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GENERAL INFORMATION

INTRODUCTION

Nanomedicine is the medical application of nanotechnology achieved by combining nano- and biomaterials with the future perspective to address pathologies in a complex and heterogeneous scenario. The challenge of nanomedicine depends on the use of nano- and biomaterials at the nanometer scale to manufacture scalable drug delivery systems and medical devices that are safe, biocompatible and non-toxic. The goal is to selectively target microenvironments and tissues through physiological and pathological mechanisms that modulate the micro- and macro environments of patients affected by rare and clinically relevant pathologies. The combination of nano- and biomaterials, the modification of interface properties, as well as the functionalization of the resulting drug delivery systems with macromolecules and active biomolecules, provides specific properties for tailoring precision medicine. This boosts the application for many diseases with suitable therapeutic options and benefits for the patients.

In this symposium, we will address the latest advances of nanomedicine for precise targeting therapies and unlock their potential for drug and gene delivery applications. The selected talks will provide valuable insights on the latest developments of translational research on nanomedicine for a personalized therapy and towards a reliable pre-clinical and clinical assessment.

SCIENTIFIC ORGANIZERS

Hélder A. Santos / University Medical Center Groningen, Netherlands

Neus Feliu / Fraunhofer IAP-CAN, Germany

Wolfgang Parak / University of Hamburg, Germany

WEBSITE

nanomedicine.beilstein-symposia.org

DATES

- **Scientific program** September 3–5
- **Traveling days** September 2nd and 6th
- **Welcome reception** September 2nd at 7 PM

CONFERENCE HOTEL

The conference, lunches and dinners will take place at:

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SCIENTIFIC PROGRAM

Tuesday, September 3

9:00	**Welcome and introduction**
	Session chair: Wolfgang Parak
9:20	**Nano-protein corona and strategies for nanomedicine** Chunying Chen / National Center for Nanoscience and Technology, China
10:00	**A discovery framework for nanoparticle shape-based adjuvants** Yan Yan / University College Dublin, Ireland
10:40	**Flash talks of posters (details on site)**
11:10	Coffee break
11:40	**Systemic homing peptides for targeted nanomedicine: discovery and (pre)clinical development** Tambet Teesalu / University of Tartu, Estonia
12:20	**A critical role for glassy states biomolecular condensates in nanoscale regulation of biology** Kenneth Dawson / University College Dublin, Ireland
13:00	Lunch
	Session chair: Helena Florindo
14:30	**Brain-targeted nanoparticles for treating Parkinson's disease** Avi Schroeder / Technion, Israel
15:10	**Gut hormone stimulation via lipid nanoparticles for the treatment of gastrointestinal disorders** Ana Beloqui Garcia / Université catholique de Louvain, Belgium
15:50	**Flash talks of posters (details on site)**
16:20	Coffee break
17:00	**The use of mRNA epitope delivery by ionizable lipid nanoparticles for the tolerogenic immunotherapy for allergic and autoimmune disease** *Online live talk* André Nel / UCLA, USA

17:40	**To be announced** Michael Mitchell / University of Pennsylvania, USA
18:20	**First Poster Session** (all posters with odd numbers)
19:30	Dinner

Wednesday, September 4

9:00	**Welcome and introduction**
	Session chair: Hélder Santos
9:05	**Strategies to promote cancer nanomedicine performance and clinical translation** Twan Lammers / RWTH Aachen University Clinic, Germany
9:45	**Engineered nanomedicines to target and reprogram the tumor microenvironment** Jai Prakash / University of Twente, Netherlands
10:25	**Exploring new approaches for surface engineering nanomaterials** Beatriz Pelaz / University of Santiago de Compostela, Spain
11:05	Conference photo
11:15	Coffee break
11:45	**Cell specific delivery of RNAs using targeted lipid nanoparticles: from vaccines to therapeutic genome editing** Dan Peer / Tel Aviv University, Israel
12:25	**Stimuli-responsive nanotools for controlled drug delivery** Pablo del Pino / University of Santiago de Compostela, Spain
13:05	Lunch
14:15	**Excursion** Wine hike along the vineyards and chair lift back to the hotel (10 EUR per person)
19:30	Dinner

