharmaceutics and Pharmaceutical Technology at the University of Santiago de Compostela.
Her lab has pioneered numerous discoveries in the field of nanomedicine and drug delivery. She has coordinated and participated in a high number of international research consortia financed by the WHO, the Gates Foundation, The World Cancer Research organization, the National Institute of Health (NIH) and the European Commission. She is the author of over 270 scientific contributions with more than

16,100 cites (H factor 71) and the inventor of 21 patent families. Because of the quality of her papers she has been among the TOP TEN in Pharmacology (Times Higher Education).
She has received 28 Awards, including the "King Jaume I Award" given to the best researcher in the area of new technologies in Spain, the "Maurice Marie Janot Award" given by APGI, the EU society of Pharmaceutical Technology and the "CRS Founders Award" of the Controlled Release Society (CRS).
She was the Vice-rector of Research and Innovation of the USC. Currently, she is President of the Controlled Release Society and Editor-in-Chief of the Drug Delivery and Translational Research jounal. She is also a member of three Academies in Spain, a member of the College of Fellows of AIMBE, a member of the College of Fellows of the CRS and a member of the US Academy of Medicine.

09h30 - 10h00 ***Mimicking the Lung Parenchyma with Organs-on-chip Technologies***
Olivier T. Guenat, AlveoliX AG & Medical Faculty, University of Bern

Olivier Guenat was born in Biel-Bienne and received his engineering degree (HTL/ETS) in Microtechnology from the University of Applied Sciences in Bienne in 1990, his MSc degree in Physics and Electronics from the University of Neuchâtel in 1995, and his PhD degree in Micro- and Nanotechnology in 2000 from the same institution. After his PhD, he was awarded an advanced fellowship from the Swiss National Foundation and pursued his postdoctoral studies at Harvard Medical School. There, he developed microfluidic systems aimed at monitoring cell signals. He then hold an Assistant Professor position at the Ecole Polytechnique in Montréal (Canada), where he founded and led the BioMEMS research laboratory until 2009. From 2009 to 2011, he returned to Switzerland and helped develop the Nanomedicine Division of CSEM SA, as Head of the Cell Systems Section. In November 2010, while still being active at the CSEM he was asked to set up the ARTORG Lung Regeneration Technologies activities within the framework of a contract between the University of Bern and the CSEM. In 2012, he decided to focus on his activities at the ARTORG Center. Today, his research aims at developing advanced in-vitro systems that better mimic the in-vivo conditions of the lung.

Brokerage Session

10h00 - 10h10 ***Horizon Europe Status and Planning: What's the Possible Situation of Nanomedicine in it?***

Heico Frima, European Commision

10h10 - 10h20 ***H2020 WP2020: Upcoming Calls Regarding Nanomedicine***
Maria Nieves Gonzalez Lopez,
NCP Spain NMBP

10h20 - 11h00 ***Contributed Talks***

11h00 - 11h30 ***Coffee-break***
Poster Exhibition & Industry Exhibition

Nanobiosensing for Personalised Medicine | **Chair: María Tomás Gamasa**

11h30 - 12h00 ***Bioorthogonal Transition Metal Catalysts for Cellular Intervention***
María Tomás Gamasa
University of Santiago de Compostela

Dr. Tomás Gamasa presents a quite unique multidisciplinary training in the fields of Organometallic catalysis, Bio-supramolecular Chemistry and Chemical Biology. She completed her Ph.D. in 2011 as FPU fellow at University of Oviedo. The doctoral studies were focused on the design and development of synthetic methodologies. She performed several research stages at different national and international institutions. In 2011, she joined Professor Thomas Carell group at LMU Munich as Post-Doctoral Research Humboldt fellow. Her work was centered on DNA modifications for the expansion of the genetic code. During her reincorporation as Juan de la Cierva fellow in the group of Prof. J. L. Mascareñas (USC, CIQUS institute) in 2015 she started working on the translation of chemical reactions to cellular environments. While this research can be ascribed to the realm of fundamental science and seeks to explore how far can scientists stress living systems to accept artificial catalytic processes, the resulting knowledge might lead to the development of new cell reactivity patterns and new tactics in biomedicine, for instance, for the regulated productions of multiple, different drugs in an orthogonal manner, opening new avenues for biological manipulation of living systems.

12h00 - 12h15 ***Molecular Phenotyping:***
Towards the Next Generation of Sequencing

Weng Kung Peng,
International Iberian Nanotechnology Laboratory
Braga, Portugal

Genetic contribution to different diseases were found to be varies and often very little, with nongenetic factors (e.g., environmental hazards) having much greater attributable risks, producing a large phenotypic variation. As a result, the current one-size-fits-all medical practices are suboptimal, leaving much room for improvement. The key goals to understanding human health and disease depend the ability to access the 'genotype-phenotype' correlogram through various omicsplatform (e.g., proteomic), and the success of translating technological innovations (-onicsplatform) into molecular medicine. Our research focuses on addressing the challenges (and unprecedented opportunities) by introducing a novel class of two-dimensional NMR-based ´molecular signature´ of biological fluids (e.g., blood) with respect to its´ various pathophysiological states. Liquid biopsy holds great promises in clinical medicine as it provides multiple global snapshot information non-invasively for disease management (e.g., prognostic, predictive treatment/recurrence) in uniquely personalized manner. We demonstrated that highly unique and specific molecular subtype can be rapidly phenotyped for disease diagnosis and monitoring (e.g., malaria1,2, hemoglobinopathies3, diabetes mellitus4) using a single drop of blood with point-of-care NMR system.

12h15 - 12h30 ***Biophotonic and DNA Sensor Interaction for Early Detection of Alzheimer's in Blood***

Diana Vasquez
International Park of Creativity

Biomolecules such as proteins, DNA, and other natural compounds are ideal markers for detecting diseases such as Alzheimer's due to their inherent biophotonic characteristics. This is important since Alzheimer's is not a predictable disease. We have developed a DNA sensor produced by using synthetic biology to detect presence of amyloid, which is one of the main proteins expressed in Alzheimer's patients. The DNA sensor takes advantage of the inherent biophotonicity of molecules such as DNA and proteins in order to detect Alzheimer's before the onset of the disease. The DNA sensor was assembled from natural and/or synthetic sequences and subsequently transformed into host cells bacteria or yeast. The efficacy of the DNA sensor was tested based on fluorescence intensity when mixed with human blood plasma using a fluorescence detector at different wavelengths. The intensity of the fluorescence was correlated to clinical parameters, tomographic images, and glycaemia results from patient blood samples. The expression of amyloid protein was also tested using standard methods including biochemical assays. The results of these correlations allowed us to group three different categories of patients. In case of Alzheimer's, patients were divided into Alzheimer's diagnosed, pre-Alzheimer's, and normal groups. The results of this investigation can predict presence of Alzheimer's as well

as the onset of the disease, which has been a great challenge since most Alzheimer's diagnostic methods are applicable only after the person has already acquired the disease.

12h30 - 12h45 ***A Field-Effect Transistor-based device for non-invasive glucose sensing in saliva***

Joanna Jankowska-Śliwińska,
Łukasiewicz Research Network-Institute of Electron Technology

The development of the methods of glucose sensing recently increased with the increase of the number of patients with diabetes globally. According to the latest World Health Organization reports the proportion of diabetes patients by the year of 2030 is predicted to be 9.7% of the general population. Taking into account the predictions and the fact that methods of glucose monitoring used so far are painful and inconvenient for the patients because of their invasiveness, there is a high demand for elaboration of a new non-invasive, selective and highly sensitive devices for glucose level monitoring. Moreover, the analysis of saliva in terms of glucose measurements seems to be attractive due to the lack of daily multiple repeated injections, easiness of taking and a positive correlation between glucose level in blood and in saliva. According to the current state of the art field-effect transistor (FET)-based biosensors due to their large current amplification and high signal-to-noise ratio are the devices of choice for non-invasive glucose biosensors. In the case of the channel material for FET construction, In-Ga-Zn-O (IGZO) has been widely used due to its high electron mobility and amorphous microstructure. By taking the advantage on the possibilities delivered by IGZO thin-film transistors we developed the device based on glucose FET biosensor featuring IGZO as a channel layer, and a Ru-Si-O Schottky gate electrode coated by Nafion® - polymeric membrane material, with embedded glucose oxidase (GOx) – the recognition element, which exhibits sensitivity to the glucose concentration in sub-millimolar concentration range corresponding to the glucose concentration in human saliva. The changes in the drain current of the IGZO FET biosensor as a function of glucose concentration were used for plotting the calibration at the VDS = 2 V. Two linear regions for glucose detection were obtained. The first one, in glucose concentration range of 0÷2 mmol/l and the second one, in glucose concentration range of 2÷10 mmol/l with regression coefficients equal to R2 = 0.997 and R2 = 0.873, respectively. The sensitivity of the fabricated IGZO FET biosensor is equal to 2.23 μA/mmol/l and 0.41 μA/mmol/l for the two ranges of glucose concentration. Importantly, the first linear range covers the glucose concentration in saliva.

12h45 - 13h00 ***Nanobiophotonics Application for the Investigation***

of Neurodegenerative Diseases in Accessible Biofluids

Silvia Picciolini, IRCCS Fondazione Don Carlo Gnocchi, Firenze | Itália

Nanobiophotonic platforms like those based on Raman Spectroscopy (RS) could be useful to detect with a fast and sensitive process all the biochemical variations of a considered sample. In the case of biofluids, RS can analyze the biochemical profile of all the soluble metabolites and also of complex circulating biomarker carriers like Extracellular Vesicles (EVs), identifying possible modifications due to a specific pathological state. Taking advantage of this technique, we tried to perform RS analysis on biofluids collected with a minimal- or not-invasive process from patients affected by neurodegenerative disorders. Our aim was to develop diagnostic, prognostic and monitoring tools for the pathological state evaluation. We analyzed saliva, serum and EVs isolated from serum. For every type of biofluid, fine tuning of the methodology was performed to obtain highly reproducible results.
Saliva is a complex mixture of biological molecules (protein, lipids, metabolites, hormones, nucleic acids) transported from different biological districts, which are susceptible to changes, in presence and concentration, during pathological states. Some salivary proteins like chromogranin A and Super Oxide Dismutase 1 were already related to ALS onset together with other stress related metabolites. For these reasons, saliva collected from patients with Amyothrophic Lateral Sclerosis (ALS) was analyzed. Concurrently, serum was considered for the evaluation of the neurodegeneration associated to Alzheimer's (AD) and Parkinson's (PD) diseases, as aggregation prone proteins like amyloid-β for AD and α-synuclein for PD are known to circulate in blood playing a role in disease progression, together with other neuroinflammatory and disease related factors. Taking advantage of silver nanoparticles and naturally secreted nanovesicles, we enhanced the differences in the serum content of AD and PD patients, respectively. The data obtained from the RS analysis on saliva, serum, and circulating EVs were analyzed using multivariate statistical analysis showing significant differences between all the three patients' groups, when compared with the respective healthy controls. These results confirmed the potential applications of RS in clinical field, being able to be used as a fast and highly sensitive diagnostic, predictive and monitoring tool opening at the same time the possibility to better understand the pathological mechanisms of neurodegenerative diseases onset an progression.

13h00 - 13h15 ***Surface-enhanced Raman scattering spectroscopy meets microfluidics: towards sensitive, automated and high-throughout integrated tools for diagnosis***

Sara Abalde-Cela,
International Iberian Nanotechnology Laboratory, Braga, Portugal

Surface-enhanced Raman scattering (SERS) spectroscopy is known to provide highly sensitive detection in fields as diverse as analytical chemistry, biomedicine, environmental or materials chemistry. However, there is no universal best SERS platform, and a careful consideration of the analytical problem is required before choosing/designing a SERS sensor. For practical quantitative analytical applications, SERS must fulfil the general requirements of an analytical technique: reproducibility of the results, linearity of the response, standardisation, molecular selectivity and a clear methodology for sample preparation. In this context, microfluidics technology offers miniaturisation, automation, high-throughput and intrinsic reproducibility. Nevertheless, for the successful use of microfluidics as sensing platform, a very sensitive detection strategy is regarded. Fluorescence has been the main detection technique integrated with microfluidics since the boosting of its development. However, SERS allows detection limits compared to fluorescence or, in occasions, even better. Furthermore, SERS offers much more information than fluorescence, as the Raman spectrum is the fingerprint of the analyte under study. Last but not least, the narrow Raman signals allow for quality multiplex direct and indirect detection. As a consequence, SERS in combination with microfluidics arises as a powerful combination able to overcome the intrinsic bottlenecks of each of the technologies towards its application in the field of medical diagnosis. At the Medical Devices group at INL we have developed different solutions that combine the advantages of nanotechnology through SERS and microfluidics. Those solutions include the design and fabrication of microfluidic devices able to isolate circulating tumour cells (CTCs – cancer biomarkers), the use of SERS for the multiplex phenotyping of the captured cancer single-cells, portable and affordable SERS devices for the profiling of different cancer cells, hybrid biomaterials for the monitoring of the metastatic microenvironment and metabolism and an in-flow and high-throughput strategy for the discrimination of pathogens,among others. During this talk, I will expand on the different strategies we are tackling to provide ad-hoc solutions to the current health challenges, and the potential of the combination of microfluidics and SERS to open new avenues towards a fast, accessible and personalised medicine.

13h15 - 13h30 ***AFM as a Nanotool to Evaluate Cardiovascular Risk in Heart Failure and Hypertension Patients***

Nuno Santos,
Instituto de Medicina Molecular, Faculdade de Medicina
Universidade de Lisboa, Portugal

Erythrocyte aggregation is an indicator of cardiovascular risk, which is influenced by plasma fibrinogen concentration. Fibrinogen levels are elevated during cardiovascular diseases. Our main goals were to understand how fibrinogen-erythrocyte binding influences erythrocyte aggregation and how it constitutes a cardiovascular risk factor in chronic heart failure (CHF) and essential arterial hypertension (EAH). Fibrinogen-

erythrocyte and erythrocyte-erythrocyte adhesion measurements were conducted by atomic force microscopy (AFM)-based force spectroscopy. Upon increasing fibrinogen concentration, there was an increase in the work and force necessary for cell-cell detachment, both for healthy donors and EAH patients. Nevertheless, higher values were obtained for the EAH patients at each fibrinogen concentration. Fibrinogen-erythrocyte (un)binding forces were higher in EAH and CHF patients, when compared with the control group, despite a lower binding frequency. Ischemic CHF patients showed increased binding forces compared to non-ischemic patients. Erythrocyte deformability (assessed as elongation index) results show that heart failure patients presented higher erythrocyte deformability than the control group at lower shear stresses, and lower deformability at higher shear stresses. This indicates that patients' erythrocytes are more deformable than those from healthy donors in blood vessels with larger internal diameters; however, in smaller-diameter vessels the opposite trend exists. Importantly, a 12-month clinical follow-up shows that CHF patients with higher fibrinogen-erythrocyte binding forces, probed by AFM at the beginning of the assessment, had a significantly higher probability of being hospitalized due to cardiovascular complications on the subsequent year. Our results show that AFM can be a promising tool for clinical prognosis, pinpointing those patients with increased risk for cardiovascular complications, for which special personalized medicine strategies should be envisaged.

EU Projects in Nanomedicine

11h30 - 11h40 ***Nobel***
Alexandre Superville, ETPN

The NOBEL Project aims at developing a European HealthTech ecosystem that will foster a healthcare revolution thanks to new emerging medical technologies. It will bring together nanomedicine, photonics, robotics, biomaterials and digital sciences into smart integrated medical devices, from academic research to the clinic. To that, it has 3 main missions: (i) to build an ecosystem, by organising multiple event and providing a space for dissemination to all European HealthTech stakeholders, thus becoming a unique meeting place; (ii) to shape a strategy, by building its common vision for the future of HealthTech in Europe, the Continuum of Care, integrating the separate roadmaps of individual technologies: (iii) to accelerate innovation, thanks to the HealthTech Translation advisory Board (TAB), a premium service offering free-of-charge tailored support to the selected innovations.

14h40 - 11h55 ***SAFE-N-MEDTECH***
Eusebio Gainza, Biopraxis

SAFE-N-MEDTECH consortium aims to bring a strong and competitive cooperation throughout the SAFE-N-MEDTECH OITB. This Consortium will offer a multidisciplinary and market-oriented innovation approach to academics, SMEs, Healthcare providers and Industries for the translation to the market of nano-enabled MTs, based on a deep understanding and knowledge of the material-nanoproperties, their advance use and applications in MTs and other aspects involved in MT safety (biocompatibility, electric compatibility, electromagnetic properties, and other properties SAFE-N-MEDTECH will build an innovative open access platform to offer companies and reference laboratories, the capabilities, knowhow, networks and services required for the development, testing, assessment, upscaling and market exploitation of nanotechnology-based Medical and Diagnosis Devices. This across the whole Life Cycle of nano-enabled MTs.

11h55 - 12h10 ***REFINE***
Adriele Prina-Mello, Trinity College Dublin

REFINE proposes a Regulatory Science Framework for the risk-benefit assessment of medical products and medical devices that are based on nanomedicines and biomaterials. The heart of our framework is the development of a product specific Decision Support System that identifies the most efficient way to deliver the data required by regulation by the best fitting methods. It will allow planning a cost-and time efficient strategy both for necessary measurements and for the advancement of methods. RERFIEN is identifying the regulatory challenges with Regulation Authorities from Europe and abroad (White Paper under publication), and design methods for tiered decision tree, guided by the latest scientific knowledge. We are studying/predicting physiological distribution of nanomedicines and biomaterials, as well as develop and validate new analytical or experimental methods and assays requested by the regulators. REFINE gathers a wide community of stakeholders in regulation, industry, science, technology development, patients, and end-users. This was recently captured and summarized in the REFINE 1st Knowledge Exchange Conference (April 2019).

12h10 - 12h25 ***TBMED***
Iraida Loinaz, CIDETEC

TBMED will establish an open innovation testing bed specialized in the development of high-risk devices (≥Class IIb). Due to a long reimbursement processes, patient access to innovative high-risk medical devices in Europe can take four times longer than in the U.S. In addition to this, the new regulations will stricter ex-ante controls

for this type of devices. This scenario represents a big challenge for European high-tech SMEs (representing 95% of the MedTech sector in Europe) to maintain their competitiveness and innovation capacity. TBMED will provide an integral service to accelerate the development of medical devices reducing time to market, covering technology development from TRL4-7 based on Quality-by-Design (QbD) concept and business management services. QbD concept enhances product and process understanding together with process control, based on robust scientific knowledge and quality risk management.

12h25 - 12h40 ***Cupido***
Daniele Catalucci, National Research Council, IRGB

The EU-funded project CUPIDO, started in February 2017, proposes an innovative solution: the application of nanotechnologies to the cardiovascular field. Cupido aims to hit the core of the cardiovascular disease, developing inhalable nanoparticles that can deliver a therapy directly to the diseased heart. Nanoparticles are extremely tiny, almost 1 million times smaller than a grain of sand in size and far too small to see with conventional microscopes. Exploiting such a tiny system as a route of administration can revolutionize the cardiovascular field, becoming the first non-invasive and heart-specific therapy. To achieve the goal, the Cupido consortium is working to develop biocompatible and biodegradable nanoparticles that can self-assemble and encapsulate drugs (novel or available) for cardiovascular disease. The nanoparticles, once inhaled, will first reach the lungs and later will translocate to the heart, where the drug will be finally released on the site of interest. The heart-specificity will be ensured thanks to chemical and magnetic guidance, reducing the chances of adverse side effects.

12h40 - 12h55 ***NeuroStimSpinal***
Paula Marques, Universidade de Aveiro, Portugal

NEUROSTIMSPINAL. Spinal cord injury (SCI) is a devastating pathology with dramatic lifetime consequences affecting thousands of people worldwide. Therefore, and considering the very limited regeneration ability of the central nervous system, in this project we propose to develop a neural tissue engineered scaffold capable of not only combining fibrous and porous topographic cues in order to mimic the morphology of the native spinal cord, but also potentiating the properties of graphene related materials (GRM) supported in a protein-rich decellularized matrix (adECM). In fact, the suggested 3D microenvironment should present electrical, chemical, mechanical and topographic features able to preserve neural cell survival and enhance neural progenitor cell differentiation towards neuronal and glial cells. Progress in this

sense will contribute to a better understanding of the key factors controlling repair in damaged neural tissues and, consequently, bring insights into new therapeutic approaches for spinal cord recovery

12h55 - 13h10 ***PANIPAC & METASTARG***
Marie de Fuente Freire, ONCOMET - IDIS

METASTARG is an innovative solution relying on nanotechnology for the early detection and treatment of occult micrometastasis to cause a direct impact in patient survival, quality of life, and health-economics. METASTARG Nanoemulsions are developed to identify OM by novel characteristic targets found in metastatic cells and Interrupt Metastasis Progression (NIMPs). This unique patient-driven approach has the potential to become a gold standard in the treatment and monitoring of lung cancer. PANIPAC proposes the development of photoactivable nanoemulsions made of bioactive sphingolipids, which via a dual mechanism of action should increase the immunogenicity of pancreatic tumours. This goal can be achieved by i) reverting the tolerogenic/ immunosuppressive tumour microenvironment of pancreatic cancer by modulating the phenotype of tumor-associated immune cells, and ii) mediating the infiltration of T effector lymphocytes to reset the immunogenicity of pancreatic tumours, and make them candidates for the development of combinatory therapies with checkpoint inhibitors and/or other immune therapies such as bispecific antibodies. If successful, the project would have an enormous socio-economic impact worldwide.

13h10 - 13h20 ***RESTORE***
Bruno Sarmento, INEB

RESTORE is a joint European initiative to find a cure for MS. In this project, researchers and clinicians from Belgium, Spain, the Netherlands and Germany have joined efforts in an attempt to find a cure for multiple sclerosis (MS), a condition that affects the brain and spinal cord. Approximately one in every thousand people are diagnosed with the disease in Europe, mostly in their 20s, 30s and 40s. Although there are many options available for treating and managing MS today, a cure is still lacking. Restore will test a new therapeutic approach based on patients' own cells. This cell therapy, a type of personalised medicine, is currently being lined up for an initial Phase 1 study in patients with MS. The researchers have developed what are known as 'tolerance-inducing' or tolerogenic dendritic cells (tolDC) – a special category of immune cells that function as the immune system's master switch. In MS, the immune system has derailed, setting a cascade of processes in motion that ultimately results in damage to the body's own cells and molecules. After being treated with vitamin

D in a laboratory, tolDC are re-administered to the patient and are then able to re-educate the patient's immune system, interfering directly with the underlying disease processes. Investigating the safety and feasibility of the vaccine will be a crucial step in the development of novel treatment strategies for MS. In order to perform this clinical trial, the researchers received support from the European Union's Horizon 2020 research and innovation programme, enabling collaboration among several EU research institutes and companies.

13h20 - 13h30 ***Radiomag COST Action***
Daniel Ortega, IMDEA Nanociencia

Multifunctional Nanoparticles for Magnetic Hyperthermia and Indirect Radiation Therapy (RADIOMAG), aims to bring together and to organise the research outcomes from the different participating network members in a practical way to provide clinicians with the necessary input to trial a novel anti-cancer treatment combining magnetic hyperthermia (MH) and radiotherapy, also identifying future research objectives upon appraisal of the obtained results. Feedback between the different working groups here is essential, and is expected that the lifetime of this Action proposal will eventually result in a compendium of best practices for magnetic hyperthermia. RADIOMAG will generate new and strengthen the existing synergies between technical advances (thermal imaging / MH), new treatment concepts (combined targeting radiosensitisation and magnetic thermotherapy) and biocompatible coating in order to achieve a breakthrough in the clinical application of magnetic hyperthermia. Due to the complexity of this aim, synergies can only be achieved on a longer time frame, by means of workshops, STSMs, joint publications, common Horizon 2020 research proposals and exchange with other COST Actions.

13h30 - 14h30 ***Networking Lunch***
Poster Exhibition & Industry Exhibition

Cell Therapies and Regenerative Medicine **| Chair: Miguel Oliveira**

14h30 - 15h00 ***Dendrimer Nanoparticles for intracellular delivery and tissue engineering application***
Miguel Oliveira, Research Institute on Biomaterials,

Biodegradables and Biomimetics of the University of Minho

Miguel Oliveira, graduated in Biochemistry at the Faculty of Sciences from the University of Porto. He holds a Post-Graduation in Biomedical Engineering from the Faculty of Engineering, University of Porto and a PhD in Materials Science and Technology, Tissue Engineering and Hybrid Materials in the Dept. Polymer Engineering, University of Minho. He is one of the editors of the book Making Research Visible to the World, In Canon Foundation in Europe, Canon Alumni Book, eds.

15h00 - 15h15 ***Glycosaminoglycans Based Scaffolds for Wound Healing***
Giuseppina Sandri, University of Pavia
Lombardi, Italy

Wound healing process is complex. Biopolymer-based scaffolds represent an attractive three-dimensional substrate for wound regeneration, for mimicking the dermal extracellular matrix. The aim of the work was the development of polysaccharide scaffolds intended for skin reparation. In particular, chitosan (CH) and pullulan (P) in association with glycosaminoglycans (either chondroitin sulfate - CS or hyaluronic acid – HA) were considered for scaffolds preparation via electrospinning. The nanofibers were prepared starting from aqueous/acetic polymer solutions to obtain insoluble membranes in aqueous fluids, able to act as a support for cell migration and proliferation. A multidisciplinary approach has been used to characterize the scaffolds including chemico-physical characterization (SEM, SAXS, thermal analysis, zeta potential) and preclinical evaluation (fibroblasts and endothelial cells adhesion/proliferation onto scaffolds and in vivo evaluation in murine burn model) that allowed to obtain integrated information. The nanofibrous scaffolds prepared were entirely based on polysaccharides, including also polymers having opposite charges (CH, cationic, and CS/HA, anionic) and citric acid was used as crosslinker (activation by heating at 150°C 1 h). The nanofibers show regular shape and smooth surface. The resistance of scaffolds to solubilization in aqueous fluids seems attributable to the creation of amide bonds (mainly in the CH scaffold, while hindered by the formation of PEC in CH/CS and CH/HA scaffolds) and felting, occurring when water is released from the electrospun scaffold, resulting in local physical multi-entanglement between fibers, that cannot be released by simple hydration. CH/CS scaffold shows the best performance in allowing the skin healing in vivo (murine burn model) and correspondingly, it evidences the best proliferation properties in vitro (fibroblasts and HUVEC). The physico-chemical analysis suggests that the CH/CS scaffold offers more adaptability in terms of swelling and fiber roughening once hydrated, thus conceivably allowing for optimal cell adhesion and migration. In addition, the macroscopic feature of a pronounced deformability pointed at the CH/CS scaffold as a good protective cover for non-flat or irregular surfaces.

15h15 - 15h30 ***Computer Vision and Machine Learning Change the Way in Which Biomedical Researchers Analyse Microscopic Images***

Eva Cernadas, CiTIUS (Centro de Investigación en Tecnoloxías Intelixentes), Universidade de Santiago de Compostela, Spain

White light microscopy imaging has a prominent role in life science and medical research. Due to the advances of the last decade in technology for microscopy, the interest on computerized methods for microscopic image analysis is growing in order to make precise quantitative characterization of slice. Microscopic study of a tissue provides detailed insight into celular morphology. For the sake of visualizing under the microscope, the tissue is normally prepared and stained following standard protocols. For white light microscope, the most used are histological and inmunohistochemical staining protocols, in which different components of the tissue are dyed with different stains. Biomedical researchers often perform manual inspection of microscopic image, but this task is very time consuming and require large effort and concentration of specialized personal. So, the quantitative analysis of biomedical images is rarely done, in spite of the useful information that they provide to diagnosis and therapeutic treatment. Quantitative analysis of microscopic images enclose the recognition, classification, measure and count of structures of interest in the image. Currently free and open source software for image analysis exists, like ImageJ (based on Java), but it is not adecuated for many biomedical analysis, because it requires specialized knowledge on computer language and programming skills for defining plugins or macros, and the recognition results provided by the automatic algorithms can not be supervised by experts before quantification. We developed software tools that combine the automatic processing of the image with a friendly graphical user interface to review the process before quantification. Some examples of these softwares, which are being used satisfactory in real environments in different fields, are: 1) LiverAnalyser, used to quantify the fat index from histological images of liver using Hematoxylin-Eosin and oil-red standard protocols in order to study obesity mechanisms; and 2) CystAnalyser, used to quatify the cystic index and cystic profile from histological images of cystic kidney and liver in the studies of the Polycystic Kidney Disease (PKD).

15h30 - 15h45 ***Planning Nanoengineered Organ Implantable, Biocompatible as Functional Corneal Substitute***

Graziella Pellegrini, University of Modena and Reggio Emilia, Italy

The long-term vision of this project is to solve the unmet medical need of restoring sight in patients, without use of cadaver corneas, risks of infectious disease transmission, high cost of long-term management of inflammation in patients and immune reaction, with consequent medium term rejection. The scientific and technological

breakthrough targeted is initiating a baseline of feasibility of a nanoengineered organ implantable, biocompatible as functional corneal substitute (nano stem-cell hemi-cornea) for GMPcompliant clinical use, colonized by autologous cell types, able to reduce inflammatory reactions by multifunctional electrodes and restoring vision through Deep Anterior Lamellar Keratoplasty, avoiding corneal donors. The novelty lies in the interdisciplinary approach, that will produce a bioengineered combined tissue (multiple autologous cells, cephalopode scaffold, integrated nanoelectrodes), through a dynamic and crossfertilizing collaboration of leading expert in several disciplines (cell/molecular biology, microbiology, nanotechnology, chemistry, biotechnology, materials science, engineering, medicine, genetics) coming from academia and industry. The achievement of the project proof-of-principle will be foundational in regenerative medicine, providing the possibility to create a 3D complex organ from phylogenetically different biomaterials, implantable in patients for long-term regeneration of diseased tissue and controlling cell adhesion, proliferation and inflammatory reactions by multifunctional electrodes, seems an hype but is a real hope, despite the risk due to high complexity of the variables of research and the manufacturing process. Tissue engineering falls within ATMPs and requires a deep scientific, technological and regulatory knowledge and expertise, well present within UNIMORE, developer of the first stem-cell based ATMP approved by EMA, expert in biomaterials, scaffolds, sensors and full characterization, with a leadership in nanotechnologies.