Thursday, September 5

9:00	**Welcome and introduction**
	Session chair: Twan Lammers
9:05	**Decoding solid tumor microenvironment to revolutionize nano-immunotherapy** Helena Florindo / University of Lisbon, Portugal
9:45	**Quantitative interaction of nano-colloids with cells** Neus Feliu / Fraunhofer IAP-CAN, Germany
10:15	**Flash talks of posters (details on site)**
10:45	Coffee break
11:15	**Nature-inspired nanotechnology for RNA delivery to immune cells** Roy van der Meel / Eindhoven University of Technology, Netherlands
11:55	**Experiments towards mapping the protein corona around endocytosed nanoparticles** Wolfgang Parak / University of Hamburg, Germany
12:25	**Comparative optimization of polysaccharide-based nanoformulations for cardiac RNAi therapy** Hélder Santos / University Medical Center Groningen, Netherlands
12:55	Lunch
	Session chair: Neus Feliu
14:10	**Polypept(o)ides in cancer immune therapy** Matthias Barz / Leiden University, Netherlands
14:50	**Polypeptide-based nanomedicine unveiling tropism and biological barrier crossing capabilities** María Vicent / Príncipe Felipe Research Center, Spain
15:30	**Flash talks of posters (details on site)**
16:00	Coffee break
16:30	**Nanoparticles in the design of tissue engineering constructs using bottom-up and top-down approaches** João Mano / University of Aveiro, Portugal

17:10	**Observations which might inspire drug delivery scientists for guiding bio-therapeutics over biological barriers** Stefaan de Smedt / University Ghent, Belgium
17:50	**Second Poster Session** (all posters with even numbers)
19:00	Dinner

ABSTRACTS

Tuesday 9:20	**Nano-protein corona and strategies for nanomedicine**
	Chunying Chen
	National Center for Nanoscience and Technology, China

Nanomaterials have enormous potential as nanomedicines for diagnosing and fighting various diseases. Researchers make every endeavor to design diverse nanomedicines with novel nanostructures and smart payloads. An inconvenient hurdle in the practice of nanomedicine is the protein corona, a spontaneous collection of biomolecular species by nanoparticles in living systems. The protein corona has shown the important roles in shaping up the transformation and transport of nanoparticles as well as in eliciting their functional (biophysical, biochemical, and biological) and adverse (immunological, toxicological, and pathogenic) effects in vitro and *in vivo*. The dynamics of intracellular release or exchange of the blood protein corona from nanomaterials following their cellular internalization are also discussed. How much of this nonspecific protein ensemble is determined by the chemistry of the nanoparticle core and its surface functionalization and how much of this entity is dictated by the biological environments that vary spatiotemporally *in vivo*? How do we “live with” and exploit the protein corona without significantly sacrificing the efficacy of nanomedicines in diagnosing and curing human diseases? The fate of nanoparticles and their biodistribution and cell internalization are regulated by the protein corona. Accordingly, manipulation of the protein corona has been a common strategy for the design and delivery of nanomedicines. This talk will discuss the chemical and biophysical signatures of the protein corona and ponders challenges ahead for the field of nanomedicine.

Tuesday 10:00	**A discovery framework for nanoparticle shape-based adjuvants**
	Yan Yan
	University College Dublin, Ireland

With the ever growing universe of nanoscale shapes, names such as “nanoflowers” and “nanostars” no longer precisely describe or characterise the distinct nature of the particles. Conversely, it is of increasing interest to investigate shape-dependent effects in biology. Here, we introduce a conceptual framework to synthesis, capture, describe the nanoscale shapes. Furthermore, we introduce a novel approach for discovering biologically important nanoscale shapes without prior knowledge. biologically relevant inductive nanoscale shape discovery and evaluation that is ideally suited to, and will ultimately become, a vehicle for machine

learning discovery. In this framework, microfluidic flow synthesis is employed to achieve reproducibility and tunability. Computational imaging analysis is used for quantitative shape analysis. In vitro high-throughput biological responses are fed into an iterative feedback to guide the search for nanoscale shape regions. Early applications *in vivo* suggest the feasibility of such framework to identify significant forms of shape-induced biological and adjuvant-like immunological control.

Tuesday	**Systemic homing peptides for targeted nanomedicine: discovery and (pre)clinical development**
11:40	**Tambet Teesalu**
	University of Tartu, Estonia

Our laboratory employs *in vivo* phage display screens to identify homing peptides binding to specific targets in the vasculature. These peptides can be used for precision delivery of drugs, biologicals, and nanoparticles into tumors to enhance their therapeutic efficacy. I will outline current approaches to mapping vascular heterogeneity using *in vivo* peptide phage display, as well as characterization and validation workflow for candidate vascular homing peptides. Additionally, I will offer a translational perspective by describing the clinical development status of the prototypic tumor-penetrating peptide, iRGD. Lastly, I will present our unpublished data on blood-brain-barrier targeting peptides, which have the potential to deliver imaging and therapeutic payloads to glioblastoma and other neurological diseases. This innovative research could help to overcome the significant challenges associated with delivering therapeutic agents to the brain and has the potential to revolutionize the treatment of these devastating conditions.

Tuesday	**A critical role for glassy states biomolecular condensates in nanoscale regulation of biology**
12:20	**Kenneth Dawson**
	University College Dublin, Ireland

Nanoscale objects are processed by living organisms using highly evolved and sophisticated endogenous cellular networks, specifically designed to manage objects of this size. While these processes potentially allow nanostructures unique access to and control over key biological machineries, they are also highly protected by cell or host defence mechanisms at all levels. A thorough understanding of bionanoscale recognition events, including the molecules involved in the cell recognition machinery, the nature of information transferred during recognition processes and the coupled downstream cellular processing, would allow us to achieve a qualitatively novel form of biological control and advanced therapeutics. Here we

discuss evolving fundamental microscopic and mechanistic understanding of biological nanoscale recognition. We consider the interface between a nanostructure and a target cell membrane, outlining the categories of nanostructure properties that are recognized, and the associated nanoscale signal transduction and cellular programming mechanisms that constitute biological recognition.

Tuesday 14:30	**Brain-targeted nanoparticles for treating Parkinson's disease**
	Avi Schroeder
	Technion, Israel

Nanotechnology holds numerous potential benefits for treating disease, including the ability to transport complex molecular cargoes, including RNA and proteins, and targeting specific tissues, including the brain. Brain-targeted nanoparticles enhance the delivery of monoclonal antibodies (mAbs) across the blood-brain barrier (BBB) and into neurons, allowing the intracellular and extracellular treatment of Parkinson's disease. 100-nm BTL cross human BBB models intact and are taken up by primary neurons. Within neurons, SynO4 is released from the nanoparticles and bound to its target - alpha-synuclein (AS), thereby reducing AS aggregation and enhancing neuronal viability. *In vivo*, intravenous administration results in a seven-fold increase in mAbs in brain cells, decreasing AS aggregation and neuroinflammation and improving mice's behavioral motor function and learning ability. Targeted nanotechnologies offer a valuable platform for drug delivery to treat brain neurodegeneration. The evolution of drug delivery systems into synthetic cells, programmed nanoparticles with an autonomous syn-bio capacity to synthesize diagnostic and therapeutic proteins inside the body, and their promise for treating disease will be discussed.

References:
[1] Z. Yaari *et al*. *Nat. Commun*. **2016**, *7*, 13325, doi: 10.1038/ncomms13325.
[2] O. Adir *et al*. *Nat. Commun*. **2022**, *13*, 2328, doi: 10.1038/s41467-022-29871-8.
[3] M. Sela *et al. Adv. Mater.* **2023**, *35*, 2304654, doi: 10.1002/adma.202304654.

Tuesday 15:10	**Gut hormone stimulation via lipid nanoparticles for the treatment of gastrointestinal disorders**
	Ana Beloqui Garcia
	Université catholique de Louvain, Belgium

Enteroendocrine cells (EECs) produce ~20 gut hormones that regulate a wide range of metabolic processes, in which most hormones have more than one physiological role and most of these physiological roles are performed by more than one hormone1. Nutrients, such

as lipids, can interact with various G-protein coupled receptors (GPCRs) expressed on EECs and induce the release of gut hormones2. Lipids are widely present in a wide range of excipients used in nanocarriers. During the last years, my group has been working on the stimulation of gut hormones secretion via lipid nanocarriers orally administered3-6. The goal of this strategy is to stimulate the secretion of gut hormones while simultaneously providing with plasmatic levels of the encapsulating peptide towards an oral delivery alternative to the subcutaneous peptide injection. By modifying the lipid nanocarrier, we can tune this effect towards a different gut hormone secretion pattern. This represents a novel promising strategy for the treatment of gastrointestinal disorders.

References:

[1] F. Gribble *et al. Annu. Rev. Physiol.* **2016**, *78*, 277, doi: 10.1146/annurev-physiol-021115-105439.
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[6] A. Beloqui, *J. Control. Release* **2024**, *373*, 31, doi: 10.1016/j.jconrel.2024.07.007.

Tuesday	**The use of mRNA epitope delivery by ionizable lipid nanoparticles for the tolerogenic immunotherapy for allergic and autoimmune disease**
17:00	**André Nel**
	University of California Los Angeles, USA

The liver makes use of a specialized tolerogenic immune environment that can be exploited for therapeutic purposes, including by nanoparticles, targeting of hepatic antigen presenting cells (APC) that specializes in suppressing systemic immunity by generating regulatory T-cells (Tregs). Key amongst the APCs are the liver sinusoidal endothelial cells (LSECs), which utilize facile endocytosis of particulate antigens and TGF-beta expression for MHC II presentation to naïve CD4 T-cells precursors to Tregs. This evolutionary conserved pathway prevents overreaction of the immune system to foreign food substances as well as endogenous proteins that could serve as autoantigens, e.g., pancreatic islet proteins involved in diabetes. We developed a liver-targeting lipid nanoparticle (LNP) platform to deliver mRNA-encoded peanut allergen epitopes to LSECs, serving as robust tolerogenic APC that can suppress allergic inflammation by FoxP3+ Tregs. The mRNA strand was constructed by including nucleoside sequences encoding for non-allergenic MHC-II binding T-cell epitopes, identified in the dominant peanut allergen, Ara h2. These epitopes were inserted in the mRNA strand downstream of MHC-II targeting sequences, further endowed in vitro with 5’ and 3’ capping sequences, a PolyA tail and uridine substitution. Codon-optimized mRNA was used for microfluidics synthesis of LNPs with an ionizable cationic lipid, also decorated with a lipid-