15h45 - 16h00 ***Carbon Nanotubes Boost Cell Alignment***

Gabriela S. Lorite, Microelectronics Research Unit,
University of Oulu, Finland

The new trend in tissue engineering and regenerative medicine is to use nanomaterials to control and/or stimulate cell growth, promoting the formation of functional tissue. Carbon nanotubes (CNTs) are recognized for their outstanding mechanical, electrical, thermal, optical and structural properties. In addition, CNTs mimic protein dimension, present high reactivity for cell adhesion mechanisms and have the potential to enhance cell proliferation. In this work, we demonstrate the use of tailored CNT micropillars templates to control orientation of cell growth. Vertically aligned micropillared multi-walled CNTs (MWCNTs) were grown on Si/SiO2 substrates by using lift-off lithography and chemical vapour-phase deposition. The MWCNTs micropillars were arranged as arrays, stripes and spirals in order to evaluate the MWCNTs capabilities to induce cell alignment. In addition to flat surfaces, Si/SiO2 micropillars were fabricated by reactive ion etching to be used as control samples. The use of CNTs to boost cell alignment was demonstrated for two distinct applications: human neurite outgrowth and chondrocytes guidance. In both applications, MWCNTs have supported longterm survival of the cells in comparison to the control templates. In contrast to tight bundles wrapping Si/SiO2 pillars, single neurite interact and anchor to MWCNTs pillars forming highly organized and seamless neuronal networks. These templates enabled guidance of human neurites

along any created patterns (such as spirals and stripes). Similarly, chondrocytes do align according to the MWCNTs micropatterned pillars. In this case, chondrocytes displayed an elongated morphology between the MWCNTs micropillars, with wrapping of the cell cytoskeleton around the pillars with multiple anchor points, while cells attach with thick bundles to the Si/SiO2 pillars.Our results strongly suggest that MWCNTs properties play a bigger role than the nanotopological cue provided by the nanostructured templates. Our hypothesis is that MWCNTs based templates provide multiple cues including electrical, mechanical and chemical cues, which cannot be achieved with other materials such as Si/SiO2 and polymer-based nanostructured templates. Further studies are taking places to clarify the role of each cue depending on the cell-application.

16h00 - 16h15 ***New Antimicrobial Coating System for Long Term Caring Facilities (LTCFs) for Elderly in Hong Kong***

Awais Farid
Hong Kong University of Science and Technology

With the second highest proportion of elderly people in Asia, censes revealed that in 30 years this number is expected to grow to more than 30% or about 2.4 million people. Numerous studies have shown that LTCFs for the elderly are an important reservoir of multidrug-resistant organisms (MDROs), and are pivotal in their spread and transmission in the healthcare network. This study aimed to reduce the environmental burden of pathogens from frequently found drugsensitive as well as drug resistant microbial stains. The research team developed a long-term multilevel antimicrobial coating (MAC) made of USEPA and USFDA approved polymeric antimicrobials that are effective for woven and nonwoven fabrics. A double blind interventional cross-over study was conducted in LTCFs in Hong Kong to collect 272 samples from 92 bedding materials (bedsheets). A set of standard operating protocols (SOP) published by AOAC and approved by the USEPA were used to investigate bactericidal activity of MAC on both drug-sensitive and drug-resistant strains of bacteria. Mean difference of log10 cfu/m² of viable total bacteria and Methicillin-resistant Staphylococcus Aureus (MRSA) was calculated from routinely washed bedding material and coated with MAC. The results were promising showing significant reduction on antimicrobial coated bedsheets showing more than 80% reduction in total viable bacterial isolates as well as MRSA with P>0.05. The colloidal MAC exhibited "contact-killing" and "anti-adhesion" properties that gave the coating the ability to rapidly disinfect the fabric of microbial contamination and prevents colonization by biofilms. The study provided an inexpensive and accessible antimicrobial technology for a rapid and sustained disinfection of surfaces rendering MDROs nonviable in the LTCF environment. This would safeguard the health of the residents by preventing infection lessening the need for medical care and hospitalization, and thus enhancing the well-being of the elderly residents.

16h15 - 16h30 ***Hydrogels Activated with mRNA for Priming Cell Differentiation***

Marcos Garcia-Fuentes, CiMUS Research Center and Department of Pharmacology, Pharmacy and Pharmaceutical Technology, Universidad de Santiago de Compostela

Priming cell differentiation is a key step for achieving tissue regeneration. While cell differentiation seems to proceed naturally for the repair of some tissues (i.e. bone, skin), others generate deficient tissues that do not match the performance of the original (e.g. cartilage). Current approaches to improve the regeneration of complex tissue mostly rely on the integration of complex signaling functionalities in the scaffolds (2). A simpler approach relies on forcing the expression of specific transcription factors that drive cell differentiation, but this is limited by the inefficient delivery of these intracellular transcription factors. We hypothesized that a matrix activated with mRNA (mRMATRIX) could efficiently induce the overexpression of these transcription factors and prime specific differentiation processes. Messenger RNA (mRNA) encoding for the transcription factors MyoD and Sox9 were purchased or synthesized by in vitro transcription from specifically designed plasmids. The mRNA was complexed with a transfecting agent and integrated in hydrogels from several biomaterials. The resulting system (mRMATRIX) were characterized for cytotoxicity, transfection efficiency and capacity to induce cell differentiation when seeded with mesenchymal stem cells (MSCs). The capacity of mRMATRIXs to prime chondrogenic and myogenic regeneration was studied by qRT-PCR, histology and immunohistochemistry. Several mRMATRIX prototypes capable of providing efficient cell transfection with no toxicity were designed based on biopolymers (e.g. collagen, fibrin, etc.). The studies indicated that, at early time points, mRMATRIXs were able to generate significantly higher levels of transcription factors than the same platforms loaded with pDNA. MSCs cultured in mRMATRIX showed >104 –fold higher expression of Sox9 and >108 –fold higher expression of MyoD as compared to control MSCs. MSCs cultured in SOX9-priming mRMATRIXs showed that >400-fold Sox9 induction is still maintained in cultures after 21 days, and that upregulation of markers of chondrogenic differentiation (aggrecan and collagen type-II) were also observed. Similarly, MSCs in MyoD-primed mRMATRIXs maintained very high MyoD levels after 15 days in culture, and significant upregulation of myogenic markers (myogenin, cadherin-15 and myosin heavy chain). The capacity of mRMATRIXs to drive tissue regeneration was confirmed by specific histological/immunohistochemistry.

Pitch-me-up! Session #2 Nanotherapeutics

14h30 - 14h45 ***Ex-vivo Testing of a Self-powered Micro Energy Harvester for Biomedical Applications***

Paula A López Martínez
Centro Singular de Investigación en Tecnoloxías da Información

14h45 - 15h00 ***NanoBioMedicine: PLGA-nanoparticles containing LIF cross the Blood-Brain Barrier and reverse paralysis in Experimental Autoimmune Encephalomyelitis (EAE)***
Su M. Metcalfe
LIFNanoRx - University of Cambridge

15h00 - 15h15 ***CancerTarg Solutions: Nanotechnologies for Hard-to-treat Cancers***
Ruth Schmid
SINTEF AS

15h15 - 15h30 ***Preclinical Development of Magnetic Nanoparticles for the Treatment of Pancreatic Cancer***
Simó Schwart
CIBBIM-Nanomedicine

15h30 - 15h45 ***NanoPyl: Smart and "Friendly" Antibiotic-free Lipid Nanoparticles for Gastric Infection Management***
M. Cristina Martins
INEB/i3S

15h45 - 16h00 ***Empty Capsids of SV40: Naturally Occurring Nanoparticles with Versatile Therapeutic Potential***
Arieh Eden
Carmel Lady Medical Center

16h30 - 17h00 ***Coffee-break***
Poster Exhibition & Industry Exhibition

Advanced Medical Imaging | **Chair: Uwe Himmelreich**

17h00 - 17h30 ***Imaging and Nanomedicine: Promises and Pitfalls***
Uwe Himmelreich
KU Leuven

Prof. Dr. Uwe Himmelreich graduated in Physical Chemistry in 1989 and obtained a Ph.D. degree in the same field from the University of Leipzig in 1994. His main field of expertise is magnetic resonance (MR) spectroscopy and MR imaging, ranging from isolated compounds to cells, preclinical disease models and patients. He was a lecturer at the University of Sydney, Australia and a senior scientist at the Max-Planck Institute for Neurological Research in Cologne, Germany before joining the University of Leuven. Since 2007, he is Professor at the University of Leuven (KU Leuven), Department of Imaging and Pathology. He heads the Biomedical MRI group and is coordinator of the KU Leuven core facility Molecular Small Animal Imaging Center (MoSAIC). His research focusses mainly on the development and optimization of preclinical imaging methods and the development and assessment of imaging contrast agents (including theranostic agents). He has extensive expertise in the field of cell labelling and cell tracking using nanoparticle based approaches. While his original focus was on MRI/MRS, he works now intensively in multimodal imaging and contrast approaches, combining MR with optical imaging, CT, PET and ultrasound. He continues to work on the validation of novel contrast agents, in particular nanoparticle based agents.

17h30 - 17h45 ***Manganese-based MRI Contrast Agents for Cancer Imaging***
Juan Gallo, International Iberian Nanotechnology Laboratory, Braga, Portugal

MRI is an imaging technique perfectly suited for human healthcare applications. To date, its main limitation is its low sensitivity. To deal with this lack of sensitivity, contrast agents (CAs) are administered in about 50% of scans.[2] Clinically used CAs are currently based on a paramagnetic ion, Gd3+. Due to safety concerns and to increase the options available, other species are being studied as potential substitutes. MnO2 nanostructures present several advantages over traditional Gd chelates, such as lower toxicity. Also, MnO2 is not particularly active by MRI, meaning that once administered won't produce significant changes in MR images. However, MnO2 is very sensitive to biologically relevant conditions such as deregulated redox or altered pH conditions. In altered redox environments, MnO2 will be easily reduced into Mn2+ which significantly enhances T1w MR signal. In this talk, the synthesis and functional characterization of several MnxOy nanostructures, as well as their versatility to be combined with reporter molecules for other imaging techniques will be discussed.

17h45 - 18h00 ***MRI Study of the Influence of Surface Coating Aging on the In Vivo Biodistribution of Iron Oxide Nanoparticles***
Natalia Herrero-Alvarez,

Magnetic Resonance Imaging Laboratory,
CIC biomaGUNE, Donostia-San Sebastián, Spain

Medical imaging is an active field of research in need for novel multimodal probes. In this line, Magnetic Resonance Imaging (MRI) is a non-invasive imaging technique with great capacity to image soft tissues at high resolution and contrast regardless of the depth. Nanoparticle-based contrast agents are of special interest since they can host functional entities either within their interior, reducing potential toxic effects of the tracers, or on their surface, providing high payloads thanks to a large surface-to-volume ratio. The long-term stability of these particles in solution is an aspect usually under-tackled during probe design in research laboratories which may jeopardize a later translation into practical medical devices. To better understand the effects of nanoparticle aging in solution, with respect to their behavior in vivo, iron oxide stealth nanoparticles were used at two stages (3 weeks vs. 9 months in solution) and their biodistribution in mice was analyzed. Both sets of nanoprobes showed similar sizes, zeta potentials, and morphology, as observed by dynamic light scattering (DLS) and transmission electronic microscopy (TEM). However, fresh nanoparticles accumulated in the kidneys after systemic administration, while aged ones accumulated in liver and spleen. This confirmed an enormous effect of particle aging on their in vivo behavior, despite barely noticeable changes were perceived on a simple inspection of their structural integrity.

18h00 - 18h15 ***Raman Imaging as a Tool for the Chemical and Spatial Characterization of Breast Cancer Microcalcifications to Improve Lesion Assessment***

Carlo Morasso
ICS Maugeri IRCCS, Pavia, Italy

Microcalcifications (MC) are common findings on screening mammography and are among the earliest signs of breast cancer. 1 At the same time, from the use of well-known radiographic risk score systems, which include MC assessment, such as Breast Imaging-Reporting and Data System (BI-RADS), only 20% of screened patients are further associated with malignancy. 2 This leads to repeated biopsies and to unnecessary surgeries with discomfort for patients and increased costs for the healthcare system. Raman microscopy (RSM) is a photonic approach capable to provide detailed chemical information of analysed samples without complex tissue preparation or staining. RSM has a proven ability to distinguish different crystal structures, including those commonly present in MC. In this context some studies based on RS suggested a correlation between MC chemical features and pathology. We analyzed 30 patients with breast calcifications detected on mammography with radiological classification BI-RAD 3-5. By using a Raman microscope we thus obtained Raman maps of the MC present in the samples with a lateral resolution between 5 and 10 µm. After pre-processing steps the Raman maps were analysed by both

clustering and multivariate analysis approaches used to produce false-colour images and to perform automated features identification. The obtained results confirm that hydroxyapatite is the prevalent form of calcium phosphate in MC and that MC composition correlates with lesion malignancy. 3 These evidences suggest that the characterization of MC by Raman imaging is a potential tool for the definition of new diagnostic signatures of breast cancer, especially if we consider that these evaluations can be performed by the simple and relative fast scanning of dewaxed slices, without altering the clinical workflow and without the need of staining or antibodies. Further studies with a larger cohort will be done to validate these results are currently on course.

18h15 - 18h30 ***Molecular Contrast–based OCT for Cardio-intravascular Imaging***

Mariana Carvalho,
International Iberian Nanotechnology Laboratory, Braga, Portugal

The ability of Optical Coherence Tomography (OCT) to render high-quality 3D structural images of biological tissues has propelled its rapid clinical applications, most notably in intravascular imaging. OCT is the optical equivalent of ultrasound, generating reflectivity-based images by measuring the returning echo response of the sample. Therefore OCT cannot identify molecular changes in the samples, like inflammation. For that to occur, molecular targets should be highlighted allowing them to be detected. This approach exploits the high-resolution and penetration depth of OCT with a smart optical nanoprobe to enhance imaging capabilities. This co-localization would help clinicians and biomedical researchers to track biochemical distribution and changes in the samples. In this work, we demonstrate a molecular contrast–based OCT that can detect and localize molecular targets. We achieve it thru binding a nanoprobe to specific areas in the sample, which are highlighted in the OCT images. With this approach, chemicals or proteins, otherwise undetectable, can be mapped. Our nanoprobes are air-filled core stabilized microbubbles with a Poly(n-butyl cyanoacrylate) (PBCA) shell, conjugated with antibodies for the molecular target. These microbubbles are widely used as contrast agents for ultrasound imaging, thus are biocompatible. For the initial tests, we developed a tissue phantom composed by a malleable Polydimethylsiloxane (PDMS) substrate embedded with TiO2 particles. The phantom was engineered to mimic the optical properties of a highly scattering tissue, as the human arteries. The samples were imaged using a clinical OCT system and the results are shown. The signal provided by the microbubbles has a dual characteristic (creates a shadow and shows high reflectivity) that allows its unequivocal location in the sample. We will also show results on preliminary test using post-mortem human arteries. Our goal is to establish an innovative medical tool to obtain more information on the atherosclerotic plaques. We are combining nanotechnology with Intra-vascular OCT to obtain molecular and morphological information of the plaques. Through the support of medical doctors, we are developing a new protocol to evaluate morphological and inflammatory features of coronary plaques with high precision, sensitivity and specificity, aiming to

create a protocol to accurately predict plaque rupture in patients.

18h30 - 18h45 ***Clinically-applicable Nanoparticle Platform for Cell Tracking and Other In Vivo Imaging Applications***

James Simon
Cenya Imaging B.V.

Personalised medicine is becoming increasingly complex, multifaceted and expensive. In order to truly optimize such therapies, we need to be able to effectively monitor the therapy with earlystage, quantitative metrics. In vivo imaging is an excellent candidate for this, but, often no single imaging modality is "perfect". Thus, there is a need for translatable, customizable imaging agents that can be applied to multimodal imaging. We have developed nanoparticles consisting of polymer-entrapped perfluorocarbon (PFC) that are suitable for 19F MRI, ultrasound, and photoacoustics, and have an unusual internal structure. In addition, they can be modified with a fluorescent dye, or radionuclide for PET or SPECT. The nanoparticles are translatable, can be produced at GMP-grade and are approved for use in a clinical trial to measure the biodistribution of a dendritic cell vaccine. We have applied them to in vivo cell tracking of various dendritic cell subsets, including simultaneous imaging of two different cell subsets using 19F MRI and fluorescence with different PFCs and different dyes. We also show SPECT imaging data. In addition, the nanoparticles show an excellent clearance profile for PFCs, and are shown to clear from dead cells in vivo. This allows for quantitative 19F MRI to assess cell numbers in a region of interest. We show labelling of several cell types, both primary human and murine cells, including dendritic cells, T cells and stem cells. We have also applied the nanoparticles to other applications, such as the imaging of bone matrices in vivo. The particles are produced as a dry powder and can be stored for extended lengths of time (years). They can be stored in suspension for shorter lengths of time (weeks, or longer if frozen). The nanoparticles are reconstituted by simple mixing in water or buffer of choice. Extremely high doses in vivo have not shown any toxicity. Finally, we are able to produce these nanoparticles at small scale (suitable for a small clinical trial) at GMP grade, and at a large scale at lab grade (suitable for preclinical use).

19h00 - 19h15 ***Closing***
Talk and Poster Award

Posters

SECTION A | *Smart Therapeutic Nanosystems*

A1 TITLE: All-in-one protein therapeutic co-formulation and co-delivery via PLGA nanovehicles: a model study

Authors: Cláudia Martins1,2,3, Veeren M. Chauhan2 , Amjad A. Selo2 , Mohammad Al-Natour2 , Hongyu Zhang4 , Paul A. Dalby4 , Jonathan W. Aylott2 , Bruno Sarmento1,5*

Affiliations: i3S - Instituto de Investigação e Inovação em Saúde, Universidade do Porto, Porto, Portugal 2 School of Pharmacy, Boots Science Building, University of Nottingham, Nottingham, United Kingdom 3 ICBAS - Instituto Ciências Biomédicas Abel Salazar, Universidade do Porto, Porto, Portugal 4 The Advanced Centre for Biochemical Engineering, Department of Biochemical Engineering, University College London, London, United Kingdom 5 CESPU - Instituto de Investigação e Formação Avançada em Ciências e Tecnologias da Saúde, Gandra, Portugal

Abstract: Co-formulation of multiple therapeutics into polymeric nanovehicles has been investigated to simplify delivery to patients, realize synergetic bioresponses, and allow a simultaneous targeting to the biologic hotspot [1,2]. A yet poorly explored field is the co-formulation of proteins, which are a multibillion-dollar class of medicines known to provide high therapeutic potency. This study proposes nanovehicles of poly(lactic-co-glycolic acid) (PLGA) for co-formulation and co-delivery of two model proteins, consisting of bovine serum albumin (BSA) conjugated with fluorescein (FITC) or tetramethylrhodamine (TRITC) isothiocyanates, which endow proteins with unique fluorochrome-dependent physicochemical profiles [3]. PLGA nanovehicles co-formulated with BSA-FITC and BSA-TRITC were manufactured through a novel microfluidic technique. Microfluidic parameters were optimized in order to obtain protein coformulated nanovehicles of around 100 nm diameter, low polydispersity index (70%). The cellular association of the proteins was around 2-times higher when co-formulated into nanovehicles, compared to the free protein controls. Moreover, the cellular association of the co-formulated proteins was 4-times higher than the physical mixture of nanovehicles individually-loaded with each protein type. This work therefore demonstrated the effectiveness of PLGA nanovehicles for co-formulating and enhancing the cellular association of co-delivered model proteins, hopefully providing a proof-of-concept foundation for future protein combinatorial nanotherapies.

A2 TITLE: Nanotechnology as a strategy to help therapeutic and antigenic proteins overcoming biological barriers

Authors: José Crecente-Campo, Matilde Durán-Lobato, Sergio del Río-Sancho, María J. Alonso

Affiliations: 1 - Center for Research in Molecular Medicine & Chronic Diseases (CIMUS), Universidade de Santiago de Compostela, Campus Vida, 15706, Santiago de Compostela, Spain

Abstract: Antigens, therapeutic peptides and proteins and, particularly, monoclonal antibodies, are gradually gaining space in the pharmaceutical industry pipelines [1]. Despite their high potential, the difficulties of these macromolecules for overcoming biological barriers and reaching the intracellular targets have limited their exploitation. Our laboratory, with a long-track experience in the formulation of macromolecules using nanoparticles, has significantly contributed to this field. Currently, our focus goes into the following areas:

Oral peptide delivery: within the context of the TRANS-INT European consortium we learnt about the key drivers for the development of local and systemic formulations of peptides intended for oral administration. We produced two categories of nanocarriers, ones that facilitated the high accumulation of the associated protein within the intestinal mucosa and others that promoted the systemic absorption of insulin [2].

Vaccine delivery: we have been involved in an NIH Canadian-US-Spanish consortium that has designed a multi-peptide HIV vaccine. The novel vaccine, consisting of 12 nanopackaged peptides, provides significant protection to SIV-infected macaques [3]. These encouraging results have led us to pursuit the scaling-up of this vaccine prototype within the frame of a European project (NANO-PILOT). The antigens can be associated to multilayer nanocapsules where immunomodulatores can be co-encapsulated.

Delivery of oncological drugs: we have developed a mAb delivery carrier, which is expected to shift the paradigm of mAb-based targeted therapies. So far, our delivery strategy, which uses hyaluronic acid polymer nanocapsules, has made possible the targeting of mAb against intracellular oncoproteins. The success of this strategy has been validated using both in vitro tools and cancer animal models (data not published).

Dermal drug delivery: we are now exploring the combination of nanotechnology and pressure waves produced by laser technology as an approach to increase the penetration of drugs, including proteins, across the stratum corneum. The results obtained so far have led us to the possibility to tailor the disruption of the stratum corneum to allow drug penetration.

A3 TITLE: Synthesis of cationic pullulan derivatives for the development of miRNA-loaded nanoparticles by polyelectrolyte complexation

Authors: Fernanda C. Moraes, Joana C. Antunes, Cédric Chauvierre, Frédéric Chaubet, Didier Letourneur

Affiliations: 1 - INSERM, U1148, CHU X Bichat Hospital, University Paris Diderot and University Paris 13, Paris, France

Abstract: The development of injectable and minimally invasive systems is an innovative strategy to try to reduce the harmful effects of acute myocardial infarction (MI), one of the leading causes of death and disability worldwide. Different types of polymers have been used in gene delivery systems, including the use of cationic polymers. They are able to form particulate structures through the creation of polyelectrolyte complexes (PECs): a simple, versatile and inexpensive method to produce nanosystems. The objectives of this work were (i) the synthesis of a polycation from pullulan, a natural and neutral polysaccharide derived from the black yeast Aureobasidium pullulans, in order to (ii) form nanosized PECs with small regulatory RNAs (miRNAs) (drug) and fucoidan (targeting moiety), a sulphated polysaccharide able to vectorize the nanosystems to the pathological vascular wall1. MicroRNAs (miRNAs) are non-protein-coding RNA molecules involved in post-transcriptional gene regulation. miR-155-5p can specifically target messenger RNAs and modulate generation of reactive oxygen species (ROS), main contributors to the progression of the disease2. A quaternary ammonium pullulan was successfully synthesized by alkylation of pullulan with GTMAC. Elemental analysis showed the success of incorporation of positive charges (N content of 2.01 ± 0.02 %). Similarly, FTIR spectra showed the presence of peaks in the region of 1430-1470 cm-1 due to C-H bending of methyl groups of the quaternary amine grafted onto the pullulan chain. The direct mixing of the cationic pullulan and anionic fucoidan and miR-155-5p in aqueous media resulted in spontaneous formation of round-shaped PECs (210.47 ± 4.56 nm) with a monodisperse size distribution (PdI = 0.07 ± 0.04) and negative ⍰ (-14.62 ± 5.06 mV) (Figure 1). The miRNA loaded was detected by agarose gel electrophoresis, which confirms the ability of cationic pullulan to form complexes with anionic miRNA via electrostatic interactions. In vitro tests performed onto hCAECs did not evidenced cytotoxicity (100-200 µg/mL) up to 1 day of incubation. PEC formation of cationic derivatives of pullulan with miRNA provided an easy and versatile method for nanoparticle production for gene therapy of myocardial infarction using polysaccharides. The association of miRNA-loaded cationized pullulan with fucoidan would allow a specific delivery of gene materials into the ischemic area thanks to fucoidan's capacity to bind to P-selectin-activated hCAECs.

A4 TITLE: Epicardial-derived exosomes in hypoxia and normoxia: a primary characterization

Authors: Cláudia Patrícia da Costa Oliveira, Juan Antonio Guadix, John Pearson, Juan Félix López Téllez, José Maria Pérez-Pomares

Affiliations: 1 - University of Málaga; 2 - BIONAND

Abstract: Exosomes are small cellular vesicles (30-150 nm in diameter) functioning as cell-to-cell message carriers [1]. These nanovesicles, whose contents (miRNAs, mRNAs and divers proteins) are thought to be an indicator of the physiological status of the cell they derive from and, ultimately, play a key role in many biological processes. Exosomes also provide instructive information to the surrounding cells on relevant changes in their environment, and therefore they are regarded as potential markers of organ disease [1]. Conditions as ischemic cardiomyopathy-myocardial infarction (MI) are usually diagnosed at advanced stages of the disease, and early subclinical diagnostic markers have not yet been identified. It has been shown that cardiac fibroblasts involved in post-MI ventricular remodeling are derived from the embryonic epicardium, and that these cells are specifically activated (proliferation, migration, collagen synthesis) upon ischemic heart damage to compensate for cardiomyocyte loss [2, 3]. Our work focuses in isolation and characterization of exosomes from an epicardial cell line (EPIC) immortalized in our laboratory from embryonic epicardial cells [4]. Our aim is to characterize epicardial-derived exosomes and define the molecular composition of these nanovesicles in different physiological contexts. In order to tackle this scientific objective, EPIC cells are cultured under normoxic and hypoxic conditions and exosomes isolated from culture supernatant by ultracentrifugation and probed for confocal and TIRF imaging. Our data indicates that EPIC cells secrete a considerable amount of exosome, and that the amount of these vesicles increases in the culture medium exosome when EPIC cells are incubated in hypoxia (5% oxygen). Moreover, when EPIC-derived exosomes are co-cultured with EPIC cells, the former are fastly internalized by some of the cells of this continuous line. TEM and proteomic analysis are being performed to assess EPIC exosomes' structuctes, size and cargo in normoxic and hypoxic states Our short term objective is to evaluate the effect of EPIC-derived exosomes on the phenotype, function and transcriptomic profile of cultured cardiomyocytes.

A5 TITLE: UNIMORE Nanomedicine Platform

Authors: Tosi, Giovanni

Affiliations: 1 - University of Modena and Reggio Emilia

Abstract: Since 2017, University of Modena and Reggio Emilia officially approached to ETPN society. This poster aims to quickly remind to the skills and abilities of UNIMORE Laboratories working on nanomedicine, products, characterizing and scaling up nanomedicines, up to studying nanotoxicology and ethical aspects. Moreover, we would like to show how we are building an European Technology Platform on Nanomedicine – ETPN Association 64-66 rue des archives 75003 Paris France www.etp-nanomedicine.eu internal platform for Nanomedicine, in line with ETPN ideas and rules and developing novel ideas or competences that could find spaces in scientific teams targeting to strategic goals within the ETPN purposes. We therefore would like to present to ETPN audience our portfolio in the field of nanotherapeutics, nanodiagnostic, regenerative medicine and nanodevices and their application in target diseases as neurodegenerative disorders, cancer and inflammation.

A6 TITLE: Responsive Gels that Trigger Gold Nanoparticle Formation for Localized Photothermal Therapy

Authors: Angel Concheiro*, Sonia Cabana-Montenegro, Silvia Barbosa, Pablo Taboada, Carmen Alvarez-Lorenzo

Affiliations: 1 - Universidade de Santiago de Compostela

Abstract: Gold nanoparticles (AuNPs) are particularly suitable platforms to provide chemotherapy and phototherapy synergisms in the eradication of tumor cells and pathogen microorganisms. However, after intravenous administration AuNPs are rapidly cleared from the bloodstream hampering the distribution towards the target site. The aim of our work was to design in situ gelling systems from block copolymers that can transform a gold salt into AuNPs directly in the final injectable formulation and then self-assemble under physiological conditions to render a gel depot that allows repeated photothermal therapy cycles in the injection site. Tetronic 1307, an X-shaped poloxamine formed by four arms of PEO–PPO blocks linked by an ethylenediamine group, as chosen as main component of the temperature- and pH-responsive gels [1]. Differently to previous studies carried out with low molecular weight PEO–PPO–PEO copolymers as gold reductants at concentrations below CMC [2], we used Tetronic 1307, of relatively high molecular weight, at concentrations suitable for undergoing the sol-to-gel transition at physiological temperature. Varying the copolymer and NaCl concentrations allowed a fine tuning of nanoparticles shape from spherical to triangular nanoplates, which determined that the surface plasmon resonance showed a maximum intensity at 540 nm or at 1000 nm, respectively [3]. The information gathered on the effects of the poloxamine concentration on the

AuNPs size, shape and photothermal responsiveness during successive irradiation cycles may help the rational design of one-pot gels with built-in temperature and light responsiveness.