anchored mannose ligand for LSEC targeting. Biodistribution to the liver was confirmed by *in vivo* imaging, while ELISpot assays demonstrated an increase in IL-10-producing Tregs in the spleen. Prophylactic administration of tandem-repeat or a combination of encapsulated Ara h2 epitopes induced robust tolerogenic effects in C3H/HeJ mice, sensitized to and subsequently challenged with a crude peanut allergen extract. In addition to alleviating physical manifestations of anaphylaxis, there was suppression of Th2-mediated cytokine production, IgE synthesis and mast cell release, accompanied by increased IL-10 and TGF-β production in the peritoneum. These exciting results have now also been extended to the development of tolerogenic multi-epitope mRNA nanoparticles that can be used for treatment of 1 diabetes. Moreover, a reverse approach can also be used, to reprogram liver APCs with LNPs that delivers innate immune stimuli for reprogramming the protective hepatic environment for pancreas cancer spread to the liver and spleen, using a combination of stimulatory immunomodulators plus nucleotide epitopes from pancreas cancer neoantigens.

Tuesday	**To be announced**
17:40	**Michael Mitchell**
	University of Pennsylvania, USA

Wednesday	**Strategies to promote cancer nanomedicine performance and clinical translation**
9:05	**Twan Lammers**
	RWTH Aachen University Clinic, Germany

Nanomedicines are extensively explored for cancer therapy. By delivering drug molecules more effectively and more selectively to pathological sites, and by attenuating their accumulation in healthy organs and tissues, nanomedicines assist in improving the balance between (chemo)therapy efficacy and toxicity. The tumor accumulation of nanomedicines is traditionally ascribed to the EPR effect, which is highly variable, both in animal models and in patients. To address issues associated with tumor targeting heterogeneity, and to advance cancer nanomedicine clinical translation, we are working on systems and strategies to monitor and modulate tumor-targeted drug delivery. In the present lecture, several of these strategies will be highlighted, including image-guided interventions to prime tumor blood vessels and the microenvironment, as well as the use of imaging and tumor tissue biomarkers for patient stratification. These efforts help to establish rational and realistic ways forward towards promoting the clinical translation and performance of cancer nanomedicines.

References:

[1] S. Bhatia *et al. Nat. Rev. Cancer* **2022**, *22*, 550-556, doi: 10.1038/s41568-022-00496-9.
[2] I. Biancacci *et al. Adv. Sci.* **2022**, *9*, e2103745, doi: 10.1002/advs.202103745.
[3] A. Dasgupta *et al. Proc. Natl. Acad. Sci. U.S.A.* **2023**, *120*, e2218847120,

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Wednesday	**Engineered nanomedicines to target and reprogram the tumor microenvironment**
9:45	**Jai Prakash**
	University of Twente, Netherlands

The tumor microenvironment (TME) is inherently highly heterogenous and complex, encompassing a variety of cell types including cancer-associated fibroblasts (CAFs), tumor-associated macrophages (TAMs), other immune cells as well as extracellular matrix. The TME is largely considered immune cell rich or immune cell deserted/exhaustive. These features of the TME determines the fate of the tumor progression as well as efficacy of chemo-/immune-therapy. CAFs and TAMs are key cell types in the tumor stroma which contribute to the tumor progression, metastasis and development of resistance.
In our lab, we are designing engineered technologies based on actively targeted nanomedicine to deliver therapeutics to these stromal cells specifically and re-program them, in order to improve the therapeutic efficacy of cancer therapeutics. We have designed “tail-flipping” nanoliposomes to target TAMs in breast tumors [1]. We have shown that TAMs can be either eradicates or re-programmed TAMs *in vivo* by delivering different therapeutic molecules using these nanoliposomes. For example, delivery of a bacterial cell wall component muramyl tripeptide reduced the tumors by 70% without any chemotherapy. Furthermore, the combination with immune checkpoint inhibitor anti-PD1 enhanced its efficacy significantly. We have also developed nanosystems for targeting CAFs using our proprietary peptide (AV3) *in vivo* [2]. We found that AV3-conjugated polymeric nanoparticles accumulated into the pancreatic tumor significantly more than non-targeted nanoparticles *in vivo*. Within the tumor, AV3-PEG-NP showed higher CAF accumulation compared to non-targeted nanoparticles. Furthermore, delivery of anti-stromal compounds resulted in reduction in the tumor stroma and enhancement of therapeutic efficacy of chemotherapy gemcitabine in pancreatic tumor model *in vivo*.

In conclusion, targeting of nanoparticles to specific cells within the TME is achievable using engineered nanomedicine which allows for modulating them and inhibiting their pro-tumoral function as well as improve the efficacy of cancer therapeutics.

References:
[1] P.R. Kuninty *et al. Nat. Commun.* **2022**, *13*, 4548, doi: 10.1038/s41467-022-32091-9.
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Wednesday	**Exploring new approaches for surface engineering nanomaterials**
10:25	**Beatriz Pelaz**
	University of Santiago de Compostela, Spain

Nanotechnology empowers the fabrication of versatile materials through composite self-assembled structures, incorporating inorganic/organic nanoparticles (NPs) and biomolecules, which serve as multifunctional tools for drug delivery. Challenges include elucidating and managing biomolecule-NP interactions (biomolecular corona), preventing intracellular degradation, and achieving precise cell/tissue targeting. Effective nanomedicine design necessitates traits such as evasion of the mononuclear phagocytic system, reduced accumulation in the liver/spleen, prolonged circulation, specific targeting, and functional efficacy.

The traditional wet chemistry methods often fall short in several aspects: 1) Control Over Ligand Number and Spatial Disposition. Precise control over the number and arrangement of ligands on the nanoparticle surface is challenging, leading to variability in targeting and functionality. 2) Complex surface engineering: Creating surfaces that mimic the multifaceted abilities of natural cellular systems, such as multivalency and specific recognition, is difficult using conventional methods.

Here, we will discuss some novel approaches to tune the surface of solid nanomaterials such as the use of DNA origami or biomimetic systems. These approaches will serve to develop the next generation of nanomedicines by improving their specificity, efficacy, and safety.

Wednesday	**Cell specific delivery of RNAs using targeted lipid nanoparticles: from vaccines to therapeutic genome editing**
11:45	**Dan Peer**
	Tel Aviv University, Israel

Objectives: Modulating gene function that can lead to cell death in the case of tumors or to change of expression of desired proteins for vaccines or editing of cells for knockout or knockin of genes is a major challenge and an unmet need.
Methods: We have developed several strategies to target unique cell surface receptors and as well as a very large library of ionizible lipids with two special features: highly immunogenic or less immunogenic (that can be used for repeated doses). In addition, we developed novel mRNA sequences with more potent immunogenicity that can be utilized against bacterial infection.

Results: In this talk, I will share several examples ranging from new therapeutic targets identified for cancer (e.g. CKAP5) to the first bacterial mRNA vaccines and will also share some data on gene editing for cancer therapeutics.
Conclusions: Cell specific delivery of RNA payloads is feasible and represent the next generation milestone in personalized medicine.

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[2] S. Chatterjee *et al. Sci. Adv.* **2023**, *9*, eade4800, doi: 10.1126/sciadv.ade4800.
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[9] S. Qassem *et al. Mol. Ther. Nucleic Acids*, **2024**, *35* doi: 10.1016/j.omtn.2024.102224.
[10] R. Masarwy *et al. Adv. Drug Deliv. Rev.* **2024**, *211*, doi: 10.1016/j.addr.2024.115359.

Wednesday	**Stimuli-responsive nanotools for controlled drug delivery**
12:25	**Pablo del Pino**
	University of Santiago de Compostela, Spain

Nanomaterials, activated by both endogenous and exogenous triggers, can perform complex functions within living cells, such as releasing active compounds and activating pro-drugs. Typically, upon exposure to living cells, engineered nanomaterials are stored in lysosomes or phagolysosomes—hydrolytic organelles responsible for degrading biomolecules, pathogens, and microorganisms. Circumventing this storage in hydrolytic organelles through external triggers or endogenous mechanisms presents a significant challenge and opportunity in nanobiotechnology. This talk will discuss innovative strategies using self-assembled active nanomaterials, including plasmonic nanoMOFs (nanocrystals based on microporous metal-organic frameworks), catalytic nanoMOFs, and cell-derived nanocarriers, highlighting their potential to revolutionize targeted cellular therapies.

Thursday	**Decoding solid tumor microenvironment to revolutionize nano-immunotherapy**
9:05	**Helena Florindo**
	University of Lisbon, Portugal