A7 TITLE: Synthesis, characterization and evaluation of magnetic doped ferrites as potential therapeutic tools

Authors: Alberto Pardo, Beatriz Pelaz, Pablo del Pino, Francisco Rivadulla, Manuel Bañobre, Wolfgang Parak, Silvia Barbosa, Pablo Taboada

Affiliations: 1 - Universidad de Santiago de Compostela; 2 - International Iberian Nanotechnology Laboratory; 3 - University of Hamburg

Abstract: In last decades, magnetic nanoparticles (MNPs) have been intensively studied due to their potential technological and biomedical applications. This work presents the synthesis and characterization of MNPs obtained by thermal decomposition with different metallic compositions. Metallic acetylacetonates were used as precursors, oleic acid and oleylamine as the stabilizing agents, 1-2 hexadecanediol as reducing agent and benzyl ether as the liquid medium. We analyzed the influence of molar ratios between iron, manganese, cobalt and zinc acetylacetonates and different parameters of the synthetic process on MNPs formation, structure and physical properties. In this manner, we have achieved an optimization of the synthetic process of doped MNPs with full control over their composition, size and magnetic properties in order to choose those particles with best hyperthermia and contrast imaging properties for biomedical applications. The characterization of the obtained MNPs was carried out by transmission electron microscopy, inductively coupled plasma mass spectrometry, superconducting quantum interference device and X-ray diffraction. The MNPs were transferred to aqueous solution via anin situ polymer coating process with dodecylamine-grafted poly(isobutylene-alt-maleic anhydride) (PMA) in order to check their potential cytotoxicity, cellular uptake profiles and therapeutic capabilities in vitro in different cell lines.

A8 TITLE: Inhalation of drug-loaded nanoparticles improves heart failure treatment

Authors: Catalucci Daniele

Affiliations: 1 - National Research Council

Abstract: CUPIDO is an EU-funded project that aims to develop an innovative and patient-friendly drug delivery system for the treatment of heart diseases: inhalable nanoparticles that can carry and release therapeutic molecules directly to the myocardial cells. Peptides or small RNAs represent efficacious tools for cardiovascular diseases treatment. However, their therapeutic use towards the clinic is hampered by compound instability and low cardiac-specific delivery, which is currently carried mainly via invasive methods. In CUPIDO, we demonstrated that inhalation of small (<50 nm), biocompatible and biodegradable self-assembled calcium phosphate nanoparticles (CaPs1) allows for rapid translocation of CaPs from the pulmonary tree to the bloodstream and to the myocardium, where their cargo is released. Currently, two therapeutic biomolecules have been successfully tested:
1) The MP mimetic peptide2 able to improve myocardial contraction through restoration of altered cardiac channel protein. Inhalation of MP-loaded CaPs restores cardiac function in a rodent model of diabetic cardiomyopathy3.
2) The cardiac-enriched miR-133, which level is inversely related to failing heart conditions. A replacement therapy via inhalable miR-133-loaded-CaPs prevents the pathological cardiac remodeling in a mouse models of left ventricular pressure overloadunpublished.
The therapeutic validations in small animal models, together with the evidence for a rapid accumulation of inhaled CaPs in the heart of healthy pigs encourages the next coming application of CUPIDO therapeutic approach in a large animal model of cardiac disease.
Altogether, CUPIDO results demonstrate that inhalation of drug-loaded nanocarriers represents a pioneering approach for heart failure treatment.

A9 TITLE: Safety Evaluation and Up-Scaling Challenges towards Development of Safe Nanomedicine for Fabry Disease

Authors: FALK Andreas1, SCHIMPEL Christa1, RESCH Susanne1, ALTENDORFERKROATH Thomas2, BIRNGRUBER Thomas2, MERLO-MAS Josep3, SALA Santi3, GONZÁLEZMira Elisabet4,5, PEDERSEN Jannik N6, PEDERSEN Jan S.6 , DANINO Dganit7, KESSELMAN Ellina7, INBAL Ionita7, CORCHERO Jose Luis4,8,9, ABASOLO Ibane10,9,4, CRISTÓBAL-LECINA Edgar4,11, PULIDO Daniel4,11, ROYO Miriam11,4, FONT Albert12, SOLDEVILA Andreu12,TOMSEN-MELERO Judit5,4, GONZALEZ Ramon5,4, VENTOSA Nora5,4, CÓRDOBA Alba3

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Engineering, Technion - Israel Institute of Technology; 8 - Institut de Biotecnologia i de Biomedicina (IBB); 9 - Universitat Autònoma de Barcelona (UAB); 10 - CIBBIM-Nanomedicine, Vall d'Hebron Institut of Research (VHIR); 11 - Institut de Química Avançada de Catalunya – Consejo Superior de Investigaciones Científicas (IQAC-CSIC); 12 - LeanBio S.L.

Abstract: Nanomedicine development has improved over traditional approaches as new nanomaterials emerge at the nano-scale. Translation still faces challenges along Up-Scaling and safety evaluation to be addressed in a structured way. In addition to favorable pharmacokinetics, which improves bioavailability, solubility and therapeutic index of their payload, nano-scaled pharmaceuticals can offer controlled release at the disease site. Smart-4-Fabry project aims to improve therapy of Fabry-Disease (FD), a rare lysosomal storage disorder. The development of liposomal nanocarriers improves the transport of encapsulated α-Galactosidase A (GLA) to target tissues such as kidney, liver, heart and brain. In addition, a major aim is to assess the safety profiles of ENMs at low, nonlethal dosages and extended repetitive exposures, which could help us to understand long-term consequences of ENMs' interactions with biological environments and further expedite their translation into clinical applications. (a) Safety evaluation: Pharmacokinetic parameters of nanoliposomes with different surface modifications (PEG length, targeting peptide) are assessed by cerebral Open Flow Microperfusion (cOFM) sampling technology. This valuable tool allows to evaluate the transport characteristics of nanoliposomes through the blood-brain barrier (BBB) into the brain tissue of rodents and thus permits identification of the most efficient candidate at an early stage of nanocarrier development. In order to develop a safe and legally compliant product, potential negative side effects are evaluated and Safe-by-Design strategies for prevention will be implemented.(b) Up-Scaling and prototype optimization: Quality by Design approach, a methodology that is encouraged by the FDA and the EMA, is applied to develop a robust process for the preparation of GLA-loaded nanoliposomes with DELOS-susp platform. These mechanistic understandings would help to design safer nanomedicines with improved therapeutic efficacy in clinical settings, involving considerable reduction on the FD treatment cost and a substantial improvement in the life-quality of FD patients.

A10 TITLE: The Effect of liposomes Cholesterol Content on the Release of Liposomes-Entrapped Calcein From Pluronic® F-127 Liposomal Gel

Authors: Mansoor Al-waeel, Sophia G. Antimisiaris

Affiliations: 1 - University of Paris Descartes, France; 2 - Alfasigma S.P.A, Italy

Abstract: Introduction: DSPC / poloxamer liposomal gels were prepared in order

to develop a sustained release system to control the delivery of the encapsulated drug over a longer period of time. The liposomes were unstable in the gel and calcein, so we studied the effect of cholesterol content on the in vitro release profile of a hydrophilic dye (calcein) from DSPC liposomal in thermosensitive hydrogels composed of Pluronic F-127 (P). liposomes dispersed in PBS pH 7.4 were used as control formulations. Materials and Methods: Three types of DSPC liposomes were prepared by thin film hydration method (plain DSPC, DSPC/ Chol ratio 2:1 and 1:1) and then hydrated with a calcein solution. Three different concentrations of poloxamer gels 15%, 18%, and 20% (w/v) were prepared by the cold method. Each sample was measured for their particle size, zeta potential, and latency. The in vitro release of calcein from liposomal gels and control was studied for 72 h.
Results: The in vitro release studies showed a rapid release of calcein from plain DSPC liposomal gels as more than 50% of the encapsulated dye was released in less than 24% in all samples. The addition of Chol significantly reduced the release to less than 30% after 72h for liposomal gels with ratio 2:1. A sustained release was achieved when the content of Chol was 50% mol as less than 17% was released after 72 hours. The liposomes dispersed in buffer showed the slowest release in all cases which was 4-5 times lower as compared to liposomal gel, which could suggest that gel affects the liposomes stability.
Conclusion: This study showed that the cholesterol content has a direct effect on the release profile of drugs encapsulated in liposomal gels, as Chol amount increases the release slows down. Additionally, The slow release of calcein from control liposomes in PBS pH 7.4 as compared to liposomal gels suggests that the gel affects the rigidity and the mechanical strength of the liposomes and break them.

A11 TITLE: Smart skin-responsive peptide based nanocapsules for the treatment of damaged skin

Authors: Damien Dupin§ , Aitziber Lopez§ , Beatriz Palla§ , Natividad Diaz§ , Adrián Pérez-San Vicente§ , Iraida Loinaz§ , Gaëlle Le Fer† , Elisabeth Garanger† , Sébastien Lecommandoux† , Dror Cohen‡ , Meital Portugal-Cohen‡ , Ze'evi Maor‡

Affiliations: 1 - CIDETEC; 2 - Bordeaux-INP, Laboratoire de Chimie des Polymères Organiques; 3 - AHAVA the Dead Sea Laboratories

Abstract: Sun exposition, contact with allergens or irritant products and other external factors drastically affect skin health, making damaged skin one of the main characteristics of actual lifestyle. Early stage treatments can prevent the onset of more serious pathologies and for that purpose, new strategies, such as new delivery platforms for active ingredient localized and triggered release, are necessary. Due to the specific physiological conditions of damaged skin, the design of delivery systems with triggered active ingredient release is well extended. Liposomes, hydrogels or

other type of micro-emulsions with stimuli-responsiveness, i.e. pH and temperature, are well known. Nevertheless, nano-emulsions offer additional features such as targeted localization, improved versatility in terms of active encapsulation and active protection due their size. Taking advantage of damaged skin physiological changes (enzymes overexpression and increase of skin pH), a new generation of polypeptides based stimuli-responsive smart nano-capsules for the encapsulation of both hydrophobic and hydrophilic type compounds have been developed . For that purpose, specifically designed bio-mimicking amphiphilic block copolypeptides are used as emulsifiers to form both oil-in-glycerol and glycerol-in-oil type nano-emulsions with enhanced properties. In the presence of pH changes or the overexpression of certain enzymes, the macroemulsifiers are desorbed from the oil-glycerol interface, leading to capsules break up and active ingredient release. This mechanism, together with the small size of the capsules, permits a targeted and triggered release of different type of active ingredients able to treat skin conditions such as UV-damaged skin or allergic contact dermatitis. In a first approach and with the aim of treating very initial stages of several diseases, the new technology has been validated with active ingredients for skin care. Ex vivo experiments carried out in human skin models have demonstrated that active ingredient performance, measured in terms of cell viability, antiinflammatory and anti-apoptosis effect, is increased in a minimum of 30% in comparison to free actives. This is due to the increased bioavailability of the active in the epidermis (more than 50%), the enhanced protection of those actives (minimum of 50%) and the already mentioned targeted and triggered release.

A12 TITLE: Quality-by-Design for the safe development of medical devices containing nanomaterials. A study case in Photodynamic Therapy

Authors: Deleforterie, Jeanne1; Kolasa, Yael1; Batista, Levy1; Hutin, Julie1; <u>Bastogne, Thierry</u>1

Affiliations: 1 - CYBERNANO

Abstract: Background. According to the new medical device regulation (MDR 2017/745), devices employing advanced materials containing nanomaterials will be classified as class III and will have to undergo (re-)assessment of risks. To that aim, the Quality-by-Design approach, as defined in ICH Q8-Q11 (QbD), is indisputably accepted and strongly recommended by the FDA and EMA for risk assessment during drug development. Some papers have emphasized the possible implementation of QbD in the medical device industry. Nevertheless, to date no real and effective adaptation of this risk-based quality management approach has been adapted to biomedical devices manufacturing.
Objectives. Our goal is to develop both a new QbD paradigm and a web-based tool devoted to the safe development of class-III medical devices containing nanomaterials.

This objective is pursued in the context of the European H2020 project TBMED (An Open Innovation test bed for the development of high-risk medical devices).
Methods. A six-step QbD approach is proposed. The first four stages are devoted to the preclinical development while the next two steps concern the industrial implementation. Three categories of risk-assessment methods are used at different development steps: failure mode and effects analysis based on prior knowledge, statistical designs of experiments and Bayesian inference. To assess its applicability, we applied the integrated QbD approach to the development of a new medical device devoted to the realtime control of light during photodynamic therapy using nanoparticle-based photosensitizers.
Results. The new SaaS platform, entitled "Nanologic", is available at: (www.i-nano.eu). Four key documents for regulatory agencies are established during the preclinical study: the target product profile, the list of critical quality attributes (quality/safety descriptors), the list of critical material attributes and process parameters (risk factors associated with the design and production phases) and the design space: a key concept of risk assessment in QbD.
Conclusion. We show how the QbD best practices can be adapted to the development of medical devices containing nanomaterials. Moreover, new questions still have to be investigated such as the solutions to be developed to better predict risks associated with the clinical proof of concept.

A13 TITLE: A nanoinformatic contribution for the Safety-by-Design in Nanomedicine

Authors: Roulette, Valentine1; Delplanque, Guillaume1; Deleforterie, Jeanne1; <u>Bastogne, Thierry</u>1

Affiliations: 1 - CYBERNANO

Abstract: Background. The complexity of nanomaterials, their physico-chemical properties and their interactions with biological and environmental systems, leads to uncertainty in the applicability of experimental data for regulatory purposes that demand sound scientific answers. A major challenge is the establishment of common languages, standards and harmonized infrastructures with applicability to the needs of the different stakeholders. Nanoinformatics is the science and practice of determining which information is relevant to compare characterization of nanomaterials and to design optimized and safe nanodevices.
Objectives. Our goal is to bring a new contribution in nanoinformatics by proposing a text mining solution for the quasi-automatic collection of nanomaterials descriptors suited to the preparation of Quality-by-Design studies.
Methods. The first step relies on the construction of a non-structured database composed of scientific articles in PDF format. Secondly, the Quality-by-Design (ICH

Q8-Q11) terminology is used to represent the main descriptors of nanomaterials with three main categories: (i) Critical Quality Attributes to describe the key physical, chemical and biological properties, (ii) Critical Material Attributes and (iii) Critical Process Parameters to characterize both design and manufacturing key variables. In a third step, a text mining algorithm, implemented in the Python language, is used to automatically detect CQA, CMA and CPP in articles and to build up a SQL database. A data curation step is then performed to complete and clean up the QbD database. The proposed approach was applied to a set of 30 scientific articles in Nanomedicine and identification performances were finally assessed.
Results. In total, 1740 words were automatically analyzed, and we obtained an average response of 83,9% (1459/1740) correct identifications, decomposed as follows: 82,2% (235/286) of accuracy for the CQA descriptors, 92,9% (604/648) for CPP and 76,9% (620/806) for CMA.
Conclusion. The proposed nanoinformatic solution has shown promising performances and allows to automate a very time-consuming task related to the collection of relevant scientific data for risk assessment in QbD studies. Short-term perspectives will be focused to the automatic extraction of more complex descriptors.

A14 TITLE: The use of nanomaterials for cancer and neurodegeneration: nanocarriers, biomembranes and immunosensors

Authors: M.J. Ramalho Δ , J.A. Loureiro, M.A.N. Coelho, M.C. Pereira °

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Abstract: Nanotechnology has proven to be extremely advantageous in creating breakthroughs in diagnosis and treatment of several diseases. Our group focus in enabling early diagnosis and the controlled delivery of drugs for cancer and neurodegenerative diseases. Our research is oriented for the design, preparation and characterization of different types of nanoparticles (NPs) as drug delivery systems. Depending on the drug to be encapsulated and the final application, different types of nanomaterials can be used for the NPs' design. During the last years our group prepared and evaluated the therapeutic potential of gold NPs, liposomes, solid lipid NPs and polymeric NPs, for the delivery of anticancer agents and therapeutic molecules for neurodegenerative disorders such as Alzheimer's (AD) and Parkinson's (PD) diseases. Tools such as experimental design allow us to optimize the synthesis of the NPs to achieve high encapsulation efficiencies and optimal physicochemical properties. The modification of the surface of the NPs with different types of monoclonal antibodies to target membrane receptors is also conducted to enhance the transport across biological barriers such as the blood-brain barriers, increasing the bioaccumulation in the target tissues. Nanosystems can also be used as tools

for drug screening during novel therapeutics development phase. Drug-membrane interactions regulate drug's pharmacokinetics and biodistribution profiles, so the study of these interactions is essential. We developed lipid bilayer models using liposomes for the drug-membrane interaction studies, mimicking the cell membrane composition and physiological environment. There is a high demand for fast and simple analytical methods for the determination of many clinical and biochemical parameters. Our group is developing nanomaterial-based biosensors such as electrochemical immunosensors with high sensitivity and efficiency to be used in diagnostic procedures for PD and AD.

A15 TITLE: Calcium alginate and gellan nanofibers embedded in polymeric matrices for the treatment of spinal cord injuries

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Abstract: Spinal cord injury (SCI) is a highly debilitating pathology that results in devastating sensory, motor and autonomic dysfunctions. Huge emotional, social and financial costs of SCI solicit the development of more effective therapeutic strategies, ensuring both neuroprotective and neuroregenerative outcomes. The present work aimed at developing novel drug delivery systems for neuroprotective sigma 1 receptor-agonist RC-33 to be locally implanted at the site of SCI; two nanofibrous membranes (NFMs), containing alginate (ALG) or gellan (GEL) and a mixture of poly (ethylene oxide) (PEO) at two molecular weights (MWs), were produced by electrospinning. ALG and GEL were selected as main fiber components since they are anionic polysaccharides forming electrolyte complexes with the cationic RC-33, while PEO as adjuvant the electrospinning process. A 1% w/w ALG solution containing 0.15% w/w high MW PEO (h-PEO) and 2% w/w low MW PEO (l-PEO), and a 1.5% w/w GEL solution with 0.15% w/w h-PEO and 2.2% w/w l-PEO were prepared in deionized water with the addition of 2% w/w poloxamer P407 and, then, electrospun (20 cm as spinneret-collector distance - 20 kV as applied voltage). Both NFMs were cross-linked with CaCl2 water/ethanol solutions in order to make the systems insoluble in aqueous media. In an attempt to produce a drug delivery system more flexible, easier to handle and characterized by regenerative properties, the cross-linked ALG-NMF was incorporated in a chitosan (CS) film, obtained through the solvent casting method. The cross-linked GEL-NFM, instead, was embedded in a GEL sponge-like dressing, produced by freeze-drying, able to absorb and, thus, to drain the cerebrospinal fluid at the site of injury, reducing intrathecal pressure. Meanwhile, dialysis equilibrium studies were performed to evaluate the maximum binding capacity of both ALG and GEL for RC-33. In the context of SCI, the drug delivery systems developed could represent a promising therapeutic platform due to their morphological and

mechanical properties. Further studies are on-going to produce and characterize RC-33-loaded NFMs. Moreover, the incorporation of cross-linked NFMs in both CS film and GEL sponge should provide a pro-regenerative support to axonal re-growth, while the RC-33 loading could modulate the SCI secondary sub-acute phase, by exerting neuroprotective and/or restorative effects.

A16 TITLE: Metallothionein Nanotransporter for Targeted Cancer Therapy

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Abstract: Metallothionein (MT) is a small protein discovered in 1957 by Margoshes and Vallee. MT consists of a single polypeptide chain of 61-62 amino acids containing 20 cysteine residues which contain several bivalent cations (Zn, Cu, Cd, Hg) bound through metal-thiolate linkages. Higher MT levels were found in proliferating cells which reflects the importance of the involvement of MT in the cellular regulation and proliferation processes. Carbon nanoparticles are very suitable carriers for antitumor drugs due to their non-toxicity and considerable binding capacity. Other types of nanoparticles, such as silver nanoparticles (AgNPs), exhibit anti-tumor effects. In patients with breast cancer metastases, anthracycline antitumor antibiotic doxorubicin (DOX) is most used. Its clinical use, however, is limited by dose-related heart muscle damage, most frequently occurring in the case of cumulative dose. Therefore, development of new approach such as nanocarriers for targeted drug delivery to tumor cells is an urgent challenge. The aim of this work was to propose a nanotechnological construct to increase the safety of treatment and maximum targeting to the tumor cell. Silver nanoparticles (AgNPs) were prepared by green synthesis method from different plant species. In addition, used AgNPs showed significant antiproliferative activity (growth inhibition by 20–40%) on S. cerevisiae. Created nanoconstruct A exhibits growth inhibition for S. cerevisiae by more than 50%. The construct was designed as two separate nanotransporters. The

nanotransporter A was formed by an antibody-modified AgNPs and multi-walled carbon nanotubes (MWCNTs) with encapsulated DOX (AgNPs/Ab1/MWCNT/DOX/ODN1). The nanotransporter B was engineered with superparamagnetic iron oxide nanoparticles (SPIONs) modified with antibody and with bound MT (SPION/Ab2/MT/ODN2). MT was bound to the SPION surface using EDC/NHS polymer. The presence of bound MT was monitored electrochemically by Brdicka method in connection with the transfer technique (AdTSV) [6, 7]. Characteristic MT signals RS2CO (-1.15 V), Cat1 (-1.25 V), Cat2 (-1.45 V), Cat3 (-1.75 V) were observed at the accumulation time of 120-180 s [8]. SDS PAGE confirmed the presence of MT on SPIONs at sizes 7 to 15 kDa. The DOX signal was spectrophotometrically and fluorometrically monitored. AgNPs size was 15-20 nm, and the size of SPIONs was in the range of 20–50 nm.

A17 TITLE: Study of Albumin Protein Corona Interaction with Cytostatics by Modified Carbon Nanotubes

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Abstract: Cancer is the second leading cause of death in developed countries. Worldwide, over 200,000 people die annually of pancreatic cancer. Conventional anti-cancer therapy is known to exhibit a number of serious undesirable effects such as a lack of selectivity for tumor tissue leading to significant drug adverse effects. The relatively low therapeutic concentration of the active agent usually results in resistance and multi-resistance of cancer cells to the drugs . Nanotransporters such as multi-walled carbon nanotubes (MWCNTs) are considered modern and effective tools of personalized treatment. Cellular uptake of functionalized carbon nanotubes (CNTs) is independent of the functional group attached to their surface and cell type. It has been reported that shorter and thicker nanotubes are safer. Chemically modified CNTs are much better soluble in the aqueous solution and evince higher stability in the physiological environment. Nanoparticles that are able to stay in the bloodstream for a long time are more likely to penetrate into tumor cells. In addition to passive targeting methods based on the EPR effect mechanism and specific acidic environment in the tumor, strategies of active targeting to a selected tumor using

ligands or antibodies that enhance the specificity of the nanotransporter are also investigated. It has been found that protein corona composition depends on the structure and physicochemical properties of the nanoparticles. However, the effect of surfactants in the structure of CNTs on the composition and formation of the protein corona has not yet been studied. It is known that proteins form a series of colored complexes with different complexing agents. We used spectrophotometry methods to study the albumin protein corona onto CNTs. The concentration dependences of BSA (0-60 g/L) in PBS solution on the absorption signal were prepared. In the case of the pyrogallol red method, the linearity of R2 = 0.9987 in the concentration range of 0-60 g/L with a detection limit of 2.6 g/L (RSD = 1.3%) was found. The linearity of the biuret calibration curve was R2 = 0.999. The detection limit was 8.8 g/L (RSD = 4.3%). These methods proved the presence of albumin onto the MWCNT surface. In other experiments, we studied the protein corona on SDS PGE. The nanotransporter was bound by BSA at various concentrations and accumulation times. It was possible to analyze the protein corona onto the surface of the MWCNTs very well

A18 TITLE: From proving amoxicillin deleterious gastric effects to enhancing its efficacy against Helicobacter pylori

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Abstract: Amoxicillin (AMX) is a worldwide used antibiotic, classified as a first-line drug against Helicobacter pylori gastric infections. However, the current treatment of these infections has several limitations, such as its side effects, the protective location of the bacteria in vivo, the low residence time of antibiotics at the stomach and the low therapeutic compliance. AMX has been associated with gastrointestinal and renal side effects, with higher toxicity when the pH is lower. By considering this association and the well-known pH gradient of the gastric mucosa, we studied the influence of pH on the toxicity of AMX. For that purpose, 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) monolayers were used as mimetic models of gastric cell membranes. The results showed that AMX induces the formation of pores in the lipid film of DPPC at both pH 1.2 and pH 5, possibly due to dimerization of AMX under acidic conditions . The disturbance of biological barriers and, ultimately, the formation of pores may be related with in vivo toxicity. Thus, the encapsulation of AMX in lipid nanoparticles can increase the retention time at the site of infection (gastric mucosa) while protecting the drug from the harsh conditions of the stomach lumen. In this context, AMX-loaded lipid nanoparticles were optimized by a Box-Behnken design. The composition encompasses a surfactant and a fatty acid with antimicrobial

properties and dioleoylphosphatidylethanolamine to bind to H. pylori receptors (active targeting). Permeability studies showed that the lipid nanoparticles were able to be retained at the site of infection once they did not permeate gastric cells. Furthermore, they interacted with mucins, which enhances their retention time at the stomach. In vitro studies show that the lipid nanoparticles killed H. pylori through the detachment of the bacterial membrane and the release of the cytoplasmic content. Moreover, due to the targeting agent, the lipid nanoparticles were bound to the bacteria within the first 15 min, which decreased the adhesion of the bacteria to gastric cells.

A19 TITLE: Cabazitaxel-loaded Poly(2-ethylbutyl cyanoacrylate) nanoparticles improve treatment efficacy in a patient derived breast cancer xenograft

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Abstract: The effect of poly(2-ethyl-butyl cyanoacrylate) nanoparticles (PEBCA NPs) containing the cytotoxic drug cabazitaxel (CBZ) was studied in three breast cancer cell lines and one basal-like patient-derived xenograft model grown in the mammary fat pad of immunodeficient mice. NP-encapsulated CBZ had a much better efficacy than similar concentrations of free drug in the basal-like patient-derived xenograft. Quantification of CBZ in mice plasma and selected tissue samples was performed by mass spectrometry. Drug encapsulated in NPs had a longer circulation time in blood. There was approximately a three times higher drug concentration in tumor tissue 24 h after injection, and two times higher 96 h after injection of NPs with drug compared to the free drug. The tissue biodistribution obtained after 24 h using mass spectrometry analyses correlates well with biodistribution data obtained using IVIS® Spectrum in vivo imaging of NPs labeled with the fluorescent substance NR668, indicating that these data also are representative for the NP distribution. Furthermore, immunohistochemistry was used to estimate infiltration of macrophages into the tumor tissue following injection of NP-encapsulated and free CBZ. The higher infiltration of anti-tumorigenic versus pro-tumorigenic macrophages in tumors treated with the NPs might also contribute to the improved effect obtained with the NP-encapsulated drug. Tumor infiltration of protumorigenic macrophages was four times lower when

using nanoparticles containing CBZ than when using particles without drug, and we speculate that the very good therapeutic efficacy obtained with our CBZ-containing NPs may be due to their ability to reduce the level of pro-tumorigenic macrophages in the tumor. In summary, encapsulation of CBZ in poly(2-ethyl-butyl cyanoacrylate) NPs seems promising for treatment of breast cancer.

A20 TITLE: Nanomedicine for siRNA delivery

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Abstract: Over years small interfering RNA (siRNA) has been developed as a competent therapeutic agent for many diseases as silencing a specific gene can bring the diseased cell to death. Nanomedicines based on Elastin like polypeptides (ELPs) provide targeting with less cell toxicity. ELPs are pentapeptide repeats of (VPGXG) n from human tropoelastin protein where X, named the guest residue, can be any amino acid except proline. They are thermosensitive, upon change in lower critical solution temperature (LCST) they undergo a transition from a water-soluble state to a collapsed hydrophobic state. The transition temperature (Tt) varies by changing the guest residue, the number of repeats, and the protein and salt concentrations. ELPs can form nanoparticles with either micellar or vesicular structures depending on the hydrophobicity of the used repeats. In order to conjugate siRNA, cationic carrier of Oligo(lysine)s were conjugated genetically at the N-terminus of the ELP hydrophobic blocks. Using high cell density fermentation, the diblock [I1H4-60]-[A3G2-60] harboring different oligo-L-Lysine fragments has been expressed and purified.

A21 TITLE: Novel ultra-short peptide hydrogel as a potential drug nanocarrier

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Abstract: Hydrogels endow a pristine soft-materials class of a large variety of

applications, ranging from magnetic resonance imaging and drug delivery to catalysis and as template materials. Particularly, self-assembled biocompatible peptide-based hydrogels have shown promising properties and results as nanocarriers for antitumor drugs. Here, a stimulus-responsive selfassembled intertwined fibrillar structure is achieved through the cooperative effect of different noncovalent intra- and intermolecular interactions. Lately, the development of hydrogelators with optimum drug delivery and mechanical properties at low cost and minimalist length has been a main challenge, which requires a cautious structure design. Such properties are not only desired for the pristine material hydrogel but also in the combination with composites. Hereby, considering structural aspects required to favour hydrogelation and empirical knowledge on the self-assembly of dipeptides, a new hydrogelator comprising a dehydrophenylalanine residue and a methionine residue N-protected with a benzyloxycarbonyl group was designed and synthesised through a low-cost synthetic route. The hydrogel was characterized using fluorescence-based techniques (fluorescence emission, excitation and anisotropy). A critical gelation concentration of 0.1 wt% was obtained, which is very advantageous for a hydrogelator with only two aromatic moieties. The developed nanosystem exhibited promising results as a competitor to current available ultra-short hydrogelators.

A22 TITLE: Development of chitosan/PEG hybrid coated PLGA nanoparticles to improve entacapone therapeutic efficacy

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Abstract: Entacapone is an orally active catechol-O-methyltransferase (COMT) inhibitor authorized in the United States and Europe as an adjunct to levodopa and carbidopa for the treatment of Parkinson's disease. Despite effective in terms of peripheral COMT inhibition, Entacapone clinical effectiveness has been questioned due to its poor bioavailability, short plasma half-life and lack of blood-brain barrier permeability. Nanomedicine emerged as a solution to overcome several drug drawbacks by using functionalized nanomaterials that prolong blood circulation, reduce toxicity and enable brain targeting. Within this framework, the use of polymeric nanoparticles bearing a combinatorial coating with PEG and chitosan as colloidal long-circulating drug delivery systems has been reported as a promising tool to overcome the pointed out drawbacks. In that regard, entacapone-loaded chitosan/PEG blended PLGA nanoparticles were synthesized after a brief optimization process by the nanoprecipitation method. The development process and the data related to physicochemical and morphological properties of the new nanoformulation will be presented in the communication.

A23 TITLE: Speeding up the Development of Targeted Precision Therapies

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Abstract: There is a growing interest in the development of nanotechnology-based medicines and diagnostics and intense research and patents, but all this research activity faces difficulties in the translational process and has produced only a limited number of new medicines on the market, partly due to the lack of appropriate characterization methods. Here we will show the results of the SPEED-THERAPY project where we have developed improved methods for in-depth characterization of tar-getd nanodrug delivery systems for combination therapies. We have developed targeted PLGA-PEG nanodelivery systems able to carry both hydrophobic and hydrophilic drugs. Our system uses Substance-P as the targeting moiety towards neuroblastoma cells. Here we will show the methods that we have developed for controlling the quality of the starting polymers (based on NMR and 1H-DOSY experiments), for the accurate measurement of the particle size distribution (based on SEC-DLS), and for the atomic-level control of the binding of Substance-P to the PLGA-PEG nanoparticles (based on 2D-NMR).

A24 TITLE: Polyaminoacid based Copolymers for self-assembling of colloidal carrier for Doxorubicin Tumor Delivery

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Abstract: Doxorubicin (DOXO) is reference therapeutic agent for the treatment of a variety of cancers . Its use is limited by unfavorable biodistribution which causes severe systemic effects. We aimed here at developing amphiphilic diblock copolymers including a PEG block and a hydrophobic poly-aminoacid block bearing Doxorubicin molecules through pH-cleavable bonds. These conjugates assemble in micelles that were studied in vitro and in vivo for delivery of DOXO. Methods. A set of block co-polymers from mPEG-SH by Ring Opening Polymerization (ROP) of N-Carboxy Anhydride (NCA) derivatives of glutamic acid (Glu) and leucine (Leu).

The γ-carboxylic group of glutamic acid was converted in hydrazide that was then conjugated to DOXO. A set of polymers with different leucine/glutamic acid ratio was generated. The critical micellar concentration (CMC) of the conjugates and DOXO release from micelles was studied at pH 7.4 and pH 5 as proxy conditions for blood and lysosomes, respectivelly. In vitro cytotoxicity was investigated on murine CT26 colorectal carcinoma and 4T1 mammary carcinoma. Mouse tumor models were generated by subcutaneous injection of the two cell lines and used to assess the therapeutic activity of the DOXO carriers. Results. Four mPEG5kDa-(Glum-r-Leun) block copolymers were generated with different Glu/Leu ratio: 16:0, 8:8, 6:10, 4:12. Doxorubicin conjugation to Glu was affected by the Glu density in the polymer block. The mPEG5kDa-(Glu[DOXO]6-r-Leu10) assembles in colloidal systems with a 9.5 ± 0.4 μM CMC and a size of about 30 nm. Micelles released only 4% of DOXO at pH 7.4 in 8 days while ~30% was released at pH 5. DOXO loaded micelles displayed IC50 on CT26 and 4T1 cells comparable to free DOXO. The administration of micelles to tumor bearing mice showed good therapeutic index and safety profile which prolonged the survival of the animals compared to a commercial liposomal formulation.
Conclusions. PEG-polyaminoacid amphiphilic block copolymers loaded with DOXO resulted a successful platform with tunable biopharmaceutical properties in term of loading capacity, self-assembling profile, release rate. Indeed, the physico-chemical features of the materials can be modulated by using different Glu-/Leu ratio or replacing Leu with semisynthetic hydrophobic aminoacids.

A25 TITLE: B-SMART

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Abstract: B-SMART is a H2020 project that started in 2017. The project's acronym stands for Brain-Specific Modular and Active RNA Therapeutics. We focus on neurodegenerative diseases such as Alzheimer's or Spinal and bulbar muscular atrophy (SBMA) for which, currently, no effective treatment exists. We explore a novel gateway into the brain (the blood-cerebrospinal fluid barrier) to enable RNA-based nanomedicines to reach the brain cells affected by the disease. We use a modular delivery system: based on three essential components which can only be successful if combined: RNA payload, nanocarrier materials, and nanobody-based targeting ligands. Within the consortium all partners us the same microfluidic platform for the controlled synthesis of nanomedicines. Using this easy to operate microfluidic assembly system will ensure quality-bydesign: uniform nanocarriers across research sites, excellent control over the physico-chemical parameters, linear scalability, and compatibility with GMP production. Over the past years we have made substantial progress in each of the three pillars that constitute the framework of the project:

1. Nanoparticle assembly of RNA payload, carrier material and targeting ligand, 2. quality control and scale-up and finally 3. Performance regarding safety and efficacy. This presentation will describe the recent highlights of the research between the 9 partner institutes and the lessons learned for the second half of the project.

A26 TITLE: Lympho-targeted polymeric nanoparticles as carriers for preventive and therapeutic vaccines

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Abstract: Nanotechnology has contributed to revolutionize the development of modern vaccines in the last decades. Nanoparticles allow the use of purified or synthetic antigens, such as protein, peptides and polynucleotides, enhancing the immune response towards these safe but poorly immunogenic macromolecules. Besides, these nanosystems facilitate the administration through mucosal routes and may avoid the need of multiple administrations. Recently, we and other authors, have hypothesized that lympho-targeted antigen nanocarriers would be beneficial, considering the high concentration of immune cells concentrated in the lymph nodes. With that aim, we have developed a panel of polymeric nanocapsules and we have evaluated their dissemination towards the lymphatics after subcutaneous administration. Negatively charged nanocapsules with particle sizes lower than 100 nm showed the highest accumulation in the mice popliteal lymph nodes. In the frame of an NIH project, and in collaboration with the University of Manitoba, we have developed a HIV vaccine consisting of 12 different nanopackaged peptides. The vaccine, administered through the nasal route, protected the treated macaques against multiple SIV challenges. Besides, we have evaluated different types of linkages between the peptides and the polymers of the nanoparticles, and the inclusion of polyIC to produce an optimized version of the vaccine. Apart from protective vaccines, our technology is also suitable for therapeutic vaccines. In the NANOT-AID project, we have included different TLR-agonist in lympho-targeted

polymeric nanocapsules. The nanosystems, loaded with a peptide antigen, showed an important protection in an autoimmune encephalomyelitis model compared with the coadministration of the antigen and the immunostimulants alone. In summary, the rational design of polymeric particles allowed us to develop systems with high capacity to interact with different types of immune cells and with an adequate biodistribution profile to function as a nanovehicles for modern antigens, both for prophylactic and therapeutic vaccines.

A27 TITLE: Changes in chromatin organization by Ag3-AQCs: effects on chemotherapy

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Abstract: Silver atomic quantum clusters (Ag-AQCs) are stable species formed by a small number of atoms of silver, with sizes below 1 nm. The small size of these Ag-AQCs, produces quantum confinement effects whose main signature is the loss of metallicity and the emergence of molecular-like properties making them unique and susceptible to be used in multiple applications. Due to their small size and to their properties, Ag-AQCs deserve consideration as potential pharmacological agents. It has been previously described that Ag3-AQCs interacts with DNA trough intercalation, resulting in the local unwinding and the elongation of the double helix. In vitro studies demonstrate that Ag3-AQCs disrupt the binding of proteins such as bacterial topoisomerase IV and DNA gyrase or the human recombinant topoisomerases I and II to the DNA. Moreover, Ag3-AQCs affect nucleosome assembly in an in vitro model. The objective of this work was to study effect of Ag3-AQCs in chromatin organization using the human lung adenocarcinoma cell line

A549. Super-resolution stochastic optical reconstruction microscopy (STORM) and a chromatin accessibility assay allowed us to confirm that Ag3-AQCs alter chromatin organization in cells. Ag3-AQCs lead to a massive decompaction of chromatin in Ag3-AQCs treated cells compared to control. Moreover, enhancement of chromatin accessibility is restricted to proliferating cells. This increase in chromatin accessibility mediated by Ag3-AQCs enhances the binding of drugs that target DNA, such as cisplatin (CDDP) potentiating their cytotoxic effect. The effect of Ag3-AQC was also investigated in vivo in mice bearing orthotopic lung tumor derived from A549. Chromatin accessibility assay corroborate the results obtained in A549 cells, showing that chromatin accessibility is increased in highly proliferating cells from tumor tissue but not in normal tissue, whose cells are mostly quiescent. Moreover, the co-administration of Ag3-AQCs increases the amount of CDDP bound to the tumor DNA by fivefold without modify CDDP levels in normal tissues. Finally, we assessed if this specific accumulation of CDDP in tumor tissue had a real effect on tumor growth. The co-administration of Ag3-AQCs and CDDP significantly reduces tumor growth in primary tumor and metastasis. In conclusion, co-administration of Ag3-AQCs increases the therapeutic index of DNA binding drugs.

A28 TITLE: SPOROPOLLENIN AS DELIVERY PLATFORM FOR ORAL PEPTIDE NANOTHERAPEUTICS

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Abstract: In the present work we developed biodegradable polymeric nanocarriers (e.g. chitosan, protamine) and evaluated their suitability for oral peptide delivery.1,2As a further step, we have associated these nanocarriers to a bioinspired, sporopollenin-based delivery platform with specific morphological characteristics to further enhance their mucointeraction. These integrated delivery systems were evaluated regarding their performance in an ex vivo intestinal model and upon oral gavage to healthy rats. Sporopollenin-derived delivery platforms were prepared from different plant pollen species by sequential washing and purification steps with organic solvents, surfactants or combinations of these. The complete elimination of contaminants/allergens and the preservation of the original 3D surface morphology were confirmed by electron microscopy, FT-IR and elemental analysis. The optimized nanocarrier prototypes showed high association efficiency of a model peptide drug and were stable in biological media for at least 6 hours. Selected nanocarriers were fluorescently labeled and were associated at different weight ratios to the sporopollenin-based platform by freeze-drying. Fluorescence and TGA measurements confirmed high loading efficacy of the nanocarriers as well as their sustained release from the porous sporopollenin

structure.Ex vivo mechanistic studies in a non-everted rat intestinal sac model showed that the combination of nanocarriers with the sporopollenin platform could largely enhance mucointeraction. This effect was also observed in vivo following oral administration, where the intact vehicles could be detected together with a high number of associated nanocarriers in the duodenal and jejunal regions of the rat intestine for extended periods of time. On the contrary, no significant fluorescence could be detected for the same concentration of control nanoparticles when administered alone. In addition, microscopic observation and histological staining showed that the integrity of the tissue was preserved throughout all experiments.
Conclusions: Our results suggest that the developed delivery system could be a promising strategy for improving the efficacy of oral peptide nanomedicines.

A29 TITLE: Tri-mannose bearing lipid polymer shell mRNA nanoparticles combine strong antitumor T-cell immunity with improved inflammation safety

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Abstract: In these last years, we are witnessing the emergence of new class of biopharmaceutics based on messenger RNA (mRNA). It can encode any antigenic protein, which allows the development of preventive and therapeutic vaccines. It offers a strong safety compared to DNA because it cannot be integrated in host genome. The translation machinery being located in the cytosol, mRNA expression does not require nuclear import, which is of benefit for hard to transfect cells such as dendritic cells (DC). We developed an advanced hybrid histidinylated lipid polymer shell mRNA nanoparticle (lipopolyplexes, LPR) endowed a tri-antenna of α-D-mannopyranoside (triMN-LPR). Wvaluated (i) their binding to human and mouse DCs able to recognize specifically mannose ligands, (ii) the nature of induced immune response after immunization and (iii) their therapeutic anti-cancer vaccine efficiency. TriMN-LPR provided high induction of a local inflammatory response after intradermal injection to mice, followed by DCs recruitment and activation in draining lymph nodes. When evaluated in therapeutic pre-clinical murine tumor models, triMN-LPR carrying mRNA encoding the respective antigens significantly exert curative responses in mice vaccinated seven days after initial tumor inoculation. Interestingly, triMN-LPR were also extremely effective in conferring antitumor T-cell

immunity upon intravenous injection. They target and activate splenic DC antigen presenting cells to subsequently prime T cells. When benchmarked against ex vivo generated DCs electroporated with mRNA, triMN-LPR treatment was superior in controlling tumor growth. TriMN-LPR have a low inflammatory feature allowing modified mRNA nucleosides use for effective T-cell immunity by contrast to liposomal formulations.. Altogether, our data provide evidence that triMN-LPR give rise to an efficient stimulatory immune response allowing for therapeutic anti-cancer vaccination in mice. TriMN-LPR could be low inflammatory alternatives to the mRNA lipoplexes currently explored in early phase clinical trials.

A30 TITLE: Epicardial-derived exosomes in hypoxia and normoxia: a primary characterization

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Abstract: Exosomes are small cellular vesicles (30-150 nm in diameter) functioning as cell-to-cell message carriers. These nanovesicles, whose contents (miRNAs, mRNAs and divers proteins) are thought to be an indicator of the physiological status of the cell they derive from and, ultimately, play a key role in many biological processes. Exosomes also provide instructive information to the surrounding cells on relevant changes in their environment, and therefore they are regarded as potential markers of organ disease. Conditions as ischemic cardiomyopathy-myocardial infarction (MI) are usually diagnosed at advanced stages of the disease, and early subclinical diagnostic markers have not yet been identified. It has been shown that cardiac fibroblasts involved in post-MI ventricular remodeling are derived from the embryonic epicardium, and that these cells are specifically activated (proliferation, migration, collagen synthesis) upon ischemic heart damage to compensate for cardiomyocyte loss. Our work focuses in isolation and characterization of exosomes from an epicardial cell line (EPIC) immortalized in our laboratory from embryonic epicardial cells. Our aim is to characterize epicardial-derived exosomes and define the molecular composition of these nanovesicles in different physiological contexts. In order to tackle this scientific objective, EPIC cells are cultured under normoxic and hypoxic conditions and exosomes isolated from culture supernatant by ultracentrifugation and probed for confocal and TIRF imaging. Our data indicates that EPIC cells secrete a considerable amount of exosome, and that the amount of these vesicles increases in the culture medium exosome when EPIC cells are incubated in hypoxia (5% oxygen). Moreover, when EPIC-derived exosomes are co-cultured with EPIC cells, the former are fastly internalized by some of the cells of this continuous line. TEM and proteomic analysis are being performed to assess EPIC exosomes' structuctes, size and cargo in

normoxic and hypoxic states Our short term objective is to evaluate the effect of EPIC-derived exosomes on the phenotype, function and transcriptomic profile of cultured cardiomyocytes.

A31 TITLE: Rifabutin-loaded Nanostructured Lipid Carriers for intracellular anti-mycobacterial therapy

Authors: <u>Rouco, Helena</u>1; Diaz-Rodriguez, Patricia1; Gaspar, Diana P.2; Gonçalves, Lidia M.2; Remuñán-López, Carmen1; Almeida, Antonio J.2; Landin, Mariana1

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Abstract: Nanostructured Lipid Carriers (NLC) are considered the second generation of lipid nanoparticles.They present a lipid matrix composed by a blend of solid and liquid lipids, characterized for being solid at both room and body temperatures. This work is focused on the in vitro performance of blank and rifabutin (RFB)-loaded NLC designed to treat mycobacterial intracellular infections. NLC's composition and formulation procedure were previously optimized by Artificial Intelligence tools in terms of physicochemical properties and drug payload. On this study, NLC thermal stability was evaluated by subjecting them to heating and cooling cycles evaluating particle size every 0.5°C by Dynamic light scattering. Cell studies were carried out in THP-1 human monocytic cell line. Cell viability assessments were performed by WST-1 assay after 24 hours of incubation with several nanoparticle concentrations (from 0.3 to 0.03 mg/mL). THP-1 derived macrophages NLC uptake was evaluated both quantitatively and qualitatively after 2 hours of incubation with coumarin 6 (C6)-labelled NLC. The experimental data showed particle size remained within the nano-range during thermal analysis, indicating NLC stability in harsh conditions, such as occurring during moist-heat sterilization. Cell viability studies pointed out a concentration-dependent cytotoxic effect for both blank and RFB-loaded NLC. A concentration of 0.12 mg/mL was selected as appropriate for uptake studies, with a cell viability of 83 ± 8 and 70 ± 8 %, for empty and loaded formulations respectively. Cell uptake by macrophages was visualized by confocal microscopy after fluorescent labelling of cytoplasm and nuclei of cells. The highest fluorescence of C6 observed in blank NLC could suggest higher NPs uptake. However, identical internalization percentages were reported (3.8 ± 0.5 and 3.8 ± 0.6 %, for blank and loaded NLC), indicating that drug payload does not affect cell uptake but decreases the C6 incorporation efficiency of NLC. Therefore, RFB-loaded NLC constitute a promising drug delivery system to treat intracellular pathologies.

A32 TITLE: Nanostructured lipid nanoparticles for delivery of antimicrobial peptides

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Abstract: Dispersions of nanostructured cubic liquid crystalline phases, also known as cubosomes, have shown great promise as delivery vehicles for a wide range of drugs. Due to their ordered structure, comprising alternating hydrophilic and hydrophobic domains, cubosomes possesses unique delivery properties and compatibility with both water soluble and insoluble drugs. However, the drug delivery mechanism and cubosome interaction with human cells and bacteria are still poorly understood. In this work we reveal how cubosomes loaded with the human cathelicidin antimicrobial peptide LL-37, a system protecting from the peptide from proteolysis and with high bacteria killing effect, interact with the bacterial membrane to provide new insights into the eradication mechanism. Combining the advanced experimental techniques neutron reflectivity and quartz crystal microbalance with dissipation monitoring a mechanistic drug delivery model for LL37 loaded cubosomes on bacterial mimicking bilayers was constructed. Moreover, the cubosome interaction with Escherichia coli was directly visualized using super resolution laser scanning microscopy and cryogenic electron tomography. We could conclude that cubosomes loaded with LL-37 adsorbed and distorted bacterial membranes, providing evidence that the peptide-loaded cubosomes function as an antimicrobial unit.

A33 TITLE: P-Selectin targeted polysaccharide nanoparticles for thrombolytic therapy

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Abstract: Atherosclerosis is an asymptomatic disease which may lead to acute and severe cardiovascular events due to the atherosclerotic plaque rupture. The intravenous injection of recombinant tissue plasminogen activator (rtPA), the gold standard for thrombolytic therapy, possesses high risks of intracranial hemorrhages and neurotoxicity. Hence, there is an unmet medical need for safe nanomedicine-based

thrombus targeting solutions. The objective is to create a non-toxic, biocompatible, and biodegradable nanocarrier which is functionalized with a targeting agent and suitable for thrombotic diseases. Fucoidan, a natural anionic polysaccharide, holds thrombus targeting properties due to its strong affinity for P-Selectin, an inflammatory adhesion molecule.In this study, nanoparticles (NPs) were elaborated by a water-in-oil emulsification protocol based on the use of a vegetable oil combined with a crosslinking of natural polysaccharides: dextran and fucoidan (Fuco) with sodium trimetaphosphate (STMP). Spherical and homogeneous NPs exhibited a size 662 ± 27 nm and zeta potential -30.3 ± 0.1 mV. The NPs remained stable for 4 weeks at 4°C. The NPs were visualized by TEM and E-SEM. The presence of the fucoidan was confirmed by elemental analysis by quantification of Sulfur content: 9% (w/w). NPs were cytocompatible at concentrations from 0.1 to 1.5 mg/ml by Resazurin assay on HUVECs. A clinically available rtPA (Actilyse®) was loaded onto NPs by adsorption. Loading efficiency (70% w/w) and release profile of rtPA, monitored by BCA protein assay, proved stable conjugation. The enzymatic activity of rtPA-loaded NPs was assessed with fluorogenic substrate PefaFlour® rtPA. The targeting strategy of FucoNPs was validated with a microfluidic experiment in vitro on recombinant human P-Selectin and activated platelet aggregates. Fuco-NPs bounded significantly more to P-Selectin coating than Control-NPs (**$p<0.01$); Fuco-NPs accumulated at the surface of platelet aggregates (***$p<0.001$) in arterial conditions. To conclude, novel biocompatible and biodegradable NPs were elaborated using natural polysaccharides.Their functionalization with fucoidan and loading with active rtPA allowed the thrombus targeting due to specific P-Selectin interactions. This ex vivo proof of concept study underlines the potential of these new functionalized polysaccharide NPs for safe preclinical thrombolytic therapy

A34 TITLE: Cell-penetrating peptides as non-viral carriers for gene therapy

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Abstract: Gene-based therapy constitutes one of the most promising tools for the next generation of therapeutics and the future of human health.1,2 Nucleic acids (siRNA, pDNA, mRNA, etc.) and geneediting nucleases (CRISPR/Cas9, etc.) can be applied to repair the malfunction or lack of an essential protein inside cells. However, the delivery of the required genetic cargo is limited by the lack of stable and effective delivery vehicles, which can be either a viral or a non-viral vector.1 The use of viruses as vectors covers the majority of the current literature on this topic, as could be expected from their higher in vivo transfection efficiency. Although they have considerably advanced the field of gene therapy, several limitations are associated

with viral vectors. In this regard, non-viral vectors (peptides, polymers) have been used for gene therapy as a safer, more reproducible and inexpensive alternative. We here report a supramolecular strategy for the direct delivery of Cas9 by an amphiphilic penetrating peptide prepared by hydrazone bond formation between a cationic peptide scaffold and a hydrophobic aldehyde tail.3 The hydrazone formation is carried out in fully biocompatible conditions and the resulting peptide amphiphiles can be directly complexed with the bioactive cargo and added to cells without any special requirement or further purification. Upon the peptide/protein interaction, noncovalent nanoparticles (≈200 nm) were formed and delivered its cargo inside cells with similar efficiency and less toxicity than one of the best methods described to date (i.e. lipofectamine). We confirmed that the hydrazone modification of cell-penetrating peptides comprises an appropriate and straightforward approach for the efficient delivery of Cas9 inside cells. To the best of our knowledge, this is the first report of a non-covalent strategy for the delivery of Cas9 with a penetrating peptide vehicle, avoiding any protein engineering or covalent fusion to the peptide carrier. Therefore, this work verifies the versatility of the strategy for the screening of adaptable carriers for different cargos in different cell lines.

A35 TITLE: UNIMORE NANOFACTORY: Technology Platform for Nanotherapeutics , Nanodiagnostics and Nanotheranostics

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Abstract: Nanomedicines (NMed) devised for therapeutic, diagnostic and theranostic aims must be carefully planned on the basis of proper technologies able to assure a balanced and rational development. UNIMORE team focused the major effort in exploiting unique and innovative technologies in all the fields connected to design, preparation, optimization and testing of NMed, to be potentially applied in human pathologies. All the technologies used are in the hand of UNIMORE team, starting from pre-formulation studies based on modeling relying, multiscale computational simulations, design of experiment able to identify key parameters as temperature and pH of work, drug loading, stable encapsulation, on-command release and adsorption of blood proteins in order to correctly set up the nanoformulation task. This phase is followed by production of NMed in which adaptable methodologies could be applied to optimize chemico-physical and technological properties of therapeutics, of diagnostic and theranostic NMed Standardized procedures are exploited to furnish full and complete characterization, acquired data are exploited

by on the edge multivariate data analysis. The process of development is improved by analytical studies, based not only on conventional (e.g. advanced microscopy, photon correlation and micro Raman spectroscopy, calorimetrical analyses etc) but also on innovative technologies as biosensoring, optical tweezers and nanoimaging. In vitro/ex vivo expertise consolidate the complete screening of NMed in terms of efficacy and safety: in this way, cell culture and co-cultures, (cell cycle, apoptosis, signaling pathways, metabolic and oxidative studies) , organ-on-chip, live imaging on cells or explanted organs, could be applied depending on the aim, leading to a complete evaluation and to furnish preliminary data for the following in vivo steps, also useful for sample refinement. In vivo preclinical studies are developed, with availability of several in vivo disease models; also innovative models for assessing efficacy in treatment and diagnosis, biodistribution and safety profiles. By exploiting these unique technologies, UNIMORE could be considered a real platform for NMed development in the field of treatment and diagnosis of neurological disorders, cancer and inflammatory/ infectious diseases.

A36 TITLE: Polymeric particles and membranes for cell encapsulation, proliferation and release

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Abstract: Polymeric nanoparticles, films and scaffolds are exceptional materials for biomedical applications such as drug delivery, tissue engineering and repair, etc. However, the synthesis of these materials or their surface modifications commonly involves the use of toxic solvents and additives, sacrificial templates that have to be removed at post-production stages, the use of sophisticated instruments, and/ or the need of extreme working conditions compromising both the integrity of the materials and the process yield. We here describe some biologically-friendly strategies to produce millimeter-size hollow polymeric particles and cross-linked polymeric membranes for tissue regeneration. Hollow particles were produced from DNA droplets and methacrylamide chitosan aqueous solutions (CH:MA), which were assembled and hardened by a double ionic/covalent superficial crosslinking proocess on top of superhydrophobic surfaces, leading to liquid-core particles with a hardened hydrogel shell. The obtained particles are soft and have an outstanding structural stability against manipulation. Cell staining, fluorescence microscopy, and MTT proliferation assays revealed that the liquid DNA guaranteed a biocompatible medium for efficient cell encapsulation and survival followed by a superior release

and proliferation of viable cells, as compared to solid CH:MA particles. Polymeric membranes were obtained by solvent casting followed by a cross-linking step mediated by the chemical vapor deposition (CVD) of glutaraldehyde (GA). The membranes were characterized against non- and solution cross-linked membranes in terms of their mechanical/surface properties and biological performance. The CVD membranes proved to be more mechanically stable against cell culture and sterilization than membranes cross-linked in solution, and to prompt the adhesion and sustained proliferation of healthy cells to levels higher than commercial tissue culture plates. The produced hollow particles and polymeric membranes hold then promise for tissue regeenration, in which manipulable and biocompatible synthetic carriers are often needed to supply living cells and other sensitive bioactive compounds. The described strategies are also of interest as are simple, reproducible, cost-effective, and totally biocompatible, not requiring the use of sophisticated experimental set-ups and/or potentially harmful procedures.

A37 TITLE: Therapeutic applications of extracellular vesicles as vehicles for lysosomal enzymes

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Abstract: Extracellular Vesicles (EVs), comprising microparticles, exosomes and other secreted vesicles, are naturally occurring delivery systems produced by most cell types. EVs have been described as fully biocompatible submicron-sized vehicles with high transfection capacity and low immunogenic responses, especially when produced in autologous systems]. In this work, we propose the use of EV as direct delivery platforms for proteins defective in lysosomal storage disorders (LSD). LSDs are congenital rare diseases caused by the lack or malfunction of a lysosomal

protein, mainly enzymes. Noteworthy, most LSDs are currently treated with enzyme replacement therapy (ERT). This approach is based in periodic intravenous infusions of human recombinant enzymes. Nevertheless, as in other protein therapeutics, ERT has important drawbacks, including poor biodistribution, low enzyme half-life, inability to cross the blood brain barrier and high immunogenicity, which often limits it efficacy. In this scenario, we isolated and characterized EVs from a CHO cell line overexpressing the alpha galactosidase A (GLA), the defective enzyme in Fabry disease, with a myc-his tag (cmychis). In our hands, EVs containing GLA (EVs-GLA) were rapidly uptaken by target cells and driven directly to the lysosomes, where the exogenous GLA enzyme could restore lysosomal functionality. Indeed, the GLA in EVs had a higher specific activity (6-fold) and greater efficacy reducing the Gb3 substrate accumulations (3-fold) than the free GLA enzyme or the Algasidase alfa in clinical use. Tolerability and biodistribution assays showed that intravenously administered EVs accumulate mainly in liver and spleen but also in kidneys, without causing any deleterious side-effect. Finally, GLA knock-out mice treated with EV-GLA showed a greater Gb3 reduction in plasma and kidneys compared to those receiving free GLA or Algasidase alfa. Additional assays are being conducted to test the blood brain barrier crossing and the generation of auto-antibodies in EV-GLA treated mice compared to their controls. Studies have been also extended to other LSD, such Sanfilippo, with promising results. Overall, our results demonstrate that EVs from cell lines overexpressing lysosomal enzymes work as natural drug delivery systems for LSD, improving the stability and the efficacy of the cargo.

A38 TITLE: Electrospun poly (vinyl alcohol)/cellulose acetate wound dressing formulations: potentialities and synergistic effect with antimicrobial agents

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Abstract: Chronic wounds are unable to heal in a predictable time due to physiological conditions of the patient and/or bacterial infections, resulting in high mortality rates. A new generation of wound dressings has been uncovered in order to aid with the healing/management of the wound and prevent possible infections. Various polymers have been applied to design electrospun nanofibrous mats. Calcium acetate (CA) has shown a great capacity to enhance fibroblasts interaction and poly(vinyl alcohol) (PVA) is known to enhance the spinnability of polymeric blends, by conferring suitable mechanical properties, while still assisting with regeneration process. In this work, PVA and CA were processed in the form of nanofibrous mats and functionalized with immunoregulatory AMPs (Tiger 17 and pexiganan) for prospective applications in wound dressings. Non-functionalized AMPs display a

broad spectrum of activity against pathogens and reduce the likelihood of bacteria to develop resistance. However, when surface-functionalized, that activity may be altered and even decrease. Here, that phenomenon was observed. PVA/CA mats were successfully produced via eletrospinning. These were characterized in terms of flexibility (tensile strength), degradability, swelling capacity, thermal and physical stability and morphology. Preliminary testing revealed a superior antimicrobial activity (particularly with pexiganan) of the functionalized mats compared to the bare mats. Data suggests that the developed AMPs-loaded PVA/CA nanofibrous mats could be ideal for wound dressing applications.