Among immunotherapeutic approaches, immune checkpoint inhibitors (ICI), have revolutionized the treatment of several cancers. However, limited efficacy has been obtained for cancer vaccines and severe immune-mediated side effects have been related to ICI under clinical development [1]. Looking into solid tumors, such as breast cancers and melanoma, the highly immunosuppressive tumor microenvironment (TME), and the immune evasion mechanisms have limited the infiltration by immune cells and therapeutics [2]. Here we show the synergistic therapeutic effect of nanomedicines [3] in combination with ICI in preclinical models of breast carcinoma (BC) and melanoma. Polymeric nanoparticles (NP) were designed to target dendritic cells (DC) and the TME by incorporating tumor-associated antigens, toll-like receptor ligands CpG and Poly(I:C), and regulators of potent immune suppressor molecules within the TME. NP surface was modified to promote DC activation, but also to potentiate their delivery to the TME. Cy5.5-labeled NP were extensively internalized by DC and triggered their activation *in vivo*. Higher levels of DCrelated co-stimulatory molecules such as CD80, CD86, and CD40, were observed when compared with non-carbohydrate carriers. The anti-tumor immune-mediated effect was evaluated *in vivo* in melanoma/BC-bearing mouse models. NP successfully induced a potent immune-mediated anti-tumor response against melanoma and BC. Synergistic anti-tumor effects were observed when NP was combined with ICI. The multifunctional nanomedicines re-shaped the immune profiling within the TME of

melanoma and BC, which correlated with the overall anti-tumor effect obtained in this combinatorial scheme. 4T1 and E0771 tumorbearing animals treated with the multifunctional nanomedicine combined with αOX40 showed a noteworthy tumor remission, with prolonged overall survival. In conclusion, the developed nanotechnology-based system induced a strong antigen-specific immune response and unlocked melanoma and BC to standard immunotherapeutic approaches, as immune checkpoint modulators.

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Thursday 9:45	**Quantitative interaction of nano-colloids with cells**
	Neus Feliu
	Fraunhofer IAP-CAN, Germany

When cells internalized nanoparticles via endocytosis this involves also proteins bound to the surface of the nanoparticles. After internalization, the original protein corona may be partly exchanged. In case the proteins bear labels providing contrast for imaging, the *in vivo* distribution of the originally bound proteins as well as the one of the nanoparticles can be determined. This can be done for example with fluorescence or X-ray fluorescence based method. Colocalization analysis then provides information about the degree in which the original protein corona is retained.

Most studies about the interaction of nanoparticles (NPs) with cells are focused on how the physicochemical properties of NPs will influence their uptake by cells. However, much less is known about their potential excretion from cells. In order to control and manipulate the number of NPs in a cell however both, cellular uptake and excretion need to be studied quantitatively. Monitoring the intracellular and extracellular amount of NPs over time (after residual non-internalized NPs have been removed), enables to disentangle the influence of cell proliferation and exocytosis, which are the major pathways for the reduction of NPs per cell. Proliferation depends on the type of cells, and exocytosis depends in addition to the type of cells also on the properties of the NPs, such as their size. Examples are given on the role of these two different processes for different cells and NPs.

Thursday 11:15	**Nature-inspired nanotechnology for RNA delivery to immune cells**
	Roy van der Meel
	Eindhoven University of Technology, Netherlands

Nucleic acid therapeutics are revolutionizing healthcare via silencing, expressing, or editing genes. However, nucleic acid-based drugs require chemical modifications and sophisticated nanotechnology to prevent their degradation, reduce immunostimulatory effects, and ensure intracellular delivery. Lipid nanoparticle (LNP) technology is the current gold standard delivery platform technology that has enabled the clinical translation of the first siRNA drug Onpattro and the COVID-19 mRNA vaccines. Nevertheless, currently approved LNP systems are mostly suited for vaccine purposes following local administration or hepatic delivery following intravenous administration.
Here, I introduce a nanodelivery platform based on natural lipoproteins, which prevents premature degradation of small interfering RNA (siRNA), ensuring its targeted and intracellular delivery to hematopoietic stem and progenitor cells (HSPCs) in the bone marrow. After establishing a prototype apolipoprotein lipid nanoparticle (aNP) that stably incorporates siRNA in its core, we built a comprehensive library of which we thoroughly characterized the individual aNPs' physicochemical properties. Following the in vitro screening of all formulations, we selected eight siRNA-aNPs that are representative of the library's diversity, and determined their capacity to silence lysosomal-associated membrane protein 1 (LAMP1) in immune cell subsets in mice, using an intravenous administration regimen.
Our data show that using different aNPs, we can achieve functional gene silencing in immune cell subsets and their bone marrow progenitors. Beyond gene silencing, the aNP platform's inherent capacity to engage immune cells provides it with considerable potential to deliver other types of nucleic acid therapeutics to HSPCs.

Thursday 11:55	**Experiments towards mapping the protein corona around endocytosed nanoparticles**
	Wolfgang Parak
	University of Hamburg, Germany

When cells internalized nanoparticles via endocytosis this involves also proteins bound to the surface of the nanoparticles. After internalization, the original protein corona may be partly exchanged. In case the proteins bear labels providing contrast for imaging, the *in vivo* distribution of the originally bound proteins as well as the one of the nanoparticles can be determined. This can be done for example with fluorescence or X-ray fluorescence based method. Colocalization analysis then provides information about the degree in which the original protein corona is retained.