A39 TITLE: Effect of shape on the biodistribution profile and toxicity of gold nanoparticles in Wistar rats

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Abstract: Gold nanostars are considered promising nanoplatforms for drug therapy, diagnostic, and imaging. The presence of the multiple sharp branches on the surface provides large surface areas and very intense plasmon bands in the near infrared, where absorption of light by biological matter is minimal. However, the shape of the gold nanoparticles (AuNPs) not only influences its applications in biomedicine but can also influence their biological effects, thus an in vivo comparison study between the gold nanostars vs. well described spheres was deemed necessary. The current work aimed at synthesizing and characterizing star- and sphere-shaped AuNPs (40-50 nm) capped with 11-mercaptoundecanoic acid (MUA) that were administrated i.v to Wistar rats at a dose of 1.33*1011 AuNPs/Kg body weight. The animals were euthanized 24h after administration and several organs, tissues, urine and blood were collected. Graphite furnace atomic absorption spectroscopy analysis (GFAAS) was used to quantify gold and evaluate the biodistribution of the AuNPs. Energetic balance and oxidative stress were evaluated by quantifying ATP, GSH and GSSG in the liver, spleen, kidney, heart and brain. The biodistribution data showed a higher accumulation for nanospheres than stars in the liver ($P \leq 0.05$). The other two organs with high uptake were the spleen followed by lungs (no shape-effect noted). Although the gold content in heart, kidneys and brain were less than LOQ, a shape-dependent disturbance in the energetic status and glutathione levels was noticed. This suggests the high sensitivity of this organs at low levels of exposure to AuNPs.
The total glutathione level in liver was altered for both spheres ($P \leq 0.05$) and stars ($P \leq 0.001$) while in spleen just for the sphere-shaped AuNPs ($P \leq 0.05$). Differences were

found for the ATP levels both in liver (P=0.1068) and the spleen (P=0.0528) between the two shapes. In conclusion, the gold nanostars presented a different profile after i.v administration to rats regarding both biodistribution and toxicity as compared with nanospheres. This proves the need for detailed toxicological investigation for different-shaped AuNPs to be used for biomedical applications. This work was supported by FEDER (POCI/01/0145/FEDER/007728, POCI-01-0145-FEDER-029584, POCI/01/0145/FEDER/007265) and by national funds (FCT, Fundação para a Ciência e Tecnologia) through UID/MULTI/04378/2013, UID/QUI/50006/2013 and the grants PD/BD/109634/2015 (PDQS) and SFRH/BD/107708/2015. To all financing sources the authors are greatly indebted.

A40 TITLE: Peptide targeted nanovesicles for the α-galactosidase A enzyme delivery in Fabry disease

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Abstract: Fabry Disease is a rare lysosomal storage disorder characterized by a lack of a metabolic enzyme, α-galactosidase A (GLA), affecting mainly the kidneys, heart and nervous system, and causing an early death in patients. Current treatment of Fabry patients, known as enzyme replacement therapy (ERT), involves the intravenous administration of the recombinant GLA. Although the ERT has significantly improved life-quality of patients, it shows some serious drawbacks such as the poor biodistribution, limited efficacy and relatively high immunogenicity of the GLA protein. In this scenario, encapsulation of GLA in nanocarriers could improve the ERT. Indeed, we have recently demonstrated that nanoliposomes functionalized with RGD peptide protect and deliver specifically the enzyme to lysosomes of target cells. Nonetheless, this GLA-nanoliposomal system still needs to increase its colloidal stability and drug loading capacity. In the present study we explore the use of two different RGD-targeted nanovesicles for GLA entrapment: Quatsomes and Hybrid-Liposomes, containing high and low amounts of Miristalkonium chloride (MKC),

respectively. Both formulations were prepared in a single-step procedure using compressed CO2. Physicochemical parameters such as size, morphology, charge, stability, GLA loading and enzyme activity were carefully characterized in order to select the best prototype for further experiments. In both systems, Quatsomes and Hybrid-liposomes, the presence of the cationic surfactant MKC increased the loading capacity and improved the colloidal stability by preventing vesicle aggregation. However, in Quatsomes, the use of high quantities of MKC provoked a considerable reduction in the GLA enzymatic activity, while HybridLiposomes with 0.4 to 4% of MKC retained high enzymatic activities. Biocompatibility of HybridLiposomes with intermediate MKC content (2.2%) was assayed in vitro and in vivo, demonstrating that repeated administrations of the carrier caused no harm to mice. In terms of efficacy, 2.2% MKC Hybrid-Liposomes were even more efficacious than the commercial GLA enzyme reducing the deleterious Gb3 deposits in Fabry cellular models. Overall, our results show that the incorporation of controlled quantities of MKC within the liposomal membrane improve several critical quality attributes of GLA-containing nanovesicles that allow their in vivo preclinical testing.

A41 TITLE: Plug and play decoration of gold nanoparticles with recombinant proteins

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Abstract: The functionalization of gold nanoparticles with active proteins has been extensively exploited in nanomedicine and beyond, due to the broad availability and easy synthesis of the nanoparticles and their ability to passively adsorb proteins. However, passive adsorption, or physisorption, does not necessarily lead to functional conjugates, as the protein function can be compromised by denaturation or inaccessibility of active sites. On the other hand, chemisorption, which is the conjugation by bespoke chemical modification of the nanoparticles, the protein or both, is unpractical to obtain routinely and universally, due to the vast biochemical diversity of proteins, which forces to undergo tedious optimizations for each individual protein-nanoparticle pair. To facilitate covalent and oriented conjugation of recombinant proteins on standard citrate-capped gold nanoparticles, we designed and synthesised a fusion protein with the function of "universal adaptor". This protein consists of two recombinantly fused polypeptides: 1. glutathione S-transferase (GST), which rapidly forms stable gold-sulphur bonds with the gold nanoparticle and 2. SpyCatcher, a well-established synthetic protein that spontaneously forms an iso-peptidic bond with any protein tagged with SpyTag. This highly modular approach provides a convenient method to covalently bind a SpyTag-modified protein to gold nanoparticles by simple mixing.

A42 TITLE: Enhanced NK cell activity by IL15 functionalized Iron Oxide Nanoparticles

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Abstract: Nanoparticles (NPs) have a clinical interest because of their usefulness for non-invasive imaging, diagnosis and therapy. Important challenges are targeting the therapy to specific locations and the induction of specific cell mediated cytotoxic activity against cancer cells; one example with very promising results are Natural killer (NK) cells. The reason of the success is that they are antigen independent and do not cause graft versus host disease (GvHD) while they exhibit a potent graft versus tumor effect. It is known that the activation of NK cells with cytokines such us interleukin (IL)15 enhances their effector function, which is very important for their anticancer effect. NPs coated with activating molecules could be an effective new approach to generate robust and potent effect against cancer through the innate immune response; conferring this formulations larger half-life and more potent activity. In this study we show a nanoformulation, based on a biocompatible, biodegradable and traceable nanomaterial, which is able to selectively enhance NK cell function. This nanoformulation mimics the transpresentation of IL15 to NK cells by monocytes or Dendritic Cells (DCs); as evidences support that this is the mechanism to present the IL15 to NK cells in vivo. Spherical hydrophobic Iron Oxide NPs (IONPs) were used. Recombinant human his-tagged IL15 (hIL15HIS) expression was optimized to obtain larger amounts of protein in the active monomeric form; it was observed that the protein tends to dimerize into an inactive form. Activation of human NK cells from Pheripheral Blood Mononuclear Cells (PBMCs) was explored by incubating the cells with the activation molecule. We are able to demonstrate in vitro that hIL15HIS is able to enhance NK cell mediated-activity in a similar way, or even slightly stronger, compared with the commercial hIL15. Now that we have already synthesized the nanoformulation, we are able to prove the transpresentation of IL15 using the IONP@hIL15HIS, compared with soluble IL15. It is expected IONP@hIL15HIS will significantly enhance NK cell mediated-activity against cancer cells.

A43 TITLE: The nose-to-brain transport of polymeric nanoparticles: A novel insight into key cellular pathways

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Abstract: Different nanoparticles of therapeutic interest were shown to cross multiple biological barriers (blood-brain, liquor-tissue, blood-liquor). One of the most appealing pathways is the nose-to-brain (N-to-B) transport through the olfactory epithelium (OE) that enables the direct delivery of nanoencapsulated drugs to the brain. However, the mechanism for this transport is not fully understood at the cellular level. Previous research suggested the involvement of olfactory neurons. Others utilized nasal epithelial cells completely deprived of olfactory cells. This model does not recapitulate well the complex structure of the olfactory system. Aiming to shed light into key cellular pathways, in this work, we investigated for the first time the interaction of polymeric nanoparticles in a broad size (17-483 nm) and surface-charge (from negatively-to positively-charged) range with nasal cells and characterized by immunostaining and western blot. After demonstrating the good cell compatibility of the different nanoparticles, we monitored the nanoparticle uptake by confocal laser scanning fluorescence microscopy. Our interesting findings will be presented. Overall, these findings represent the first unambiguous evidence of the possible involvement of specific cell types in N-to-B transport of nanoparticles and they emerge as a valuable experimental tool to screen the biocompatibility and transport of nanomaterials from the nasal mucosa to the brain.

A44 TITLE: Blood brain barrier opening and Therapeutic Effect of Free and Nanoencapsulated Cabazitaxel in an Invasive Patient-Derived Glioblastoma Model

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Abstract: The presentation will discuss a recently performed study that has been published in the journal Nanotheranostics. Glioblastoma (GBM) represents one of

the worst prognoses among cancers, with a median survival of 14.6 months. GBM typically exhibits rapid, invasive growth, insensitivity to radiation and chemotherapy and often an intact blood brain barrier (BBB). The standard therapy for GBM consists of a combination of surgery, radiotherapy and temozolomide. Partly due to the intact BBB, the selection of effective drugs is highly limited. Cabazitaxel (Jevtana®, Sanofi-Aventis) is a highly potent taxane used as a second line treatment in docetaxel-resistant prostate cancer presumably due to its lower affinity to the efflux transporter P-glycoprotein (P-gp). One of the most promising methods for targeted drug delivery across the BBB is sonopermeation where focused ultrasound (FUS) and exogenous microbubbles are used to enable permeation through the BBB. We have previously presented an inhouse technology consisting of biodegradable poly (alkyl cyanoacrylate) (PACA) nanoparticles (NPs) that can be used to stabilize microbubbles , and used them to deliver the NPs both to solid tumors and across the BBB. In the current study we aimed to treat mice with a patient derived invasive and non-angiogenic xenograft (PDX)-model of GBM. We hypothesized that cabazitaxel can be a useful drug in the treatment of GBM and investigated the effect of sonopermeation of the BBB on both the delivery of cababazitaxel and cabazitaxel-loaded NPs (cab-NPs). We show for the first time that cabazitaxel can give a significant therapeutic effect in a patientderived orthotopic model of glioblastoma. Also, we show that the drug crosses the blood-brain barrier more effectively in the tumor than in the healthy brain due to reduced expression of pglycoprotein efflux pumps in the vasculature of the tumor. Surprisingly, neither sonopermeation nor drug formulation in polymeric nanoparticles could increase either accumulation of the drug in the brain or therapeutic effect. Nonetheless, our study shows that cabazitaxel is a promising drug for the treatment of brain tumors.

A45 TITLE: Custom biocoating of nanomaterials with recombinant proteins

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Abstract: Nanomaterials have a high surface/volume ratio, a fact that triggers biomolecular coating upon exposure to biological contexts. This protein coating,

known as biocorona, provides a biological identity to nanomaterials, directing their activity, biodistribution and clearance. To improve targeting of nanoparticles in diagnosis and therapy, one of the most important applications of nanomaterials in the field of nanomedicine, it is very critical to understand how proteins interact with the nanomaterials surface and how the unspecific biocorona can interfere with the targeting. A common strategy to enhance the targeting is coupling antibodies to the nanoparticle surface. However, antibodies coupling to the nano-surface can lead to low accessibility to their recognition sites. Moreover, antibody active sites can be hidden with the unspecific biocorona proteins, losing their target ability. In addition, the interaction of the protein with the surface of nanomaterials can trigger protein polypeptide unfolding, and this represents a warning signal recognized by some circulating proteins and macrophages, shortening the nanomaterial circulation time. Here we design a new one-step strategy to endow nanomaterials with a custom-designed bioidentity. This method, applicable to a wide array of nanomaterials, uses genetic engineered proteins to ensure precise and stable ligand orientation on the nano-surface, preventing inactivation, unspecific biofouling and mistargeting, thus controlling the evolution of the biocorona upon exposure to the biological medium. Furthermore, this model leads to the orientation of the protein on the nanomaterial surface and thus protein exposes the active site of the ligand to interact with the cellular surface receptor.

A46 TITLE: Core-shell polymer-based nanoparticles to deliver miR-155-5p to endothelial cells

Authors: Antunes, Joana C.; Benarroch, Louise; Moraes, Fernanda C.; Juenet, Maya; Gross, Marie-Sylvie; Aubart, Mélodie; Boileau, Catherine; Caligiuri, Giuseppina; Nicoletti, Antonino; Ollivier, Véronique; Chaubet, Frédéric; Letourneur, Didier; Chauvierre, Cédric

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Abstract: Heart failure occurs in over 30% of the worldwide population, being most commonly originated from cardiovascular diseases such as myocardial infarction. microRNAs (miRNAs) target and silence specific messenger RNAs thereby regulating gene expression. Since the endogenous miR-155-5p has been ascribed to vasculoprotection, loading it onto positively charged core-shell poly(isobutylcyanoacrylate)-polysaccharide nanoparticles (NPs) was attempted. NPs showed a decrease ($p<0,05$). This study is a first proof-of-concept that miR-155-5p can be loaded onto NPs, remain intact and biologically active in endothelial cells. These nanosystems could potentially increase an endogenous cytoprotective response and decrease damage within infarcted hearts.

A47 TITLE: Multifunctional liposomes for application in dual cancer therapy

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Abstract: Liposomes have been known as ideal nanoencapsulation systems, as they can overcome many pharmacokinetics problems of the loaded drugs. The combination of liposomes with magnetic and plasmonic nanoparticles enables the development of promising multifunctional nanosystems for dual and synergistic cancer therapy. The magnetic nanoparticles component allows magnetic guidance capabilities to the therapeutic site of interest (using external magnetic field gradients) and local heating capabilities (under AC magnetic field). Likewise, plasmonic nanoparticles can produce local heat due to their strong photothermal properties (under irradiation). Hence, the combination of magnetic and plasmonic nanoparticles makes possible the development of multifunctional nanosystems with the capabilities of magnetic guidance, thermotherapy and controlled drug delivery, with promising applications in dual cancer therapy. In this work, liposomes based on magnetic MnFe2O4 nanoparticles and magnetic/plasmonic MnFe2O4/Au nanoparticles were developed. The nanoparticles were synthesized and the structural, spectroscopic and magnetic properties were investigated. The prepared nanoparticles were entrapped in liposomes (aqueous magnetoliposomes, AMLs) or covered with a lipid bilayer (solid magnetoliposomes, SMLs. The magnetoliposomes were evaluated as nanocarriers for promising antitumor drugs, which exhibit very low growth inhibitory concentration values (GI50), between 0.09 and 5.67 μM, when tested in vitro against several human tumor cell lines. The local heating capabilities of the systems containing magnetic/plasmonic nanoparticles were assessed through the fluorescence quenching of rhodamine B incorporated in the lipid layer of SMLs, when excited with a light source]. A promising application of these multifunctional liposomes in oncology is anticipated, allowing a combined therapeutic approach, using both chemotherapy and magnetic hyperthermia/phototherapy.

A48 TITLE: Versatile biocompatible and biodegradable sphingomyelin nanosystems for personalized medicine

Authors: Bouzo BL, Vázquez-Ríos AJ, Díez-Villares S, Lores S, Alijas S, Jatal R, Nagachinta S, Piñeiro R, Hurtado P, López R, de la Fuente M*.

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Abstract: In line with the personalized medicine concept, which mainly applies in cancer but also in many other scenarios, there is a growing interest towards the development of versatile nanosystems that can accommodate more than one type of drug depending on every patient's needs. This includes not only conventional low molecular weight drugs, but also biotechnological drugs such as gene therapies, antitumoral peptides, aptamers, proteins, and mononuclear antibodies. From a translational perspective, we believe it very important to develop nanosystems that are easy to prepare and could adapted to industrial requirements, simple in composition, stable, biodegradable, biocompatible, and highly versatile so that they can be useful for the association of different drugs and molecules increasing their therapeutic potential. Bearing this in mind, we decided to develop a new type of nanosystems based on only two components, sphingomyelin, a major component of cell membranes that has already been used in the preparation of nanoformulations, and vitamin E, an antioxidant and a widely used excipient with a well-known safety record. Sphingomyelin nanosystems (SNs) were produced by adapting the ethanol injection method, widely described for the preparation of liposomes. SNs with a mean size around 100nm forming a monodisperse population, and with a slightly negative surface charge were obtained, demonstrated to be highly stable over time and upon incubation with biologically relevant media. Importantly, in vitro experiments demonstrated that they are non-toxic and can efficiently interact with cells to deliver the therapeutic cargo. In vivo experiments, such as those carried out in zebrafish embryos, proved that SNs are highly compatible upon injection. Importantly, SNs are promising systems in therapeutics, leading to the efficient association of different types of drugs and biomolecules (from small hydrophobic drugs to RNAs, aptamers, peptides and proteins), and can also be efficiently radiolabelled for PET and MRI imaging, therefore being of application for the management of cancer and other disease conditions. Finally, we have carried out experiments proving that SNs can be decorated with peptides and antibodies for targeting purposes to specific cell subpopulations. Altogether, we believe we have developed a technology that is highly versatile and can hold a great potential in the era of personalized medicine for the management of cancer and other prevalent diseases.

A49 TITLE: Opens issues in cell targeting. The case of CD44-targeted hyaluronic acid nanoparticles

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Abstract: We have a long-standing interest in the use of hyaluronic acid (HA) as a key component of targeted nanocarriers. HA-mediated cell targeting is made possible by

the interactions between HA and its most important cell surface receptor (CD441,2). Recently, we have mostly focused on using HA nanoparticles to deliver nucleic acids 3-4, but we have also tackled more fundamental issues: is there a fixed stoichiometry (how many receptors cluster around each particle) for nanocarriers when they bind to the cell surface5? What is the interplay between binding (e.g. to CD44) on the cell surface and internalisation6? In the first case, our most important conclusion is that for HA/CD44 the stoichoiometry is variable and depends on the affinity of the interaction; high affinity (possibly beneficial for targeting) can therefore be detrimental because a larger number of receptors may cluster around each particle, and – when all receptors are saturated, this decreases the number of particles bound to each cell. For the interplay between binding and endocytosis, this may strongly depend on the presence and activity of co-receptors. We have seen that in a panel of several tumour cells, CD44 expression scales with internalization rate, but not with cell binding, suggesting therefore the presence of another (yet unknown) scavenging receptor that is responsible of an initial targeting event. The overall message is that much is yet to be understood in the pharmacodynamics of nanocarriers to allow for an efficient and reproducible precision nanomedicine.

A50 TITLE: Microneedle patches for anti-TNFα therapy

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Abstract: Microneedle (MN) arrays have been investigated as mean to deliver drugs, proteins and other molecules to the epidermal and/or intradermal space, overcoming the skin stratum corneum permeability barrier. Biodegradable MNs can dissolve in the skin, avoiding biohazardous waste. The use of MN have been particularly promising in the field of vaccines. Using the skin route may bring unique advantages targeting the immune system. Tumor necrosis factor α (TNFα) is an important molecule involved in host defense processes. High levels of this pro-inflammatory cytokine have been related with different autoimmune diseases. Anti-TNFα therapy is standard of care in for rheumatoid arthritis, but it is also applied in other chronic inflammatory diseases such as Crohn's disease, or psoriasis. This project aims to prepare fully biodegradable polymeric MN arrays that can perforate the skin and release of relevant amounts of biologically active anti-TNFα to the epi-/dermal space. To obtain sharp silicon MNs, silicon wafer was patterned by lithography and MN shape was defined by sequential isotropic-anisotropic deep reactive ion etching process. 33x33 arrays (2cm) with MNs of 200μm diameter and 600μm height were obtained. These were replicated using poly(dimethylsiloxane) (PDMS). Polymeric MNs were prepared using chitosan, poly(vinyl alcohol) (PVA) and poly(vinyl pyrrolidone) (PVP). Chitosan was firstly added in the molds and subsequently freeze-dried. Mixtures of PVA:PVP were then added

onto PDMS molds. After drying (24hrs, RT), solid MN patches were peeled off. The mechanical properties were tested and perforation was assessed using skin models. Protein-loaded MN arrays were produced using bovine serum albumin (BSA), anti-TNFα. Drug loading was quantified using fluorescence-based techniques. Arrays of polymeric MN were prepared. 200 µm MN do not significantly change morphology after mechanical loading. The presence of chitosan do not significantly affect the array mechanical properties. BSA was successfully incorporated in the patches. More than 1mg of BSA could be loaded in the MN arrays. Preliminary results suggest that anti-TNFα in MN tend to decrease in MN containing chitosan. The work shows the preparation of sharp, biodegradable 600 µm MN that can accommodate and release therapeutic molecules. Further improvements in anti-TNFα encapsulation may be required to achieve the release of relevant therapeutic doses.

A51 TITLE: Microfluidics as smart nano-assemblers for drug and gene delivery lipid-based nanocarriers

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Abstract: Drug and gene delivery lipid-based vectors hold great promise for the development of therapeutics. While much progress has been achieved lately, namely in the development of stealth carriers and stimuli-responsive and passive- and active-targeting approaches, further progress in the science and technology is still needed to fulfil these hopes. In particular, some of the drawbacks of lipid-based systems result from the fact that most conventional methods to produce these particles not being very controlled, leading to significantly broad size-distributions and poor mean size control. Such lack of control hinders not only the efficiency of nanocarriers (which in many applications is size-dependent) but also hinders our ability to establish structure-efficiency relations that allow improvements in our understanding of biological barriers and how to overcome them. In recent years, microfluidic methods, have started being used for the controlled production of liposomes and lipid-DNA particles. These devices involve precise handling of fluids in microchannels. Hence, unlike traditional bulk-mixing methods, microfluidics allow controlled and reproducible mixing of substances, with control over chemical gradients at the micro- and nanoscales, yielding a much improved control over the size and structure of lipid-based nanoparticles. In this presentation we will describe our use of microfluidics with hydrodynamic focusing to improve the control over the size and structural features of cubosome nanoparticles and lipoplexes. In particular, by controlling only the flow parameters, we were able to produce cubosomes with tunable size. Ongoing work

is now aimed at achieving the same control in lipoplex systems. Nanoparticle size is a key parameter in the carriage of pharmaceuticals. Controlling cubosome and lipoplex size is therefore a relevant step towards the design of new and more efficient formulations.

A52 TITLE: Star-shaped polypeptides: versatile carriers for biomedical applications

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Abstract: To date, we have created a library of biocompatible star-shaped polypeptides incorporating different amino acid side-chains, which are capable of electrostatically complexing therapeutics including small molecules, proteins and nucleic acid cargoes. We have successfully incorporated small molecule therapeutics including anti-infectives and anti-inflammatory agents into oppositely charged star-polypeptides using electrostatic interaction. The materials have also been applied to difficult-to-deliver biotherapeutic cargoes. In one iteration, the structure of the starpolypeptide was tailored for controlled delivery of therapeutic proteins via the incorporation of poly(-glutamic) acid (PGA) into the star structure as the amino acid to produce biocompatible starshaped PGA polypeptides (star-PGAs) that successfully incorporated Vascular Endothelial Growth Factor (VEGF). In another, -lysine was incorporated as the amino acid to create star-shaped poly(-lysine) polypeptides (star-PLLs) which are capable of electrostatically complexing therapeutic nucleic acid cargoes and facilitating their efficient delivery to various cell types. We have successfully integrated these star-polypeptides into medical devices for therapeutic and regenerative applications with positive in vivo pre-clinical studies completed. Star-shaped polypeptides are therefore a versatile platform that can be tailored for efficient delivery of a range of therapeutic cargoes and can be easily incorporated into medical devices. Our expertise in this area permits the synthesis of multiple bespoke polypeptide architectures, which can be tailored for a range of biomedical applications.

SECTION B | *Nanostructured Theranostic Systems*

B53 TITLE: Co-assembled dehydropeptide-Gd3+ chelate nanostructures as Contrast Agents for Magnetic Resonance Imaging

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Abstract: Magnetic Resonance Imaging (MRI) is the leading imaging modality for clinical diagnosis, prognosis and disease management. MRI relays on the Nuclear Magnetic Resonance (NMR) phenomenon: interaction of the nuclear magnetic moment of the water protons of tissues with external magnetic fields and low energy radiofrequencies. MRI Contrast Agents (CA) are paramagnetic water relaxers (Gd3+ and Mn2+ chelates, iron oxide nanoparticles, stable organic radicals) that shorten the relaxation times (T1,2) of the water protons in their vicinity, yielding in some cases dramatic contrast enhancements. The parameter relaxivity (r1,2, mM-1s-1) measures CA efficacy - enhancement of the water protons relaxation rates R1,2 (R1,2= 1/T1,2) normalized to 1 mM of paramagnetic centres. Gd3+ chelates are the most efficacious CA for T1w MRI. Increasing the effective molecular weight of Gd3+ chelates is an established strategy for enhancing chelate relaxivity (self-assembly, non-covalent association with Serum Albumin and covalent attachment to macromolecular objects). Self-assembled peptide-based hydrogels (SAPH) are soft biocompatible materials made of entangled highly hydrated nanostructured fibres. A plethora of biomedical applications have been proposed for SAPH. Our research group introduced dehydrodipeptides N-capped with naproxen (Npx, a NSAID drug) as non-toxic, proteolysis-stable hydrogelators that undergo self-assembly into elastic hydrogels. Dehydrodipeptide-based hydrogels revealed suitable nanocarriers for drug delivery applications. Moreover, dehydrodipeptide hydrogels loaded with superparamagnetic iron oxide nanoparticles (SPION) are promising agents for MRI and Magnetic Hyperthermia. In this communication we report co-assembly studies of dehydrodipeptide hydrogelators with Gd3+ complexes. Co-assembled nanostructures were characterized by UV-Vis spectrophotometry, fluorescence spectroscopy, circular dichroism (CD) spectroscopy and by electron microscopy techniques (SEM,TEM). The efficacy of the dehydropeptide-Gd3+ chelate co-assembled nanostructures as MRI CA was evaluated by T1,2 MRI relaxation maps (120 MHz, 37 °C).

B54 TITLE: Uptake, permeability and diffusion of multifunctional mesoporous silica nanoparticles in tumor organoids for targeted therapy

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Abstract: Over the last years, mesoporous silica nanoparticles (MSNPs) became highly popular drug nanocarriers in cancer therapy because they offer many advantages such as high drug loading capacity, easy surface functionalization and high biocompatibility. A full understanding of their behavior inside the tumor is vital knowledge for their further translation towards the clinic. Unfortunately, nanoparticle uptake and efficacy studies often remain at the single cell level, hardly being representative for an actual in vivo tumor. In this research, we investigate the permeability of MSNPs into 3D cell assemblies called organoids, directly originating from cancer patient's biopsies. These ''tumoroids" recapitulate key features of the real tumor and therefore serve as a near-physiological model to study the behavior of drug delivery systems. We test uptake, permeability and diffusion of MSNPs with different polymer coatings, being polyethyleneimine and hyaluronic acid. Depending on the charge and characteristics of the polymer coatings, the uptake and penetration of the MSNPs inside the tumoroid will be altered. In addition, the polymers chosen in this research each have an their specific cellular function (cancer cell targeting and consecutive escape of the particles from the endosomes). As such, this research will provide valuable information about the action of this multifunctional drug delivery system which can be also applied to other nanocarriers.

B55 TITLE: Real-time drug release monitoring through dual T1/T2 nanostructures

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Abstract: Theranostic oncology is a new medical field that promises better cancer diagnosis and treatment by combining therapy and diagnosis in a single agent. Theranostic agents deliver drugs and imaging probes simultaneously, thus providing a clearer understanding of how a drug is delivered to tumours and how these tissues respond to treatment. Nanotechnology has become increasingly involved in cancer and theranostics, since the use of nanoparticles enables targeted delivery of large

payloads of drugs and/or imaging agents. In particular, superparamagnetic iron oxide nanoparticles (SPIONs) can be used as T2 contrast agents (CAs) in magnetic resonance imaging (MRI). MRI is a non-invasive imaging modality that provides three-dimensional images and has high spatial resolution. The contrast of MRI can be improved by the use of T1 (paramagnetic) or T2 (superparamagnetic) CAs. Recently, our group has been studying the interactions between T1 and T2 agents in the search for optimised performance in MRI and T1-T2 effects that could give an added value to the imaging process. Following on that work, we are currently working on the synthesis of a nanoparticle-f-element hybrid for theranostic oncology composed of: an antitumor chemotherapy drug, a lanthanide complex that acts as a T1 agent, and a targeted SPION-based nanoparticle that functions as both a T2 probe and a hyperthermia effector. Targeting peptides will also be integrated in these theranostic platforms. They will be tested on their ability to selectively accumulate in cancer cells and to release the conjugated drug. The drug release will be followed in real time through MRI. The theranostic agent will also be validated as a magnetic hyperthermia effector.