Thursday 12:25	**Comparative optimization of polysaccharide-based nanoformulations for cardiac RNAi therapy**
	Hélder Santos
	University Medical Center Groningen, Netherlands

Ionotropic gelation is widely used to fabricate targeting nanoparticles (NPs) with polysaccharides, leveraging their recognition by specific lectins. Despite the fabrication scheme simply involves self-assembly of differently charged components in a straightforward manner, the identification of a potent combinatory formulation is usually limited by structural diversity in compound collections and trivial screen process, imposing crucial challenges for efficient formulation design and optimization. Herein, we report a diversity-oriented combinatory formulation screen scheme to identify potent gene delivery cargo in the context of precision cardiac therapy. Distinct categories of cationic compounds are tested to construct RNA delivery system with an ionic polysaccharide framework, utilizing a high-throughput microfluidics workstation coupled with streamlined NPs characterization system in an automatic, step-wise manner. Sequential computational aided interpretation provides insights in formulation optimization in a broader scenario, highlighting the usefulness of compound library diversity. As a result, the out-of-bag NPs, were utilized for loading therapeutic RNA to ameliorate cardiac reperfusion damages and promote the long-term prognosis.
Overall, this work presents a generalizable nanoformulation design strategy for polysaccharides, offering design principles for combinatory formulation screen and insights for efficient formulation identification and optimization.

Thursday 14:10	**Polypept(o)ides in cancer immune therapy**
	Matthias Barz
	Leiden University, Netherlands

The enormous potential of polymeric nanomedicines arises from the possibility to combine desirable material properties with compartmentalized functionalities in one distinct nanoparticle, to encapsulate and deliver single or multiple drugs and limit their side of action.[1] The Barz lab has developed a novel class of materials called polypept(o)ides, which enables the design of multifunctional nanomedicines with low immunogenicity, tunable chemical functionality, size, and shape, which are completely based on endogenous amino acids.[2,3] The presentation will summarize our work on cancer (immune) therapy.
The efficiency of cancer immune therapies can be significantly improved when induction of immunity and abolishment of immune tolerance are achieved in parallel.[4] For an effective immune therapy of solid tumors local abolishment of immune suppression is essential to enable the immune system fight cancer cells.[5] On way tumor cells and related tolerogenic

immune cells use to dampen or completely silence immune responses against solid tumors is the use of cyclic adenosine monophosphate (cAMP). To break this cycle, we use PeptoMicelles containing an adenosine cyclase inhibitor, which block the enzyme producing cAMP. In combination with a non-therapeutic regulatory T cell depletion or adjuvants, PeptoMicelles achieve a complete remission of established B16-F10-OVA tumors.[6] In a second approach, iron oxide nanoparticle loaded PeptoMicelles are used to target tolerogenic macrophages to restore a tumor suppressive proinflammatory phenotype. When applied to primary murine and human macrophages, these nanoparticles display preferential uptake, sustained intracellular iron release, and induce a strong inflammatory response.[7] When nanoparticles are combined with first line therapies, drug efficacy and overall survival can be substantially improved in rodent models of lung cancer.[8]
Besides, also the modulation of the tumor microenvironment presents an interesting target in the therapy of solid tumors. Recently MFAP-5 was identified as targeted to alter the tumor stroma and improve tumor accessibility. Since LNPs failed to provide therapeutic RNAi in hepatocellular liver carcinoma (HCC model), we developed multicompartment polyion complex micelles (PICM) based on triblock copolypeptoids, which are stable in circulation, accumulate in the tumor microenvironment, and efficiently deliver siRNA against MFAP-5 into the cytosol of cancer associated fibroblasts (CAFs) to achieve regression of established liver tumors.[9] Therefore, the presentation will highlight the use of functional polymers and underline the potential of polypept(o)ide-based nanomedicines in cancer (immune) therapy.

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Thursday	**Polypeptide-based nanomedicine unveiling tropism and biological barrier crossing capabilities**
14:50	**María Vicent**
	Príncipe Felipe Research Center, Spain

Polypeptides play a crucial role in several advanced fields, particularly in nanomedicine [1]. The physicochemical characteristics and biological performance of polypeptide-conjugates are intricately influenced by multiple structural factors. This highlights the importance of conducting comprehensive structure-activity relationship studies to optimize the design of hierarchical polypeptide conjugates. Notably, the inherent structural complexity of these

conjugates offers a distinct advantage, as even minor structural modifications can lead to remarkable and unexpected biological effects [1].