B56 TITLE: Multifunctional magnetic nanocomposite hydrogels as smart delivery systems

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Abstract: The structural combination of a 3D hydrophilic polymeric network with magnetic nanoparticles (NPs) holds the promise of providing superior functionality to the composite material with high potential for therapeutic and diagnostic applications, including drug delivery, tissue engineering, magnetic resonance imaging (MRI), and magnetic hyperthermia (MH). Therefore, the goal of this work was the development of magnetic nanocomposite hydrogels for use as localized multifunctional drug delivery systems. Magnetic hydrogels were synthesized by incorporation of magnetic iron oxide (magnetite, Fe3O4) NPs with loadings varying between 0% and 10% of the weight of the polymer. Magnetite NPs used for the preparation of the magnetic nanocomposites were synthetized following a hydrothermal method and showed spherical shape with an average particle size of 10 nm. The assynthetized magnetic hydrogels were characterized by scanning electron microscopy (SEM), Fourier transform infrared spectroscopy (FTIR) and thermogravimetric analysis (TGA). The swelling properties and their functional performance in both MRI and MH were

also evaluated. The FTIR and TGA results, together with chemical analysis by ICP confirmed the homogeneous incorporation of Fe3O4 NPs into the final hydrogel formulations. The morphological properties of the hydrogels with and without Fe3O4 nanoparticles showed that both hydrogels possessed a homogeneous structure. All of the hydrogels swelled and reached equilibrium within 48 h, with the Fe3O4 loading exerting some effect in their swelling behavior. Concerning MRI contrast performance, a strong Fe-concentration dependent T2 effect was observed, showing that the magnetic properties of Fe3O4 NPs were retained after incorporation into the hydrogels. Magnetic hyperthermia temperature measurements of the magnetic hydrogels showed a pronounced heating effect that was directly correlated to the iron content. In terms of drug delivery, a hydrophilic drug model was successfully incorporated in the hydrogel that showed a prolonged drug release under tissue-mimicking experimental conditions. Finally, we will also show preliminary results regarding the interaction of the designed hydrogel nanocomposites and several cell lines. All these combined properties hold promise for the design of innovative magnetic hydrogels for theranostic applications, gathering localized hyperthermia, controlled drug release and non-invasive imaging properties.

B57 TITLE: SELF-i: Self-reporting immunostimulating formulation for on-demand cancer therapy with real time treatment response monitoring

Authors: <u>Costa Da Silva, Milene</u>1; Martins, Cátia2; Costa, Susana2; Raposo, Manuela2; Bañobre-López, Manuel1; Gallo, Juan1

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Abstract: According to World Health Organization, colorectal cancer is the third most commonly diagnosed cancer (1.8 million cases worldwide in 2018, 10.2% of the total) and the second deadliest (881 000 deaths in 2018, 9.2% of the total). Current approaches to colorectal cancer treatment rely on surgery as the first intervention line, followed by non-specific treatments like chemo and radiotherapy. These treatments result in severe side effects and are often inefficient. Therefore, there is the need to develop novel therapies that will result in a more specific and powerful anti-cancer response with fewer side effects. One of the available options for alternative cancer therapy is the combination of immunotherapy with nanotechnology. In most cancers, the immune response against cancer is suppressed and incapable of efficiently eradicating primary tumors and metastasis.Immunotherapy is emerging as one of the most promising therapies against cancer, since immunostimulating compounds are able activate immune cells and (re)program the patient's immune system to fight

cancer. In addition, the use of drug-engineered nanoparticles allows the specific delivery and controlled release of drugs to tumors, contributing to a precise and targeted therapy. In this scenario we propose the preparation and in vitro validation of nanoparticles that will be designed to specifically deliver immunostimulating drugs (IS) to the tumor microenvironment. This drug delivery system comprises a superparamagnetic iron-oxide multi-core plus a heat-responsive polymeric coating.. The superparamagnetic core plays a dual role: on one hand it enables its longitudinal non-invasive detection and tracking via magnetic resonance imaging, while on the other it will be used as magnetic hyperthermia effector for the production of heat. The second component of the system consist of a temperature sensitive pNIPAM-derivative coupled with immunostimulating drugs. After an increase in temperature, this type of polymers lose up to 90% of their volume and release their cargo, working as an efficient system for on-demand drug release. We expect to develop an efficient and localized nanoparticle-based system to be applied in colorectal cancer theranostics.

B58 TITLE: (Para)magnetic nanocarriers for early detection and treatment of solid tumors

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Abstract: In 2012, there were an estimated 14.1 million new cases of cancer in the world and in 2015 more than 50000 people were diagnosed with a cancer in Portugal. The vast majority of thesecancers appear as solid tumours. Current treatment protocols (surgery, chemo-, radio- and immunotherapy) have proved their utility in the clinic, but they are still far from being the solution to this problem. Therefore, an innovative tool against solid tumours was developed through a combination of early diagnosis and treatment (theranosis). We have recently developed a multifunctional nanocomposite including magnetic nanoparticles (NPs) dispersed in a lipid matrix. Both the magnetic and lipid components of this system combine their properties in a synergic manner to provide a powerful weapon in the fight against cancer. The lipid part of the system can be used to encapsulate chemotherapeutic agents. This encapsulation has a double effect on the outcome of the treatment, on one hand increases the local concentration of the drug in the cells, and on the other protects healthy tissues from the deleterious effect of the drug, reducing then side effects. The magnetic part brings also interesting capabilities to the system. Magnetic nanoparticles (MNPs) have been translated to the clinics as magnetic resonance imaging (MRI) contrast agents (CAs) and thus can be used for the early non-invasive detection of tumours. MNPs can as well be used to generate heat under alternating magnetic fields. This heat can be used for the direct ablation of tumours through hyperthermia, and/or for the externally controlled delivery of drugs. This work aims

at extending the capabilities of this system by incorporating also paramagnetic nanoparticles (MnxOy) with MRI and therapeutic abilities. Overall, this study consists in the creation of a nanocomposite containing a chemotherapeutic drug, doxorubicin, and a (para)magnetic core made of magnetite (Fe3O4) NPs and manganese oxide (MnO) NPs. This nanocomposite was characterized in terms of physico-chemical and functional properties, with a focus in its performance as an MRI contrast enhancer, MH effector and controlled drug delivery system. Further work will involve in vitro experiments to validate this (para)magnetic nanocomposite in different cell lines.

B59 TITLE: Magnetic Hybrid Nanocomposites a platform for cancer theranostics

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Abstract: Early diagnosis and management of cancer still remain an overwhelming challenge. Every year almost 10 million people worldwide die of cancer, and it is estimated that this number will grow annually; thus an early-diagnosis combined with effective treatment strategies is an imperative need. Current drug treatments against cancer are effective but not selective: they are able to kill not only cancer cells, but also damage healthy tissue and cause unwanted, and sometimes severe side effects. This implies a reduction of the overall therapeutic potential of such drugs. Recently, organic-inorganic hybrid nanocomposites (NCs) are being designed to synergistically combine the drug encapsulation/release capability of the organic matrix with the intrinsic physico-chemical properties coming from the inorganic component2,3. These NCs have emerged as an ideal platform aimed at overcoming the multiple barriers found in cancer treatment and advance towards a more efficient targeted diagnostic and therapeutic solution. In this work, we present the preparation of a drug-loaded magnetic hybrid nanocomposite (mNC)4,5 by a simple, versatile and scalable melt-emulsification method. Doxorubicin(DOX)-loaded magnetic hybrid nanocomposites (mNCs-DOX) have been developed and fully characterized, showing excellent features as T2-contrast agents in magnetic resonance imaging (MRI), good heating properties in magnetic hyperthermia (MH) and good performance as magnetically responsive drug delivery vehicles. mNCs-DOX have also been pre-clinically validated through in vitro and in vivo studies, suggesting a synergistic effect between heat generation and controlled DOX delivery over cancer cell growth and offering non-invasive imaging capability. This approach represents a starting point towards the design and development of an optimized second generation of drug-loaded mNC formulations able to respond to endogenous and exogenous stimuli (changes

in pH, temperature and others), which are expected to further enhance selectivity towards cancerous tissue.

B60 TITLE: Towards a standardised workflow for the characterisation of nanoparticle bioconjugates

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Abstract: The fast development of nanotechnology-based solutions in the biomedical field at a preclinical level is not always translated into its implementation in the clinics. There are a number of reasons that help to understand the different relative speeds of research vs translation, from technological, to regulatory and economic. The knowledge on nanoparticle synthesis and functionalisation keeps growing and continuously more complex nanostructures are produced. Characterisation procedures for the description of these structures have been lagging behind the fast development of synthetic nanomedicine, contributing somehow to the dysfunctional translation process. A deep understanding of the nature of complex nanostructures through an exhaustive characterisation process would help alleviate and smooth some of the concerns commonly raised against nanotechnology, e.g. safety or reproducibility. The H2020 project PANA aims at developing a nanoplatform for the early diagnosis and treatment of Alzheimer's disease. Within the consortium two groups are devoted to the preparation and bio-functionalisation of nanoparticles (mainly magnetic). This framework provided a perfect opportunity for the development of a standardised protocol for the characterisation of complex nanoparticles bio-conjugates. A wide range of technologies were considered for the full description of the properties of the nano-bio-conjugates. Considerations such as time demand, and cost were used to pinpoint techniques suitable as screening options together with strong scientific criteria to decide whether a sample was worth to move to further stages of characterisation/development. Overall, the physico-chemical, structural, magnetic and functional properties were characterised and several decision checkpoints were implemented to ensure an optimised used of resources.

B61 TITLE: Graphene-based magnetic nanoparticles as smart multifunctional theranostic nanosystems

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Abstract: One of the ultimate goals of nanomedicine is to exploit smart nanosystems potentiality to produce personalized diagnostic and therapeutic strategies by using local environment of living hosts to drive material behavior and thus, improve medicine and human health. In this work, graphene-based magnetic nanoparticles (GbMNPs) where developed and fully characterized (i.e., physicochemical, magnetic and biological) seeking to combine the excellent magnetic performance of magnetite nanoparticles with the unique properties of graphene-based materials, such as high chemical and thermal stability, high charge carrier mobility and large surface area for functionalization. These magnetic nanocomposites have the potentiality to represent a new clinical platform that fulfils all the requirements for theranostic applications (i.e., diagnosis and therapy). One of the most interesting possibilities is the use of GbMNPs as pH stimuli-responsive controlled drug release systems, triggered by the abnormal acidic pH values (around 4.5 – 5.5) that are found in tumor endosome/lysosome microenvironments combined with magnetic hyperthermia (MH) and MRI contrast enhancement capabilities. [2]. On one hand, the superparamagnetic core of these nanocomposites allows heat generation in the tumor under an alternating magnetic field that induces cancer cells death, and can simultaneously boost the drug release on those abnormal tissues. Additionally, the unique sp2 carbon structure with #-# stacking and negative surface charge, enables the adsorption of a variety of molecules, e.g., chemotherapeutic drugs, DNA and/or RNA, which can be specifically accumulated into tumors, and be released by deprotonation in the presence of acidic tumor sites. On the other hand, the magnetic interaction between the superparamagnetic magnetite core and the nuclear magnetic moments of water protons induces a T2-MRI contrast enhancement (T2 shortening – dark effect). In this work, we will show details on the synthesis and in-depth characterization of the GbMNPs, as well as preliminary results on the nanomaterial-cell interaction. The proposed strategy can represent a new way to design and synthesize stable graphene-based materials with novel structures for targeted imaging and combinatorial thermo-chemotherapy for cancer theranostics.

B62 TITLE: Therapy based on lipid nanoparticles against lung melanoma metastasis in murine models

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Abstract: According to recent studies, lung cancer is the most common cancer leading with an incidence of 1,378,400 deaths per year worldwide with an overall 5-year survival rate of 15 %. Despite clinical progress and new drug discoveries, the incidence of cancer mortality is still highly. However, due to the deficiency in early-stage diagnostics, most lung cancers are only detected at advanced stages, once metastasis have produced and are not suitable for surgery. Lung's metastasis is almost incurable owing to drug resistance. The therapy of metastatic lung cancer remains poor due to limited targeting efficiency to lung tumor cells and a low selectivity of tumor cells against normal cells. For this reason, lung metastasis is one of the diseases that demands alternative therapies using drug delivery system. In this work, we propose the nanoencapsulation of doxorubicin in solid lipid nanoparticles as an alternative treatment. Here we have transplanted melanoma cell to mice and have treated these for 20 days with NPs loaded with Doxorubicin. Our results demonstrate how the encapsulated drug decreases the amount of metastatic spots in the lung compared to control lungs, both untreated or treated systemically with doxorubicin in solution. These results suggest that the solid lipid nanoparticles encapsulated drug is more effective than conventional drug. In addition, the number of deaths in mice reduced in ca. 10% compared to mice treated with NPs with doxorubicin against mice treated with free doxorubicin. In conclusion, these experiments show how doxorubicin encapsulated in solid lipid nanoparticles are potentially useful in the treatment of lung's metastasis in mice, improving the antitumoral effect and cytotoxicity of doxorubicin while decreasing the systemic toxic effects.

B63 TITLE: Plasmonic biomimetic nanosystems

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Abstract: Engineered materials in the nanoscale are revolutionizing all areas of life science, thanks to their novel physicochemical properties. However, there are many drawbacks to be considered when designing functional nanomaterials that act when inside a biological system. The development of stimuli-responsive nanoformulations based on biomimetic materials, mimicking natural cellular structures, offers a universal tool that will allow developing a novel class of a versatile drug delivery carriers for potential application in the treatment of different diseases by selecting the specific cargo and a biomimetic coating capable of achieving in vivo targeting by evading the host defense. This work is based on designing novel biomimetic nanostructures which combine cell membrane components and inorganic nanoparticles (NPs) to create functional nano bio-inorganic assemblies with physical (e.g., plasmonic gold nanorods) and biomimetic capabilities. The photothermal heating of plasmonic NPs under resonant illumination has been used to trigger payload release from the NP-modified nano-cellular assemblies. The study of controlled delivery of the cargo by the response external to stimuli (e.g., light) is carried out inside living cells using a near-IR laser illumination system.

B64 TITLE: Nanobody-conjugated iron oxide nanoparticle for targeting applications

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Abstract: Iron oxide nanoparticles (IONPs) are known as one of the most promising innovations in the biomedical sector and applications include: medical imaging, hyperthermia treatment and drug delivery. Targeted delivery can be achieved by functionalizing the IONPs with targeting agents, which can specifically bind to biomarkers. Antibody-conjugated nanoparticles have been a hot topic over the last few years [4]. Recently, also the nanobodies seem very promising as they have a few additional advantages. The smaller size, monomeric structure and higher stability make these more useful than antibodies for biomedical applications. This work shows a new method for nanoparticle functionalization, and nanobody coupling. Monodisperse IONPs were synthesized by a thermal decomposition method. [5] The particles were functionalized with polyethylene glycol (PEG) polymers using a carboxy-silane condensation to increase the hydrophilicity. A bifunctional coating is

produced by using different PEG polymers, of which 10% have an alkene end-group and 90% have an alcohol end-group. The nanobodies are coupled to the alkene groups using a thiol-ene click reaction under UV-radiation. The nanoconjugates are physiochemically characterized using several techniques, such as: Transmission Electron Microscopy (TEM), Thermogravimetric Assay (TGA), Fourier Transform Infrared spectroscopy (FT-IR), Iron determination, Bicinchoninic Acid assay (BCA), and Cell Flow Cytometry. Bifunctional water-dispersible IONPs were synthesized to allow for coupling reactions with nanobodies. The TEM images display monodisperse nanocrystals with a spherical morphology and an average diameter of 12.9 ± 1.8 nm. The TGA measurements show a weight loss starting from 300°C attributed to the silane ligands. The FT-IR spectra demonstrate the presence of the different coatings as the vibrational bands match the functional groups in the coatings, as well as the Fe-O bonds. Furthermore, iron determination allows for calculation of the iron concentration, and the BCA assay is used for protein determination of the samples. Combining iron determination and BCA experiments we are able to estimate the amount of nanobody per particle. We have an average protein concentration of 14.6 µg/mL, which corresponds with an average of 1.6 nanobodies per magnetic core. Lastly, the activity of the nanobodies after coupling can be tested using cell flow cytometry. In vitro and in vivo are anticipated for the near future.

B65 TITLE: A bionanohybrid for drug delivery: Tobacco Mosaic Virus functionalized mesoporous nanoparticles

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Abstract: Biological and inorganic nanocarrier drug delivery systems are being

explored, each of which have their advantages and limitations in terms of cell targeting and specificity, cell internalization, efficient payload delivery, and safety profiles1,2. Combining a biological coating on top of an inorganic carrier, we hypothesized that this hybrid system would increase colloidal stability resulting in enhanced cell targeting and uptake properties compared to the bare inorganic nanocarrier. Then, we engineered a hierarchical assembly featuring the functionalization of cargo-loaded mesoporous silica nanoparticles (MSNP) with Tobacco Mosaic Virus (TMV). The MSNP provides the delivery system, as the porous structure enables high therapeutic payload capacity; and TMV serves as biocompatible coating enhancing cell interactions. The resulting MSNP@TMV nanohybrid has a wool ball-like appearance and demonstrate enhanced cell uptake, hence cargo releaseproperties. The MSNP@TMV has potential for medical applications, such as cancer drug delivery, contrast agent imaging, or immunotherapy.

B66 TITLE: Rheological Characterization of Magnetic Hydrogels as Composite Materials for Biomedical Applications

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Abstract: Currently, much research is devoted to the development of nanocomposite materials that combine the properties of polymeric matrices with those of inorganic nanoparticles, in particular magnetic nanoparticles. In particular, magnetic iron oxide nanoparticles (NPs) have become key elements in the design of functional nanostructures able to play an active role in different biomedical applications. One example is the increasing interest they are gaining to formulate magnetic nanocomposites as advanced drug vehicles for targeted and controlled release. The present work mainly focuses on the rheological characterization of a magnetic nanocomposite able to encapsulate and delivery therapeutic molecules for topical applications. The interaction with an external magnetic field will provide the material with additional functionality and will enable extra diagnostic/monitoring and therapy capabilities, such as magnetic resonance imaging and magnetic hyperthermia.
The magnetic nanocomposite consists of a biocompatible biopolymer-based hydrogel containing iron oxide (magnetite) nanoparticles. Physicochemical properties of the designed formulations have been evaluated by rheological analysis, where the flow curves and viscoelastic properties were studied. Flow analysis demonstrated that both native and cross-linked biopolymer hydrogels exhibited a shear-thinning behavior, whereas those produced through a cross-linking strategy led to a more

rigid structure compared with native formulations, which behave like a pseudo-gel structure. In addition, flow curves showed that magnetic nanoparticles have a much lower viscosity than hydrogels, whereas viscoelasticity measurements revealed a clear difference between hydrogels with and without nanoparticles. These results indicated that magnetic biomaterials obtained have rheological properties of interest for pharmaceutical and biomedical applications.

B67 TITLE: Formulation and characterization of theranostic magnetopolimeric nanoparticles

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Abstract: Colloids based on biodegradable polymeric nanoparticles (NPs) like poly(ethylcyanoacrylate) (PECA) exhibit characteristics that make them ideal as delivery systems. The inclusion of magnetic nuclei into the polymeric matrix represents an improvement in NP design. This additional magnetic functionality provides the nanocomposite with magnetic guiding properties and tissue targeting, as well as it enables medical applications such as magnetic hyperthermia (MH) and magnetic resonance imaging (MRI). The objective of this work is to formulate a theranostic polymeric nanocarrier that combines the drug encapsulation capability of the polymeric shell and the magnetic functionality of the magnetic nanoparticles (MNPs), aiming at clinical applications such as noninvasive imaging by MRI and targeted MH therapy. Special focus of this contribution has been paid on the synthesis, characterization and magnetic functionality validation of the designed nanocomposite. Maghemite cores were prepared by oxidation of magnetite particles obtained by chemical co-precipitation. Final core@shell nanocomposites were formulated through the emulsion polymerization procedure. An optimal particle size suitable for parenteral administration was obtained by adjusting the maghemite:PECA ratio to 2:4. Morphology and structure of the nancomposites were studied by TEM, whereas physicochemical properties such as hydrodynamic size, surface electrical charge, and colloidal stability were determined by DLS and #-potential. Moreover, #-pot and contact angle determinations pointed to a reproducible coverage of the magnetic nuclei by the PECA shell, since both surface electrical properties and

surface thermodynamics of the maghemite cores were found to be quite similar to those of the pure PECA NPs. In terms of application, MH measurements of the nanocomposite aqueous dispersions as a function of the frequency and magnitude of the alternating magnetic field were performed that indicated a MNPs-concentration dependent heating effect, producing temperature increases of up to 18,2 °C in 15 min at iron concentrations below 2 mg/mL.. Additionally, preliminary results will be also shown on the ability of the designed magnetic formulations to induce a T2-MRI contrast enhancement at clinical field of 3T. Finally, the nanomaterial-cell interaction will be studied in vitro through cytotoxicity assays and preliminary results will be shown in both healthy and tumor cells.

B68 TITLE: Key parameters controlling the bio/nano interface: thermodynamic characteristics of the nanoparticles/protein interaction

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Abstract: The study of the interaction of nanoparticles (NPs) with biological system (bio-nano interaction) is of significant importance for nanomedicine, as well as for nanosafety research, helping to increase effectiveness and safety use of nanostructured systems. The interaction of NPs with the biological medium modulates the surface of the nanoparticles, conferring a "biological identity" to their surfaces (referred to as "biomolecule corona"), which determines the subsequent cellular/tissue responses. The identification of energetic parameters driving the interactions between manufactured nanomaterials (MNMs) and proteins is essential for understanding the dominant contributions determining adsorption processes and the biological responses. In the present work, the complex phenomena occurring at the bio/nano interface are evaluated by assessing the thermodynamic signature associated with the interaction of five variants of titanium dioxide MNMs with one of the most abundant plasma protein, e.g. BSA (Bovine serum albumin). The NMs used in these investigations are representative nanomaterials from the JRC Repository [2] having different particle size and crystalline structures. The binding characteristics for protein-NPs systems represented by the binding constant, binding stoichiometry, enthalpy, entropy and Gibbs free energy changes were obtained by means Isothermal Titration Calorimetry. The effect of NPs on the protein stability was investigated by measuring the thermodynamic parameters for the protein denaturation (denaturation temperature, heat capacity, enthalpy, entropy and free energy changes). The information generated by the calorimetric investigation evidenced the correlation

SECTION C | *Organ-on-a-chip and Biomimetic Systems*

C69 TITLE: Cancer single-cell encapsulation and proliferation monitoring in microdroplets

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Abstract: The prevalence of cancer as a leading cause of death worldwide continues to weigh heavily on society. In 2018, nearly 10 million people died due to cancer, being the second leading cause of death globally. Circulating Tumor Cells (CTCs) are cancer cells that split from existing tumors in the body and start to travel to surrounding tissues,] and thus can give a faithful insight into a patient's stage of cancer development, since their expression is associated with the appearance of advanced tumors. However, because of their very short lifespan (with a half-life of 1 to 2.4 hours) and the difficulty to isolate CTCs from blood samples, there is a severe lack of information about their different genotypes and phenotypes, which could help understand their selective pathways of seeding into secondary organs.Nowadays, the current methods for unraveling the mechanisms behind cancer cell proliferation still rely heavily on 2D techniques, which do not replicate in detail the intricacies of formation of 3D spheroids, some of the most specialized structures of cancerous cells that can emulate in vivo characteristics of tumors.There have been numerous approaches to generate spheroids in laboratories, from 96-well plates to hanging drop cultures. However, in order to obtain conclusive results that can give a much-guided insight into the complex dynamics of cancer cell proliferation, a method to isolate single cancer cells in a simulated and controlled 3D-environment is required. Achieving this goal would allow researchers to predict the growth rates of specific tumors and their expansion potential. Microdroplets is a branch of microfluidics that enables high throughout encapsulation of single-cells for functional studies and analysis of cell behavior. This technology has the potential to advance the study of cancer cell growth dynamics from single-cells to spheroids, due to their ability to create an isolated 3D environment. To that end, this project intends to develop a microfluidic system that can enable the growth and proliferation of single cancer cells in the isolated system of a microdroplet. In this way, we aim to observe how cells with specific phenotypes proliferate and relate the findings to the known mechanisms of metastasis. Ultimately, this project can aid the understanding of cancer invasiveness

and help in patient stratification and personalized treatment.

C70 TITLE: A novel human stratum corneum mimetic model, as a tool in the optimization of skin drug formulations

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Abstract: Transdermal delivery represents a very attractive administration route that provides various advantages over other methods of administration, including enhanced patient compliance via noninvasive, painless, simple application and reduced side effects. Thereby, the research on suitable drugs for this route continues to increase. Yet, alternatives to animals and human skin are impelled by economic and ethical reasons. In this context, the main goals of this study are to develop and characterize a stratum corneum model that mimics this human skin layer, inspired on a phospholipid vesicle-based permeation assay (PVPA), and to validate it using skin drug formulations.To mimic the human stratum corneum layer, the phospholipid vesicles were prepared with a selected lipid composition, which closely corresponds to the main human lipid classes on this skin layer. The design of the developed model was optimized using dynamic light scattering, phospholipid quantification and scanning electron microscopy images. To compare the stratum corneum model developed with the porcine skin model, the storage stability was assessed as well the calcein permeation in the presence of several conditions: a pH range, co-solvents and drugs. The established stratum corneum model could be stored at -20°C for up to 2 weeks without significant changes, was stable within the pH range from 2.0 to 8.0 and with the addition of co-solvents (DMSO, cremophor® and oleic acid). The human mimicking stratum corneum PVPA model was able to detect calcein permeability differences in the presence of different drugs commonly used in the therapy of skin-related diseases. The obtained data correlated well with the well accepted pig ear model, which highlights the potential of this new human stratum corneum model. Hence, this model can thereby constitute a valuable tool to improve the process of transdermal drugs development, as it may reduce the duration and the economic costs associated, and even replace the animal testing, during early stages of drug development.

C71 TITLE: MyoChip: building an innervated and irrigated muscle-on-chip

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Abstract: Bioengineered muscles aim to reproduce contractile function of skeletal muscle in vitro, but often fail to fully mimic architecture as well as microenvironment of the native tissue. The MyoChip project aims to overcome these limitations by creating a muscle model that contains myofibers irrigated by microvasculature and innervated by neurons. The approach relies on a biochip platform, which provides appropriate biochemical and biomechanical cues that guide the differentiation of hiPSC into the three respective tissues.
During the initial stage of the MyoChip project, distinct microfabrication-based techniques were established in order to (i) generate uniformly aligned and fully differentiated myofibers and (ii) test various conditions that allow co-culture of muscle with endothelial cells. A qualitative validation of the structural development and contractile function of the bioartifical muscle was based on fluorescent microscopy.
Ultimately, the MyoChip project aims at generating a muscle-on-chip platform that reproduces the architecture and contractility of in vivo skeletal muscle. This physiological relevant organ-on-chip technology will have numerous applications in research of myogenesis, muscle building and aging, drug testing and screening, prosthetics and biorobotics.

SECTION C | *Nanobiosensing for Personalised Medicine*

C72 TITLE: Electrochemical based micro-reactor for monitoring of 3D cellular Microenvironment

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Abstract: The tumor microenvironment having the attention of oncologist and researchers, due to the relevant contribution of the non-cellular components to the mechanisms underpinning cancer progression, drug response and chemo-resistance. The use of 2D (monolayers) models are far away from the real tissue model. Standard 2D cell cultures approach are not a real imitator the multicellular organization of solid tumor tissues and usually limited with important contribution of extracellular matrix (ECM). The limitations of 2D planar cultures systems led to the development of 3D tumor models. The development of 3D tumor models offers several advantages as: 3D growth, spatial structure and organization, which mimics the native ECM, and drug sensitivity testing for personalized cancer therapy, among others The sensing

under the cell culture environment is very crucial for the development of successful 3D model system. Herein, we fabricated a low-cost microfluidic microreactor with the objective of a functional 3D cell culture model system with integrated electrochemical sensing of cell culture microenvironment. The system is based on microfabricated device containing a 3D scaffold (Gellun gum hydrogel) for supporting cell culture inside the device. The sensing micro-reactor is fabricated in Poly-methyl methacrylate (PMMA) material, suitable for micro-reactor due to its high strength, excellent optical property and low cost. The microdevice is combined with an optical window for inspection of the cells during measurement. The design of the device allowing cell growth with continuous supply of the fresh cell media. A custom developed inlet and outlet system works within an incubator. The presented data demonstrates the application of the micro-reactor with breast cancer (MCF-7) cells. A cyclic voltammetry is applied to monitor cellular metabolic activity, cellular acidification was accessed with potentiometric pH sensors using gold and platinum electrodes. The system itself provides the foundation for electrochemical monitoring systems in 3D cell culture diagnostics. We observe cell viability for day 5 and 10 day and 15 day, when MCF-7 are cultured in dynamic condition on scaffold. The pH was monitored over the time and changes in the pH were observed inside the micro-reactor over the time.

C73 TITLE: A microfluidics platform for the SERS-based detection of acute myeloid leukemia

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Abstract: Acute myeloid leukemia (AML) is the most common form of acute leukemia in adults and is frequently associated with poor outcomes to conventional therapies in the most affected population (median age of 67 years). After treatment with chemotherapy, even patients that clinically achieve complete remission (CR) can relapse through the persistence of minimal residual disease (MRD), with fatal consequences. Current diagnostic tools, based on bone marrow biopsy and flow cytometry, demonstrate early detection of the disease but have low sensitivity and are also highly invasive, painful and costly. Accurate and early diagnosis of MRD would allow the application of appropriate therapy, improving the prognosis of patients. Nanotechnology, surface-enhanced Raman scattering (SERS) spectroscopy and microfluidics are some of the key enabling technologies (KETs) that have remarkably flourished in the recent years and demonstrated to be powerful tools for cancer

diagnosis. The main goal of this work is to create a non-invasive diagnostic tool for the early and non-invasive detection of MRD in AML. For this purpose we designed, fabricated, tested and optimised a microfluidic SERS-based sensor for high efficiency isolation and analysis of leukemic blasts. First, SERS tags for cell recognition were synthesised using gold nanostars (AuNSTs), which offer an extra enhancement of the Raman signal. These AuNSTs were synthesised by following conventional wet chemistry protocols with slight modifications and codified with Raman reporters (RaRs) for indirect detection. Afterwards, a protective silica coating layer was deposited over the surface of the AuNSTs@RaR to prevent leakage of the RaRs and facilitate the bioconjugation with the membrane receptor antibodies. Figure 1 shows the optical, morphological and SERS properties of these engineered SERS tags. In parallel, microfluidic devices for the isolation and concentration of leukemic blasts from peripheral blood, will be done in order to apply the SERS tags for the analysis of the expression of blasts in the circulation of AML patients. The precise sample handling of microfluidics combined with the multiplex capacity and high sensitivity of SERS may pave the way to render a powerful tool for early detection of MRD in AML.

C74 TITLE: Validation of a microfluidic device for the isolation and characterization of circulating tumor cells towards cancer progression monitoring in metastatic breast cancer

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Abstract: Breast cancer is the second most common cancer in the world and one of the leading causes of cancer-related mortality in women. The underlying cause of morbidity and cancer-related mortality is cancer metastasis. To improve clinical diagnostic and therapeutic decisions, it is necessary to develop new blood-based biomarker detection strategies. Circulating tumor cells (CTCs) escape the primary tumor and disseminate through the bloodstream, exhibiting great metastatic potential]. The study of CTCs contained in body fluids gained great importance in cancer research, as they represent a real-time snapshot of the tumor burden and offer unique opportunity for minimally invasive sampling in cancer patients. The study main aim was to perform a head to head comparison of a microfluidic device (RUBYchip®,

PCT/EP2016/078406) designed for CTC capture based on cell size and deformability, against the only FDA-approved technology, CellSearch®, for CTC enumeration. For this purpose, whole blood samples from metastatic breast cancer patients diagnosed at the Hospital de Santa Maria, Lisbon, were collected at baseline (before starting systemic treatment) and at monitoring follow-up (~12 weeks of ongoing treatment). Prior to patient sample analysis, and to optimize the performance of the RUBYchip®, three different breast cancer cell lines, MCF-7, MDA-MB 435 and SKBR3 were used to spike 7.5mL whole blood samples from healthy volunteers. The spiked samples were introduced in the system at different flow rates and the isolation efficiency assessed. Double amount of blood specimens were collected from each patient in order to process the samples with both technologies in parallel. CellSearch® analysis was performed in the Liquid biopsy analysis unit of the Health Research Institute of Santiago (IDIS). Further to isolation, identification and phenotypical analysis of CTCs in the RUBYchip® was achieved by immunostaining with antibodies against cytokeratin, HER2 and CD45. Results can elucidate and identify CTC subpopulations which could be differentially associated with patients' clinical outcome. Comparative testing of these technologies allows to understand the prognostic value of this highly sensitive and standardized approach for CTC isolation and phenotypic characterization in peripheral blood of breast cancer patients. Implementation of CTCs as a routine tool in the clinical management of metastatic breast cancer is expected to assist in the monitoring of disease progression, improve patient stratification and prognosis.

C75 TITLE: MicroRNA sensors based on functionalized gold nanoparticles

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Abstract: MicroRNAs (miRNAs) are small regulatory RNAs, where their dysregulation has been associated with the progression of several human diseases, including cancer. These molecules can be used as biomarkers for early disease diagnosis and are stable in a variety of body fluids and tissue samples, allowing for their detection using non-invasive approaches. Currently established detection methods, however, are complex, costly, require specialized personnel and sophisticated equipment, limiting their application in point-of-care settings or resource-limited facilities. Recently, approaches based on nanotechnology, in particular gold nanoparticles (AuNPs), have emerged as promising alternatives. In this work, the unique optical properties of AuNPs are explored to develop both colorimetric and lateral flow-based sensors for miRNA detection. Such systems should allow simple, fast and low-cost detection, being suitable for handling by patients or non-specialized professionals. For colorimetric sensors, we propose the use of 10-13 nm AuNPs functionalized with

probe oligonucleotides bearing a cholesterol moiety. In our system, a thiol group is placed at one end of the oligonucleotide, to ease the conjugation with AuNPs, and a cholesterol derivative is placed at the other, to achieve target-driven modulation of the colloidal stability of the nanostructures, leading to color changes in solution. For lateral flow-based ones, the same AuNPs are functionalized with oligonucleotides bearing a thiol group in one end and a biotin derivative in the other. Target-driven exposure of the biotin on the surface of the AuNPs leads to their retention in nitrocellulose paper strips with a streptavidin line. We apply these systems in the diagnosis of uveal melanoma and Duchenne's muscular dystrophy, two rare but lethal diseases in which early and accurate diagnosis is essential. We have selected miRNAs that have been proposed as early blood biomarkers of such diseases. Our systems have shown good sensitivity (nmol to pmol range) and selectivity in vitro, allowing specific detection of target sequences with the naked eye in few hours or minutes. We are currently developing signal amplification methods to further improve the sensitivity and selectivity of the sensing systems, and detecting overexpressed miRNAs in cell extracts and biological fluid samples.

C76 TITLE: Development of a microdroplet-based optofluidics platform for the analysis of single cancer cells

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Abstract: In the context of personalised medicine, the analysis of single-cells is key in order to understand the origin and evolution of cancer towards providing an accurate prognosis. Microfluidics and microdroplets offer a perfect isolation environment for the study of single-cells.However, due to the small volumes handled in microfluidic devices, it is necessary to couple this technology to an ultrasensitive detection technique. The combination of surface-enhanced Raman scattering (SERS)spectroscopy with microfluidics offers a great potential for the development of automated and sensitive diagnostic platforms. Herein, SERS and droplet microfluidics were combined towards the analysis of single cancer cells. Initially, a set of microdroplet devices allowing droplet generation (D), single-cell encapsulation and storage (E) were designed and optimised to achieve a generation of droplets having 80 µm of diameter. The capability of these devices as SERS sensing platforms

(F) was tested using gold nanostars, which were codified with Raman reporters and functionalised with antibodies. Following, single cancer cells were encapsulated already immune-conjugated with the nanoparticles recognising specific membrane receptors expressed at those cancer cells. More specifically, for the proof-of-concept of this technology, EpCAM was used for the targeting of MDA-MB435 cancer cell line. Finally, encapsulated cells were characterised inside the microfluidics platform using Raman spectroscopy. As a result, it was possible to identify the EpCAM expression on the MDA-MB-435. In this way, a phenotypic study at the single cell level was made for a breast cancer model line. Finally, the high throughput of microdroplet technology and the multiplex ability of SERS will give access to fast and efficient single cell phenotyping. Ultimately, the development of these platforms intends to pave the way towards a more personalised handling of cancer diagnosis and monitoring, in a reproducible, automated and fast way.

C77 TITLE: Multiplex label-free biosensor based on DNA for monitoring diseases

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Abstract: Portable and rapid sensing platforms compatible with low-resource settings are great tools to assist health care personnel with disease diagnosis and selection of suitable treatments. DNA based biosensors have been increasingly used in medical diagnostics to continuously monitor health markers. The availability of these techniques increases the possibility for personalized medicine development, with especially regard to chronic, genetic and immune deficiency related diseases. DNA-based biosensors may also assist on early detection and accurate diagnosis which generally involves high sensitivity detection of multiple analytes from the same sample. Here, we present a multiplex label-free biosensor based on single-stranded DNA probes immobilized on a graphene sensor surface which measures the hybridization between target DNA strands and their complementary DNA probes. The fabricated graphene sensors are based on an electrolyte-gated field-effect transistor array, with a receded, integrated gate architecture. To read the biological events the chip with multiple graphene sensors is plugged in a home-made electronic readout platform, with the size of a credit card, to ensure portability. For the DNA detection, first the graphene transistor channel is modified with PBSE linker through π-π stacking interactions. The introduced succinimidyl ester groups are then available to react with the amine modified DNA probe sequences of 30 nucleotides long (10 μM in 10

mM Phosphate buffer, pH 7.4) to form stable amide bonds. To block the unreacted NHS-ester ligands of PBSE ethanolamine was added to the surface to prevent non-specific binding. Amine modified DNA probes immobilized on the graphene surface are fully complementary to the DNA target. Each modification step was monitored by measuring 10 transfer curves and, from the last 3 curves, computing the average of the gate voltage values at which maximal channel resistance is observed (VDirac). Preliminary results show that DNA hybridization events upon increasing DNA target concentration from 1 fM to 1 uM induce a VDirac shift to higher values and sensor linearity was obtain for the range between 1nM to 1μM. These results suggest that DNA-based biosensors could be applied for the simple, fast and point-of-care detection of cancer, genetic and infectious diseases allowing personalize medicine.

C78 TITLE: Biosensor based DNA for Dry eye detection

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Abstract: Dry eye disease (DED) is a disorder of the tear film due to excessive tear evaporation or tear deficiency, resulting in inflammation to the ocular surface which lead to dryness, ocular surface discomfort, light sensitivity, blurred vision and other uncomfortable symptoms. The major challenge on DED diagnosis is related to the absence of symptoms in 40% of the patients and/or lack of correlation between signs and symptoms. The patients have their visual functions compromise and the high impact of DED symptoms interfere with daily activities affecting their quality of life. The current diagnose is based on the symptomatology of the patients and techniques of evaluation of the tear film which are poorly standardized. Here, we developed a new strategy to diagnose dry eye disease based on the detection of diadenosine tetraphosphate (Ap4A), a biomarker molecule that is abnormally elevated in tears of patients diagnosed with DED, allowing the diagnosis with a sensitivity of 74% and a specificity of 96%. We purposed a biosensor based on gold nanoparticles (GNP) conjugated with a small peptide for colorimetric detection of Ap4A. GNP were functionalized with peptides acting as recognition elements followed by the specific interaction with Ap4A. The GNP conjugated with the small peptide were stable and disperse in solution presenting a red colour which upon Ap4A, increase GNP affinity and consequent aggregation inducing a colour change to purple. The interaction between the peptide and the Ap4A was evaluated with circular dichroism (CD) spectroscopy. The increasing concentration of Ap4A induced an increase in stability of the peptide indicating the specific interaction. Spherical GNP were synthesized and characterized by ultraviolet-visible spectroscopy and dynamic light scattering

(DLS). These GNP showed a maximum extinction of 526.41 ± 0.03 nm (red color) and a diameter between 30 nm. Upon characterization, the GNP surface was loaded with peptide though a sulfur-gold bond of the cysteine residue present at one end of the peptide. Peptide loading caused an increase of 3.87 ± 0.79 nm of the particle average size determined by DLS. At this stage GNP/peptide functionalized nanoparticles were red, which would only turn purple in the presence of Ap4A due to the aggregation of the GNP. The specificity of the system, based on peptide and Ap4A interaction, will allow the development of a colorimetric/naked eye system for dry eye diagnosis.

C79 TITLE: Picking up one thing or two about neurodegenerative disorders: probing the nanomechanics of biological systems with the AFM

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Abstract: Since its invention, the atomic force microscope (AFM) has offered unprecedented ways to investigate biological systems, complementing other existent microscopy techniques with its unique ability to image at high resolution and in physiological conditions. Lately, force measurements have supplemented AFM data with some innovative functional meaning that can shed light in molecular and cellular processes. Neurodegenerative diseases are complex multi-factor diseases: they are linked with some aberrant protein behavior, but also a series of alterations in the physiology of cells in the CNS accompany the disease and have a confirmed but have still unclear relationships with the pathologic protein aggregation. In the last years, we have used the AFM to investigate some aspects of neurodegenerative diseases. Single-molecule force spectroscopy allowed us to investigate the conformational equilibria of the prion protein and to try to simulate the initial events of protein aggregation. The ability of the AFM in measuring local mechanical properties makes it useful towards achieving new types of characterizations for live cells too. For example, invasive tumor cells have been found to have different mechanics that can enable them to infiltrate other tissues. Recently, we have performed a nanomechanical analysis of microglial cells. These are cells making the resident immune system in the brain, with the task of protecting neurons. Most neurodegenerative diseases are accompanied by neuroinflammation: microglial cells can become chronically activated and turn into neurotoxic rather than neuroprotective. We have recently shown that the morphological changes that are one of the signs of microglial activation also imply a change in the mechanical properties of these cells.

C80 TITLE: INTERACTION OF DNA SENSOR AND PHOTONICITY FOR EARLY DETECTION OF DIABETES IN BLOOD AND SALIVA

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Abstract: Molecules such as glucose, proteins, and other compounds can be ideal markers for detecting diseases such as Alzheimer's and diabetes due to their inherent biophotonic characteristics. However, the nature of the source of the sample (e.g., blood or saliva) could introduce interference since it might share similar photochemical expressions under the same wavelength. Hence, we have developed a DNA sensor using synthetic biology as well as taking advantage of the inherent biophotonicity of molecules such as DNA, proteins, and glucose in order to detect diabetes as well as predict the onset of diabetes. The DNA sensor was constructed either in bacteria or yeast using natural and/or synthetic sequences.The efficacy of the DNA sensor was tested based on fluorescence intensity when mixed with human blood plasma or saliva using a fluorescence detector at different wavelengths. The intensity of the fluorescence was correlated to conventional clinical glycemia levels from patient blood or saliva samples. Predicting degenerative diseases such as diabetes has always been a challenge due to many noncontrolled variables. In this study, the use of statistical analysis coupled with computational modeling can assist in the analysis of the experimental results. While conventional statistical analysis can predict the results of related variables, computational modeling can complement statistical results by comparing nonrelated variables. Thus, in our investigation, we were able to specifically identify, categorize, and quantify the influence of different factors such as sample type (e.g., blood or saliva), age and gender of patient, and time of sampling. Patients were able to be diagnosed and grouped in different categories based on exact predictions by integrating quantitative and qualitative variables through the neuronal computational modeling. The quantification of fluorescence has greater sensitivity than conventional techniques, which will help diagnose diabetes in patients at the early stages of the disease. Our methodology permitted the grouping of three different categories of patients. For diabetes, patients were divided into diabetic, pre-diabetic, and normal groups. A neuronal network computational modeling was designed for the analysis of the results. This investigation provides a much-needed non-invasive and very sensitive diagnostic method based on fluorescence that can identify the presence of the diabetes faster and earlier.

C81 TITLE: Phenotypic profiling of circulating tumour cells during metastatic disease progression

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Abstract: Metastasis account for 90% of cancer-associated mortality. In the face of remarkable improvements in cancer management, these numbers are revealing of our limited understanding of the pathological mechanisms driving the onset and progression of the metastatic disease. Preventing metastasis formation and targeting existing metastasis are imperative clinical challenges. Circulating tumour cells (CTCs) are tumor-derived components from peripheral blood with high metastatic potential. Their enumeration and phenotypic and genomic assessment enable continuous monitoring of metastatic disease progression. This predictive dynamic biomarker has demonstrated potential in patient stratification, therapeutic strategy, predicting relapse, prognosis, therapy resistance, and pharmacodynamics. Oncodynamics biobanking has an integrative approach to the analysis of cancer progression and relies on a prospective collection of liquid biopsies to evaluate tumor clonal evolution, host immune response, tumor heterogeneity and circulating biomarkers during disease progression under therapy. Oncodynamics Biobank includes patients diagnosed at Oncology Department of Hospital de Santa Maria, Lisbon, with de novo stage IV breast, prostate, colorectal and melanoma, referred by multidisciplinary meetings. Whole blood samples were collected at baseline (before starting systemic treatment) and at each tumor objective evaluation (every 12-16 weeks), until four consecutive progression episodes. Electronic Case report forms include demographic and real time clinical data. CTCs are isolated in a microfluidic device based on cell size and deformability (PCT/EP2016/078406). Further phenotypical analysis of CTCs was achieved by immunostaining with antibodies against cytokeratin (for the detection of epithelial CTCs), vimentin (for the detection of mesenchymal CTCs), CD45 (leukocyte common antigen, as CTC exclusion criteria) and DAPI (for nucleus). Each cell is imaged under fluorescence microscopy for phenotypical analysis. Extraction of DNA and RNA from CTCs is being optimized. This study results inform on the diversity of malignant cell populations that make up primary tumours, elucidate on the heterogeneous populations that drive tumor progression and treatment-induced resistance accountable for poor treatment outcomes. Unravelling driver events during the clinical course of the disease is a step towards precision medicine in oncology.

SECTION C | *Cell Therapies and Regenerative Medicine*

C82 TITLE: Positively charged carbon dots for safe in vivo imaging of mesenchymal stem cells

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Abstract: Stem cell therapy is one of the most rapidly developing fields of tissue regeneration and modern medicine.The homing ability of human mesenchymal stromal cells (MSCs) to migrate to the tumor or inflammatory site make them a promising candidate for targeted therapy and regenerative medicine. However, a proper understanding of the stem cell role in therapeutic function is still missing. That is why their visualization in vivo is essential, because it could answer several crucial question regarding their safe application in a tissue regeneration process. Here, we present the highly biocompatible positively charged carbon dots with quaternary ammonium attached on its surface (QCDs) as a new potential candidate for the fluorescent imaging of MSCs. We performed a complex battery of cytotoxicity tests to verify the high biocompatibility and excellent fluorescence properties of QCDs inside MSCs. Furthermore, the character of MSCs remained unchanged after QCDs treatment. We determined the safe labeling conditions (100 µg/ml of QCDs and 24 h of incubation) and additionally demonstrated the in vivo feasibility of our material. The QCD-labeled MSCs were transplanted subcutaneously into an immunodeficient mouse and visualized by optical in vivo imaging. The labeled cells were strongly fluorescent allowing semi-quantitative detection. Moreover, the unaffected homing ability of QCD-labeled MSCs transplanted intravenously into tumor bearing mouse was clearly confirmed. Therefore, we demonstrate that QCD-labeling can be highly promising approach for stem cell imaging during the regenerative therapy.

C83 TITLE: Multimodal nanoparticles for structural and functional tracking of stem cell therapy on muscle regeneration

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Abstract: Cell therapy offers promising opportunities to approach several diseases for which no effective therapies are currently available. However, the prognosis of the treatment efficacy commonly only relies on the progression of the disease symptoms. There is a need of tools to evaluate and predict the safety and success of cell-based treatments in earlier stages. The current lack of methods providing real-time tracking of transplanted cells and knowledge on their early biodistribution and viability, is one of the major weakness of the available cell-based treatments.The main goal of nTRACK is to develop a safe and highly sensitive multimodal nanoimaging agent enabling noninvasive, quantitative and longitudinal stem cell tracking and whole body biodistribution. nTRACK will also provide information on cell (long-term) viability using the combination of CT, MRI and PET, which are imaging modalities that are clinically available. The synthesis of nTRACK NPs and cellular labeling processes will be scaled up and will follow good manufacturing practice (GMP) requirements. A second goal is to establish a predictive model for early assessment of treatment effectiveness, based on short-term evaluation of the typical migration and biodistribution patterns of the stem cells. This predictive model could substantially improve overall management of the disease and will transform cell therapy treatment from "one size fits all' concept towards personalized treatment. The nTRACK technology will be demonstrated on a muscular injury sheep model, using imaging infrastructure commonly used in hospital settings. In addition, non-clinical safety studies on the nTRACK nanoparticles will be conducted following the conclusions of a series of formal interactions with regulatory authorities, to allow the prompt introduction into clinical trials after the end of the project. nTRACK will end up with fully characterized, (nano)safe and functional nano-based contrast agent proved in a clinical imaging framework with a large animal model to resemble human complexity plus a data interpretation and modelling software.

C84 TITLE: Combined atomic force microscopy and fluorescence: new opportunities for skeletal regenerative medicine

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Abstract: Life expectancy around the globe has increased dramatically in the last fifty years. However, an aged society results in an exacerbated burden to healthcare. Bone fractures and a number of age associated orthopaedic conditions, constitute a significant clinical and socio-economical problem. Thus, effective strategies to

repair and replace damaged bone and cartilage are urgently needed. Regenerative medicine aims to develop the tools and scientific knowledge to help repair/replace damaged human cells and tissues, with stem cell research a key component in the reparative paradigm. Cell therapy has emerged as a potential treatment option for skeletal diseases, although there remain challenges and questions to be addressed. Thus, enhanced understanding of the bio-physical properties of bone cells would offer a significant contribution to the elucidation of the cellular mechanisms in the fate, function and processes in tissue development. Our current work combines differential spinning disk fluorescence microscopy with atomic force microscopy, allowing the simultaneous detection of fluorescence with nano-mechanical mapping at the single cell level [1]. Relevant complementary information on heterogeneous cell populations can be retrieved, helping to distinguish different bone cell populations and accurately determine cell mechanical properties. Using an interdisciplinary study that combines physics, optics and stem cell biology, this study provides new insight on bone single-cell bio-physical properties and the development of novel tissue engineering and regenerative medicine approaches.

C85 TITLE: 3D hydroxyapatite scaffolds for ligament/tendon reconstruction

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Abstract: The aim of this work was the design and the development of 3D hydroxyapatite scaffold for ligament/tendon reconstruction. Hydroxyapatite (HP) nanoparticles were loaded into a polymeric blend to be electrospun and the resulting nanofibers were collected on drum to obtain a tubular 3D construct as hybrid system. HP should improve cell proliferation at tendon to bone interface and system mechanical properties. Chitosan citrate (2,5% w/w CH) and Pullulan (10% w/w PLL) were hydrated in 45:55 volume ratio acetic acid:water at room temperature by magnetic stirring. The polymeric blend was electrospun to obtain both flat/random scaffold (R-CH) and tubular/aligned scaffold (A-CH) using planar collector and drum, respectively. HP nanoparticles (diameter 200 nm) were added to the polymeric solution to obtain tubular/aligned hybrid scaffolds (A-CH-HP) having 0.6% w/w and 3% w/w HP concentration on dry systems. The scaffolds were crosslinked by heating for 1h at 150°C. The scaffolds were characterized by chemical-physical (SEM, FT-IR, XRPD analysis and mechanical properties) and preclinical properties (fibroblasts NHDF and osteoblasts SAOS-2 viability and adhesion/proliferation, assessed by MTT and CLSM). The SEM images showed that both R-CH and A-CH scaffolds maintained

smooth surface and thin fiber diameters while XRPD confirmed HP crystallinity in the nanofiber structure also after crosslinking. 3D scaffold (aligned nanofibers in tubular shape) was characterized by good elasticity and maximum tensile force and the system containing 0.6% HP evidenced the best performance in the range of tendon tissue1 MTT test suggested good biocompatibility for all the scaffolds and the CLSM images highlighted fibroblast cytoskeleton orientation along the nanofiber direction, more evident for the hybrid system. In conclusion 3D scaffold (aligned nanofibers in tubular shape) with a critical HP concentration of0,6%w/w presents to be a promising tool for surgical reconstruction of tendons/ligaments.

SECTION C | *Advanced Medical Imaging*

C86 TITLE: SyncRGB-FLIM: synchronous fluorescence imaging of red, green and blue dyes enabled by ultra-broadband few-cycle laser excitation and fluorescence lifetime detection

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Abstract: We demonstrate for the first time that an ultra-broadband 7 femtosecond (fs) few-cycle laser can be used for multicolor nonlinear imaging in a single channel detection geometry, when employing a time-resolved fluorescence detection scheme. On a multi-chromophore-labelled cell sample we show that the few-cycle laser can efficiently excite the multiple chromophores over a >400 nm two-photon absorption range. By combining the few-cycle laser excitation with time-correlated single-photon counting (TCSPC) detection to record two-photon fluorescence lifetime imaging microscopy (FLIM) images, the localization of different chromophores in the cell can be identified based on their fluorescence decay properties. The novel SyncRGB-FLIM multi-color bioimaging technique opens the possibility of real-time protein-protein interaction studies, where its single-scan operation translates into reduced laser exposure of the sample, resulting in more photoprotective conditions for biological specimens.

C87 TITLE: GFP fluorescence peak fraction analysis based nanothermometer for the assessment of exothermal mitochondria activity in live cells

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Abstract: Nanothermometry methods with intracellular sensitivities have the potential to make important contributions to fundamental cell biology and medical fields, as temperature is a relevant physical parameter for molecular reactions to occur inside the cells and changes of local temperature are well identified therapeutic strategies. Here we show how the GFP can be used to assess temperature-based on a novel fluorescence peak fraction method. Further, we use standard GFP transfection reagents to assess temperature intracellularly in HeLa cells expressing GFP in the mitochondria. High thermal resolution and sensitivity of around 0.26%°C−1 and 2.5%°C−1, were achieved for wt-GFP in solution and emGFP-Mito within the cell, respectively. We demonstrate that the GFP-based nanothermometer is suited to directly follow the temperature changes induced by a chemical uncoupler reagent that acts on the mitochondria. The spatial resolution allows distinguishing local heating variations within the different cellular compartments. Our discovery may lead to establishing intracellular nanothermometry as a standard method applicable to the wide range of live cells able to express GFP.

SECTION C | *Pitch-me-up*

C88 TITLE: Lipid-based nanoformulations as delivery systems for new and improved drug molecules

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Abstract: In medicine today, many diseases are faced with promising basic research results with respect to new and improved drug molecules. Development of treatment is however hampered by effective delivery of the drugs. Focus on the present work is on lipid formulations, such as liposomes, lipidic nanocapsules, solid lipid nanoparticles and lipid complexes, as delivery systems for conventional, and new drug molecules, and for antimicrobial peptides. Lipid formulations can be used for several delivery routes; nevertheless, the work herein focuses on topical applications, and on delivery to the eyes and lungs. To be able to use lipid formulations as drug delivery systems the formulations needs to be applicable in a clinical setting. Optimization for drug loading efficiency, drug release, physical and chemical stability, manufacturing

process (including scalability for clinical studies), environmental impact, and cost, needs to be considered during formulation development. Results from two large projects were RISE is a major partner will be presented, the FORMAMP project and transMed. The objective is that these formulation studies will pave the way for further development for every day therapeutic use in patients.

C89 TITLE: Centrifugal Field-Flow Fractionation hyphenated with Dynamic Light Scattering for the characterization of PLGA drug delivery particles under physiological conditions

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Abstract: Biodegradable nanoparticles as drug delivery systems (DDS) for biomedical applications have become highly attractive due to their stability in the blood stream and controlled release characteristics. However, in order to understand and optimize the parameters that control drug release, in-depth physico-chemical characterization of the DDS, ideally under physiological conditions, is mandatory to gain information about the particle size distribution and its potential alterations. Field-Flow Fractionation (FFF) is among the most promising analytical techniques, when it comes to the characterization of particulate systems in the nano-range. In recent years, due to its high flexibility, wide applicability and excellent compatibility to sophisticated detection systems such as Dynamic Light Scattering (DLS) and Multi Angle Light Scattering (MALS), FFF has become more and more popular as powerful characterization tool in the field of nanomedicine. In this study, polymer-based, non-cytotoxic PLGA-nanoparticles were characterized according to size and integrity of the particle formulation. PLGA was chosen due to its biodegradability, biocompatibility, controlled release characteristics and approval for therapeutic devices by the US Food and Drug Administration (FDA). Special focus was laid on the size and stability of these potent DDS in cell culture medium over time. Therefore, PLGA-NPs were incubated at 37 °C and characterized at distinct time intervals using Centrifugal Field-Flow Fractionation (CF3) hyphenated with DLS (CF3-DLS). In order to closely mimic the incubation condition and thus physiological conditions, CF3-fractionation was performed using cell culture medium as eluent thereby allowing a realistic insight into the behavior of such DDS under potential real-life application conditions.

C90 TITLE: Enabling simultaneous imaging of super-resolution fluorescence microscopy and scanning probe microscopy for the life sciences

Authors: <u>Gómez-Varela, Ana</u>1; R. Stamov, Dimitar2; Miranda, Adelaide1;

Alves, Rosana3; Barata-Antunes, Cláudia3; Dambournet, Daphné4; Drubin, David G.4; Paiva, Sandra3; De Beule, Pieter A. A.1
Affiliations: 1 - International Iberian Nanotechnology Laboratory, Life Sciences Department, Medical Devices; 2 - JPK BioAFM Center, Nano Surfaces Division, Bruker Nano GmbH, Colditzstr. 34-36, 12099 Berlin, Germany; 3 - Centre of Molecular and Environmental Biology, Department of Biology, University of Minho, Braga, Portugal; 4 - Department of Molecular and Cell Biology, University of California, Berkeley, Berkeley, CA, USA

Abstract: Atomic Force Microscope (AFM) has been integrated with different fluorescence microscopy techniques to correlate the AFM nano-mechanical data with biological specificity of the sample, thereby significantly enhancing the relevance of the information derived from the combined microscopy approach. However, up until this day it has not been possible to acquire data with fluorescence optical sectioning and scanning probe microscopy techniques within nearby spatial volumes at the same time. This severely limits the utility of combining both image techniques as all dynamical information is lost. We have previously circumvented existing integration problems and demonstrated simultaneous operation of aperture correlation microscopy through a Differential Spinning Disk (DSD) approach and nanomechanical mapping AFM. Obtaining data with two microscopy techniques simultaneously namely removes the need to perform both measurements independently and the subsequent data averaging required to eliminate cell to cell variation in observed signals. Here, we report for the first time a hardware set-up capable of achieving simultaneous as well as spatially overlapping super-resolution Structured Illumination Microscopy (SIM) microscopy and atomic force microscopy. SIM illumination is particularly suitable to combine with AFM as it allows to avoid induced cantilever bending due to fluorescence excitation light, a significant problem when integrating AFM with other super-resolution fluorescence microscopy schemes. We demonstrate system performance with sub-resolution fluorescent beads and report imaging simultaneous combined data of CRISPR/Cas9 genome-edited human bone osteosarcoma epithelial cells (U2Os line).

C91 TITLE: MANUFACTURING PROCESS DEVELOPMENT OF A STERILE NANOPARTICLE FORMULATION FOR AN INNOVATIVE DELIVERY AGAINST ALZHEIMER DESEASE

Authors: <u>Felici, Sara</u>1; Romagnoli, Andrea1; Maggi, Chiara1

Affiliations: 1 - IBI

Abstract: IBI, a pharmaceutical company located in Italy, is involved in the B-SMART project (deadline 2021), funded by the European Commission (Horizon 2020), with the purpose of developing a scalable nanoparticles GMP manufacturing process,

following a quality-by-design approach. The aim of the project is to create brain-targeted RNA-based nanomedicines, able to cross the blood-brain barrier, for neurodegenerative diseases, as Alzheimer. To facilitate the nanoparticles production, in a fast and reproducibility way, the preparation was performed through the microfluidic technique, using the NanoAssemblrTM instrument that realizes a laminar flow inside a specific cartridge, allowing a rapid mixing of two different solutions.The result is a nanoparticle suspension with specific characteristics of scalability and dimension/shape uniformity (< 130nm / PDI < 0.2). Two different types of nanoparticles loaded with a siRNA, lipidic and polymeric, were evaluated for GMP scaling-up. The lipid nanoparticles were prepared mixing an aqueous solution containing siRNA with an organic one of specific lipids. Polymeric nanocapsules [2] were prepared with siRNA absorbed of in an oily core. After the nanoparticles preparation, a purification was performed by a Tangential Flow Filtration System. Subsequently, a sterilizing filtration was developed using PES filters 0.22µm. By DLS analysis, no changes in size and PDI were observed, indicating that this technique is suitable for the sterilization of nanoparticles. Besides, stability studies were performed for the different nanoparticle formulations, showing a good stability in solution at room temperature for lipidic nanoparticles, while the polymeric ones resulted more stable as a freeze dried powder.

C92 TITLE: A Protocol for Wear Testing Dental Abutments Subject to Surface Modification

Authors: <u>Lepicka, Magdalena</u>1; Cortez, Hugo1; Alves, Filipe1; Gobbo, Pietro2; Cortez, Ana3; Freitas, Paulo1

Affiliations: 1 - International Iberian Nanotechnology Laboratory; 2 - Gadget Whisper, Lda; 3 - Celoplás, Plásticos para a Indústria S.A.

Abstract: In order to enhance performance of dental materials, various strategies can be introduced. Nowadays, the application of an anti-wear coating on selected parts of the implant assembly is considered one of the most reliable methods for promoting the long-term stability of a dental implant. Nevertheless, while there are multiple international standards for fatigue or corrosion testing of metallic implantable devices [3; 4], up to this day no standard that would provide the guidelines for wear testing of dental coatings has been introduced. Therefore, the aim of this study was to develop a method for wear testing of the surface modified dental implants, particularly abutments. Using the ISO 14801 standard for fatigue testing of dental implants, a tribological testing machine was designed and fabricated at INL. In the developed protocol, the dental implant is tested in the "worst case scenario" conditions, where its central longitudinal axis makes a 30°±2° angle with the loading direction of the machine, while the bone loss is simulated by clamping the implant in resin 3 mm below the nominal bone level. As the average bite forces are estimated

to fluctuate between 20 and 120 N [5], in each performed test, the load changes in a sine wave from 13 to 135 N (±5%) and 1 million of dynamic loading cycles are applied. The selected test conditions are in compliance with the literature [6; 7; 8] and are aimed to simulate one year of in vivo mastication. What is more, in order to measure the stabilizing efficacy of an applied coating, a digital torque wrench is used to tighten the abutment to the endosseous part of an implant before the loading test, as well as to loosen the assembly parts after dynamic testing. Moreover, visual examination of the dental implant parts before and after the wear test is done by the means of optical and electron microscopy techniques. The efficacy of the proposed protocol was tested on both bare and PVD TiN-coated dental abutments. According to the results, the post-loading surface condition as well as the loosening torque can be used to select the promising anti-wear coatings. The developed method provides comparison between bare and surface modified implant parts, while the collected data can be tested for statistical significance. Moreover, the wear patterns observed for the non-coatedsamples correspond to the literature data on the implant abutments used in vivo.

Speakers

Speakers

PLENARY SPEAKER: **LAURENT LÉVY**
CEO and Founder, Nanobiotix

Laurent Lévy holds a doctorate in physical chemistry, specialized in nanomaterials, from the Pierre and Marie Curie University (Paris) and from the CEA (Commissariat à l'Énergie Atomique et aux Énergies Alternatives) and a DEA (advanced studies and diplomas) in physics of condensed matter from the UPVI-ESPCI (Paris). He has extensive experience in sciences and techniques related to nanotechnologies, a field in which he worked for more than 10 years. His research at the frontier of biotechnology and nanotechnologies has resulted in the development of a number of concrete applications such as NanoXray, which could open a new method for cancer treatment.
For many years, Laurent was a consultant in the development of application of nanotechnologies with large companies such as Sanofi (pharma), Guerbet (medical imaging), Rhodia (chemistry), as well as for biotechnology start-ups. Laurent is the author of 35 international scientific publications and communications, has applied for several patents and completed his training by a post-doctoral fellowship at the Institute for Lasers, Photonics and Biophotonics, SUNY (State University of New York), Buffalo, USA.

KEYNOTE SPEAKER: **JENNIFER GROSSMAN**
Senior Scientific Program Manager at NIH/NIAID

Jennifer Grossman, PhD is a Senior Scientific Program Manager in the Vaccine Translation Research Branch (VTRB) in the Division of AIDS of the National Institute of Allergy and Infectious Disease (NIAID), one of the US National Institutes of Health (NIH). She oversees the production of a large portfolio of investigational HIV vaccine products, including nanoparticles, peptide and protein immunogens (monomers, trimers, germline-targeting, lineage-based, epitope-based), viral vectors, DNA, RNA,

and adjuvants. Jennifer provides nanotechnology subject matter expertise for R&D, manufacturing, analytics, formulation, Q/A and regulatory support for HIV vaccine products. Before joining the VTRB, Jennifer was at the National Cancer Institute (NCI), where she led alliance management for the Nanotechnology Characterization Lab (NCL). There, she established and managed productive collaborations within NCI, FDA, NIST and a network of over 150 drug development labs in industry and academia.
Jennifer's areas of scientific expertise include analytical methods for assessing drug delivery systems and modeling of nanoparticle structures and interactions. She has experience in a variety of issues related to nanotech drug development and regulation. Prior to joining the NCL in 2006, Jennifer conducted research in Physical Chemistry at the University of Maryland, where she focused on modeling and measuring protein motion under the guidance of Dr. David Fushman, Professor of Biochemistry. She holds a Ph.D. in Physical Chemistry, an M.S. in Chemical Physics and a B.S. in Physics.

KEYNOTE SPEAKER: **OLIVIER T. GUENAT**
CEO at AlveoliX AG & Head Organs-on-Chip Technologies, Medical Faculty, University of Bern

Olivier Guenat was born in Biel-Bienne and received his engineering degree (HTL/ETS) in Microtechnology from the University of Applied Sciences in Bienne in 1990, his MSc degree in Physics and Electronics from the University of Neuchâtel in 1995, and his PhD degree in Micro- and Nanotechnology in 2000 from the same institution. After his PhD, he was awarded an advanced fellowship from the Swiss National Foundation and pursued his postdoctoral studies at Harvard Medical School. There, he developed microfluidic systems aimed at monitoring cell signals. He then hold an Assistant Professor position at the Ecole Polytechnique in Montréal (Canada), where he founded and led the BioMEMS research laboratory until 2009. From 2009 to 2011, he returned to Switzerland and helped develop the Nanomedicine Division of CSEM SA, as Head of the Cell Systems Section. In November 2010, while still being active at the CSEM he was asked to set up the ARTORG Lung Regeneration Technologies activities within the framework of a contract between the University of

Bern and the CSEM. In 2012, he decided to focus on his activities at the ARTORG Center. Today, his research aims at developing advanced in-vitro systems that better mimic the in-vivo conditions of the lung.

KEYNOTE SPEAKER:
MARÍA JOSÉ ALONSO, *Full Professor,*
Pharmacy and Pharmaceutical Technology,
Univ. of Santiago de Compostela, CIMUS Research Institute

María José Alonso is Professor of Biopharmaceutics and Pharmaceutical Technology at the University of Santiago de Compostela.
Her lab has pioneered numerous discoveries in the field of nanomedicine and drug delivery. She has coordinated and participated in a high number of international research consortia financed by the WHO, the Gates Foundation, The World Cancer Research organization, the National Institute of Health (NIH) and the European Commission. She is the author of over 270 scientific contributions with more than 16,100 cites (H factor 71) and the inventor of 21 patent families. Because of the quality of her papers she has been among the TOP TEN in Pharmacology (Times Higher Education).
She has received 28 Awards, including the "King Jaume I Award" given to the best researcher in the area of new technologies in Spain, the "Maurice Marie Janot Award" given by APGI, the EU society of Pharmaceutical Technology and the "CRS Founders Award" of the Controlled Release Society (CRS).
She was the Vice-rector of Research and Innovation of the USC. Currently, she is President of the Controlled Release Society and Editor-in-Chief of the Drug Delivery and Translational Research jounal. She is also a member of three Academies in Spain, a member of the College of Fellows of AIMBE, a member of the College of Fellows of the CRS and a member of the US Academy of Medicine.

Speakers

INVITED SPEAKER: **GRAEME STASIUK**
Non-Clinical Lecturer in Molecular Imaging at University of Hull

Dr Graeme Stasiuk was appointed as Non-Clinical Lecturer in Molecular Imaging at the University of Hull, in the School of Life Science in June 2014. He has seven years of research experience, post PhD, into the design, synthesis and development of molecular imaging agents for all modalities.
Following a year at the French Alternative Energies and Atomic Energy Commission (CEA) in Grenoble, developing multimodal nanoparticle MR/Fluorescent contrast agents, he joined Professor Nick Long's group at Imperial College London in 2011 as Post-Doctoral Research Assistant, this work was focused on multimodal and targeted imaging.

INVITED SPEAKER: **MIGUEL OLIVEIRA**
Research Institute on Biomaterials, Biodegradables and Biomimetics of the University of Minho

Miguel Oliveira, graduated in Biochemistry at the Faculty of Sciences from the University of Porto. He holds a Post-Graduation in Biomedical Engineering from the Faculty of Engineering, University of Porto and a PhD in Materials Science and Technology, Tissue Engineering and Hybrid Materials in the Dept. Polymer Engineering, University of Minho.
He is one of the editors of the book Making Research Visible to the World, In Canon Foundation in Europe, Canon Alumni Book, eds.

Speakers

INVITED SPEAKER
MARIA DE LA FUENTE FREIRE
Head of the Nano-Oncology Unit, ONCOMET,
Health Research Institute of Santiago de Compostela

María de la Fuente is the principal investigator of the Nano-Oncology Unit of the Health Research Institute of Santiago de Compostela (IDIS), and member of the cancer research area of the CIBER (CIBERONC). Dr. de la Fuente obtained her PhD degree in 2006 in the field of nanomedicine and nanotechnology, and performed several research stages at different national and international institutions. In 2013, she established the Nano-Oncology Unit. Dr. de la Fuente's research is devoted to developing innovative nanotools to provide solutions to confront one of the biggest current challenges in oncology, metastases.
She is the author of more than 45 scientific articles, books and book chapters, and has more than 100 contributions to national and international congresses. She has been directly involved in more than 30 research projects, being principal investigator in 12 of them. She is inventor of five patents, and is actively participating in tech transfer and valorization initiatives to move the developed technology forward to a clinical setting (as NANOMEDTAB and the IGNICIA program of the Axencia Galega de Innovación).

INVITED SPEAKER: **UWE HIMMELREICH**
Coordinator MoSAIC at KU Leuven

Prof. Dr. Uwe Himmelreich graduated in Physical Chemistry in 1989 and obtained a Ph.D. degree in the same field from the University of Leipzig in 1994. His main field of expertise is magnetic resonance (MR) spectroscopy and MR imaging, ranging from isolated compounds to cells, preclinical disease models and patients.
He was a lecturer at the University of Sydney, Australia and a senior scientist at the Max-Planck Institute for Neurological Research in Cologne, Germany before joining

the University of Leuven. Since 2007, he is Professor at the University of Leuven (KU Leuven), Department of Imaging and Pathology. He heads the Biomedical MRI group and is coordinator of the KU Leuven core facility Molecular Small Animal Imaging Center (MoSAIC). His research focusses mainly on the development and optimization of preclinical imaging methods and the development and assessment of imaging contrast agents (including theranostic agents).
He has extensive expertise in the field of cell labelling and cell tracking using nanoparticle based approaches. While his original focus was on MRI/MRS, he works now intensively in multimodal imaging and contrast approaches, combining MR with optical imaging, CT, PET and ultrasound. He continues to work on the validation of novel contrast agents, in particular nanoparticle based agents.

INVITED SPEAKER: **RUI SOUSA**
Tech Transfer Officer & Program Manager at TecMinho

Rui Sousa is a Technology Transfer Officer with over 4 years of experience in the field. Rui is responsible the management of the innovation portfolio of the University of Minho, managing the full process from scouting new technologies until market uptake. Rui also acts as an industry liaison, establishing strategic R&D partnerships between the University and industry, solving clients' problems through innovation
Rui is also involved in project NOBEL, a Coordination and Support Action sponsored by the European Commission, managing the operation of the HealthTechTAB, Europe's first Translation Advisory Board for health technologies, overseeing all communication, brand and community-building actions for the tool.

Speakers

INVITED SPEAKER: **ELENA MARTÍNEZ**
Senior Researcher, Nanobioengineering
Institute for Bioengineering of Catalonia (IBEC)

After finishing my BSc in Physics, I started my scientific career completing a PhD in Materials Science in 2001 (University of Barcelona, Spain), focused on the study of the mechanical and tribological properties of thin films by nanoindentation technique.

Afterwards, I was engaged as a postdoc associate at the Laboratoire de la Matière Complexe, led by Prof. Francis Levy, at the École Polytechnique Fédéral de Lausanne (EPFL, Switzerland). There, I worked with nanostructured materials based on chromium nitride (CrN). In 2003, I received a "Ramon y Cajal" grant to lead a five year research project on the micro and nanostructuring technologies of polymer materials at the Barcelona Science Park (www.pcb.ub.es). It meant building the facilities and expertise in the newer micro and nanofabrication techniques for biomedical applications such as soft lithography, microfluidics, microcontact printing, nanoimprint lithography, focused ion beam nanolithography and dip-pen nanolithography. In 2008, I joint as Senior Researcher the Nanobioengineering group of IBEC led by prof. J. Samitier. There, I have been developing micro and nanopatterning techniques for cell culture applications as an independent research line.

In 2013, I was formally able to constitute my own independent research group, "Biomimetic systems for cell engineering", by being appointed as a "Junior Group Leader" at the Institute for Bioengineering of Catalonia (IBEC). Our research focuses on the technological development of cell culture systems that mimic in vivo complex signals (topographical, biochemical and mechanical) for applications in basic cell research, disease modeling and regenerative medicine. Such new technological setups are being developed by applying micro and nanofabrication techniques, including microfluidics, to soft polymer materials (extracellular matrix-like materials) in combination with tissue engineering approaches (mini-bioreactors with electrical and mechanical stimuli) to account for the 3D architecture, ligand distribution at the nanoscale, spatiotemporal biochemical gradients and mechanical properties of the living tissues. The aim of such systems is to provide reliable in vitro models of tissue-like constructs that help to fill the gap between conventional 2D cell cultures and animal experiments.

Speakers

INVITED SPEAKER: **SIMON BACONNIER**
TNA manager at EUNCL

Scientific by training (PhD in Biophysics,) has move to international and transversal collaboration. Simon has been involved in about 10 different EU projects including 4 in the nanomedicine field (NANO2LIFE, EUNCL, NOBEL, REFINE). Simon has experienced different industrial environment, including the start-up environment (Genopôle Start up cluster and Antineo) and the pharma company (OncoTherapy Science France).
His different experiences are connected by a medical innovation thread including nanotechnologies.
Simon most recent position as EUNCL operational coordinator puts him at the forefront of nanotechnology for medicine developers in a multidisciplinary environment including physics, chemistry, and biology as well as regulatory, medical development and production. With a clinical sensitivity Simon has worked and is still working at the bridging of these different community on a daily basis to serve the nanomedicine and biomedical stakeholders.

INVITED SPEAKER: **ALEXANDRE CECCALDI**
General Secretary of the ETPN
Coordinator of the NOBEL Project

Alexandre Ceccaldi is the General Secretary of the European Technology Platform on Nanomedicine (ETPN) since 2015 and coordinator of the NOBEL European Project since 2017.
He holds a doctorate in biology (University Pierre and Marie Curie, Paris) and an engineering degree from Agro ParisTech, with experience in anticancer strategies, industrial management and innovative design. Previously, he has managed INGESTEM, the first French national public consortium entirely dedicated to the R & D on induced pluripotent stem cells and their medical applications .

He is currently responsible for the management and development of the secretariat of the Paris-based ETPN, and provides strategic and operational support to a community of more than 120 member organizations of the European nanomedicine ecosystem, in coordination with the board of directors of the platform.

INVITED SPEAKER: **PRISCILLA PAGNACCO**
Marketing product manager at Pixium Vision

Graduated from Sorbonne University of Paris in health marketing and management, Priscilla starts her career working in innovative medical devices at Boston Scientific in Peripheral Vascular stents as a product manager. After one year of experience in the United States at Marketing department of EOS Imaging, a breakthrough and the leader in low dose 3D medical imaging, she enter in Pixium Vision to bring international disruptive innovations like PRIMA to the market.

INVITED SPEAKER: **IRAIDA LOINAZ**
Director of the CIDETEC Institute for Nanomedicine en Fundación CIDETEC

Organic chemistry by training, Iraida Loinaz led the Biomaterials Unit of CIDETEC since its inception. In the last years, with a high translational vocation, she manages the CIDETEC Nanomedicine Institute. The unit has created spin off companies, licenced two technologies. She's coordinator of three EU funded projects, including Nanopilot and TBMED.

Speakers

INVITED SPEAKER: **MARÍA TOMÁS GAMASA**
Research Fellow at University of Santiago de Compostela

Dr. Tomás Gamasa presents a quite unique multidisciplinary training in the fields of Organometallic catalysis, Bio-supramolecular Chemistry and Chemical Biology.
She completed her Ph.D. in 2011 as FPU fellow at University of Oviedo. The doctoral studies were focused on the design and development of synthetic methodologies. She performed several research stages at different national and international institutions. In 2011, she joined Professor Thomas Carell group at LMU Munich as Post-Doctoral Research Humboldt fellow. Her work was centered on DNA modifications for the expansion of the genetic code.
During her reincorporation as Juan de la Cierva fellow in the group of Prof. J. L. Mascareñas (USC, CIQUS institute) in 2015 she started working on the translation of chemical reactions to cellular environments. While this research can be ascribed to the realm of fundamental science and seeks to explore how far can scientists stress living systems to accept artificial catalytic processes, the resulting knowledge might lead to the development of new cell reactivity patterns and new tactics in biomedicine, for instance, for the regulated productions of multiple, different drugs in an orthogonal manner, opening new avenues for biological manipulation of living systems.

INVITED SPEAKER: **LORENA DIÉGUEZ**
Medical Devices Research Group Leader
at INL - International Iberian Nanotechnology Laboratory

Lorena Diéguez joined INL in 2014 as Staff Researcher and is, since May 2018, the leader of the Medical Devices research group. Her research is focused in the development of biomicrofluidic devices mainly devoted to Translational Medical Research

in close collaboration with Hospitals. For that purpose, her work is devoted to the development of integrated biosensing systems and nanobioengineered diagnostics microsystems for the isolation and characterization of Tumor Cells from body fluids of cancer patients, as well as the development of microfluidic organ-on-a-chip 3D models. Lorena is also very interested in translating her technology from the lab to the clinic, and she has been very active in her endeavours as entrepreneur, creating the spin-off company RUBYnanomed in the field of liquid biopsy.
She obtained her Bachelor's degree in Physics and her Masters in Optoelectronics at the University of Santiago de Compostela in 2005, then completed her Masters in Nanoscience and Nanotechnology at the University of Barcelona (UB) in 2007 and obtained her PhD in Nanosciences in optical and electrochemical biosensors at the Institute for Bioengineering of Catalonia and the ETH Zürich. Her postdoctoral research at the University of South Australia (UniSA) from 2010 was devoted to the study of rare cells from biological samples using microfluidics.

INVITED SPEAKER: **KATHLEEN SPRING**
Orogram Manager at Gesellschaft für Bioanalytik Münster

Kathleen Spring, PhD, program manager at Gesellschaft für Bioanalytik Münster e.V. in Münster, Germany. Bioanalytik Münster is a is a non-profit organisation. It's a regional network which was initiated in 2000 by local universities, research centres, enterprises, transfer institutions and investors to promote science, research and education in the field of (nano) bioanalytics in Münster region. They provide a central communication and information platform for scientists, entrepreneurs, investors and members of the public who are interested in bioanalytics. Bioanalytik Münster is involved as a partner in 3 EU-funded projects. EU-NCL and REFINE are projects initiated by the nanomedicine community, helping the nanomedicine community to prepare products for regulation and on the other hand to create a regulatory science framework for the risk-benefit assessment of nanomedical products. The third project is the Coordination and Support Action (CSA) NOBEL which wants to build a unique ecosystem in Europe for health technologies by devel-

oping a joint strategic vision of the key enabling technologies (KETs). She holds a Diploma (2007) in Human Biology of the University of Greifswald, Germany and a PhD (2012) in Molecular Biology of the University of Montreal, Canada. Since 2017, she is a program manager at Bioanalytik Münster and mainly involved in the CSA NOBEL. Since then she's also a member of ETPN. She has experience in science and research as well as in organisation and managing.

The Venue

The Venue

Welcome to INL - the International Iberian Nanotechnology Laboratory, an Intergovernmental organization (IGO) that attracts scientists and engineers worldwide to perform interdisciplinary research in Nanotechnology and Nanoscience.
INL offers a complete range of solutions created in articulation with partners from scientific research, academia and industry, aiming to become the worldwide hub for nanotechnology deployment, addressing major global challenges.

INL has witnessed a tremendous growth during the last four years from about 60 people to close to 300 from 32 countries. INL working methodology is to do cutting edge research in some well-defined areas and at the same time work with companies to provide solutions, expertise and compounded knowledge to enhance their competitiveness and market growth. INL vision is to become the best nanotechnology deployment organization worldwide - for the clear benefit of the society at large, namely in the Health and Medicine fields.

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Welcome to Braga

City Center
PRAÇA DA REPÚBLICA

Braga is a lively city, one of the oldest in the country, and is teeming with young people who study at its universities.

Built more than 2,000 years ago, "Bracara Augusta" was founded by Augustus.
It was located on one of the main Roman roads in the Iberian Peninsula, and the administrative seat of the Empire, and later given the status of capital of the Roman province of Gallaecia, present-day Galicia, by Emperor Caracalla.
The Braga Diocese is the oldest in Portugal and, in the Middle Ages, the city even competed with Santiago de Compostela in power and importance. One of the Caminos de Santiago passed through here, when this pilgrimage cult grew with the Christian reconquest and the foundation of Portugal.

Braga's Cathedral is also the oldest in the country and was built in the 12th century by the parents of Portugal's first King, D. Henrique and D. Teresa, who are buried there. Braga is to this day one of the country's main religious centres, having the Holy Week Celebrations and the São João Festival as the highlights in its liturgical and tourist calendar.

City Center
AVENIDA DA LIBERDADE

Welcome to Braga

Besides the Tesouro-Museu da Sé (Cathedral Treasure Museum), it is worth visiting the Biscainhos Museum, housed in a Baroque palace, a landmark period in the history of Braga, and the D. Diogo de Sousa Archaeological Museum, since the city also abounds in remains from the Roman era. We suggest a leisurely stroll around the historic centre to visit some of the many churches, admire the houses and historical buildings, such as the Palácio do Raio, the Theatro Circo, the Arco da Porta Nova, and to have a coffee at the emblematic Brasileira with a view of the busy Avenida Central. But Braga is considered the youngest city in Portugal and, from its contemporary landmarks, the Braga Municipal Stadium stands out, designed by Souto Moura, one of the most prestigious Portuguese architects and winner of the Pritzker Prize.
Every visitor to Braga must see the Bom Jesus Sanctuary, a city icon, with its monumental staircase. Amid an expanse of greenery, it offers an excellent panoramic view of the city, as do two other churches nearby: Nossa Senhora do Sameiro Sanctuary, an important place of Marian worship, and Santa Maria da Falperra Church. Over the last few years, the University and the quality of contemporary architecture have instilled an atmosphere of youthful vibrancy which has brought this ancient city an unexpected modernity.

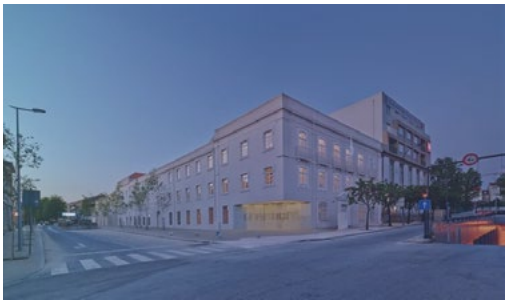
6Km from the City Center
BOM JESUS

The most impressive religious sanctuary in Portugal, Bom Jesus do Monte lies 6km east of Braga in a verdant park draped over the western slopes of Monte Espinho and remains one of Portugal's most compelling visitor attractions.

A site of worship for pilgrims from around the world, the shrine at the summit can be reached via the hydraulically operated funicular railway or on foot by climbing the steep stairs. The effort of the climb is rewarded by a spectacular panoramic view.

City Center
THEATRO CIRCO

Theatro Circo was conceived for the first time in 1906 by a group of people from Braga, led by Artur José Soares, José António Veloso and Cândido Martins. In 1911, the project began to take shape at the hand of the architect João de Moura Coutinho and on April 21, 1915, the Theatro Circo was opened, coinciding with a period of great economic and social development in Braga. Over the decades, the space was readapted to new requirements imposed by the evolution of the times and acquired new qualities, most notably the installation of sound cinema. Great artists of international renown at the time graced its stage such as cellist Guilhermina Suggia, violinist Isaac Stern and pianist Arthur Rubenstein, the National Orchestra of Florence, Prague, Madrid or Vienna, the Opera House of London, among many others.

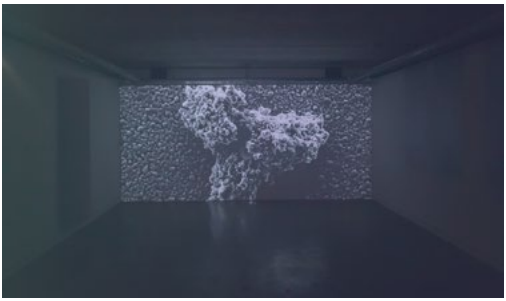


City Center
GNRATION

A result of Braga 2012 – European Youth Capital, gnration was born. This is a space for creation, performance and exhibition within the domain of contemporary music and the relationship between art and technology.

Welcome to Braga

Through a sustained strategy of permanent openness to the community, it wishes to assert itself as a unifying center for cultural and creative dynamic, taking up its position as a space geared towards the awareness and formation of new audiences, exposing them to relevant artistic practices, in light of a contemporary and cosmopolitan perspective.
Together Gnration and INL created a collaborative program on Art and Nanotechnology - Scale Travels. This unique programme was launched in 2015 is part of INL mission to articulate art and nanotechnology and it allows to reach the community and specifically an audience that would not be so likely to engage with Science, INL, and nanotechnology in particular. It is one of the many ways we at INL try to convey Science and Nanotechnology.
Artists who engage with INL are often surprised to realize that science demands creativity and that the scientist has to be creative to overcome many different challenges.
The relation that is established with the artistic object is as rich, diverse and complex as use that can be given to nanotechnology.



Located in the ground floor of gnration, the INL gallery hosted unique work by artists such as Tarik Barri, AGF, Ryoichi Kurokawa or Joanie Lemercier.

GNRATION
Address: **Praça Conde de Agrolongo, 123 Braga**

Sponsors & Exhibitors

Sponsors

UK Headquarters
Email: info@endomag.com
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Jeffreys Building, St John's Innovation Park
Cambridge, CB4 0WS, United Kingdom

Endomag is a medical device manufacturer, headquartered in Cambridge, UK, with a US office in Austin, Texas. Originally incorporated as Endomagnetics Ltd, the company was founded in 2007 and produces surgical guidance products which assist surgeons in locating and removing cancerous tumors, predominantly for breast cancer surgery.[1] The company's products are distributed by Leica Biosystems in North America and Sysmex Corporation across Europe.

The company's location at the St John's Innovation Park in Cambridge is central to Silicon Fen, the cluster of high-tech businesses in the area which form one of the most important technology centres in Europe.

Headquarters
Phone: +33 (0)1 40 26 04 70
60 rue de Wattignies,
75012 Paris
France

Nanobiotix is a late stage clinical company pioneering nanomedicine for more than a decade. We intend to significantly change the outcomes for cancer patients following a different path than other Pharma or Biotech companies: a new way to treat patients thanks to nanophysics at the heart of the cell.

Nanobiotix is a spin-off from the State University of New York (SUNY), Buffalo and was incorporated in 2003. Nanobiotix is listed on the regulated market of Euronext Paris on 29 October 2012.

Nanobiotix operates worldwide from the headquarters based in Paris, France and affiliate office in Cambridge, MA, USA. Nanobiotix has partnered with PharmaEngine for clinical development and commercialization of NBTXR3 in Asia.

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The NOBEL H2020 Project (www.nobel-project.eu) aims at developing a European HealthTech ecosystem that will foster a healthcare revolution thanks to new emerging medical technologies. It will bring together nanomedicine, photonics, robotics, biomaterials and digital sciences into smart integrated medical devices, from academic research to the clinic. To that, it has 3 main missions: (i) to build an ecosystem, by organising multiple event and providing a space for dissemination to all European HealthTech stakeholders, thus becoming a unique meeting place; (ii) to shape a strategy, by building its common vision for the future of HealthTech in Europe, the Continuum of Care, integrating the separate roadmaps of individual technologies: (iii) to accelerate innovation, thanks to the HealthTech Translation advisory Board (TAB), a premium service offering free-ofcharge tailored support to the selected innovations.

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