In our research group, we have addressed traditional challenges in polypeptide synthesis by employing highly controlled reactions, followed by exhaustive characterization. This approach has allowed us to create well-defined polypeptide architectures through NCA polymerization techniques [2]. Additionally, post-polymerization modifications enable the introduction of various functionalities, resulting in a spectrum of orthogonal reactive attachment sites [1,3]. By applying these methods and a bottom-up strategy, we have successfully synthesized polypeptides with diverse architectures, such as diblock copolymers and star-shaped structures, which have the capacity to self-assemble into supramolecular nanostructures [4]. These nanostructures exhibit remarkable properties, including tissue specificity [4], subcellular compartment tropism [5], and even potential for brain delivery [6]. This strategic approach, combined with the careful design of polymer-drug linkers [3, 5-7], has enabled comprehensive in vitro and *in vivo* evaluations. The results demonstrate a lack of toxicity, an improved in vitro cell internalization rate, and significantly increased terminal and accumulation half-life *in vivo*, along with notable accumulation in lymph nodes [4], mitochondria [5], or the brain [6].
These promising findings suggest that these systems could serve as effective nanocarriers for therapeutic and/or diagnostic applications [8].

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Thursday 16:30	**Nanoparticles in the design of tissue engineering constructs using bottom-up and top-down approaches**
	João Mano
	University of Aveiro, Portugal

Bioinspired engineered microenvironments provide cells with a holistic “instructive niche” that offers the adequate entourage for cellular control both in space and time. In bottom-up tissue engineering approaches small elements can be used as building blocks to be assembled into large constructs to produce macroscopic tissues. We have been proposing different strategies for generating such basic unit elements with well-defined combinations of cells and biomaterials. We can use, for example, nanoparticles to form colloidal gels that could be used to produce injectable or 3D printed hierarchical constructs. Another possibility is to fabricate cell-rich membranes using magnetic force based tissue engineering. The cells internalised initially magnetic nanoparticles and are forced to accumulate into non-adherent surfaces using external magnetic fields. After maturation, cell constructs can be obtained with different geometries, sizes and stratified heterogeneous organizations. In particular, we developed thin cell micro-stamps that could be assembled into more complex structures. These magnetic cell membranes could be also magnetically stimulated to trigger some biological effects, including stem cell differentiation. We are also developing top-down tissue engineering solutions to produce hybrid scaffolds. Hollow channels in tissue engineering constructs are crucial for mimicking physiological environments and facilitating the rapid delivery of nutrients and oxygen to cells. We develop enzyme-based microparticles incorporating magnetic nanoparticles able to engrave channels by the action of an external magnetic field. This new concept could open new avenues in creating fully controlled channels, in a single, wireless, top-down and biocompatible step, in hydrogels or soft-materials, even with complex tortuosity.

Thursday 17:10	**Observations which might inspire drug delivery scientists for guiding bio-therapeutics over biological barriers**
	Stefaan de Smedt
	University Ghent, Belgium

Over the past 30 years protein bio-therapeutics experienced major clinical successes and nowadays represent the fastest growing category of drugs. Meanwhile, progress in the understanding of RNA biology has led to the identification of new RNA classes with unanticipated functions. Following these advances new therapeutic strategies based on nucleic acids have been introduced. Examples are single stranded antisense oligonucleotides, double stranded small interfering RNAs and microRNAs, all for inhibiting gene expression and,

most recently, single guide RNAs for the editing of mutated genes. In addition to nucleic acids which silence or edit genes, there is a major interest as well in the therapeutic potential of messenger RNAs to express proteins in cells for e.g. vaccination to protect the population against infections or to treat cancer patients. As 30–40 years ago, when recombinant proteins and monoclonal antibodies began to be developed as therapeutics, it seems that with nucleic acid bio-therapeutics we are again on the brink of a revolution in drug development. Today the majority of clinically advanced nucleic acids are systemically injected and tested for liver associated diseases. However, numerous potential targets for nucleic acids are present in tissues beyond the liver, like in the lungs and the eyes, which can best be reached through local administration of the nucleic acids, like through inhalation in the lungs or local injections in the eye.

Fulfilling the therapeutic expectations of nucleic acids will however require to overcome major delivery hurdles, including how to keep them stable and how to deliver them across cellular, extra- and intracellular membranes into the cytosol. Currently, the packaging of nucleic acids into well designed nanoparticles (‘nanomedicines’) is the major approach taken to deliver them in cells. While major successes have been achieved, it remains however of paramount importance not to forget that only a very low percentage (~1%) of systemically injected nucleic acids packaged in nanoparticles become successfully delivered at their targets. The lack of safe and efficient delivery technologies remains the major bottleneck for the breakthrough of nucleic bio-therapeutics.

At Ghent University we undertake efforts for a better understanding of the physicochemical and biopharmaceutical understanding of nanomedicines, including lipid nanoparticles (LNPs) loaded with nucleic acids, this to improve their delivery performance and safety. Though, while the clinical introduction of mRNA vaccines has boosted the interest and investments by the pharmaceutical industry into the further development of LNPs, at university we consider it our role to keep on trying to identify new concepts and strategies which might open new avenues for the delivery of bio-therapeutics to targets which cannot be reached through nanoencapsulation. This lecture will explain our recent efforts in finding strategies to deliver nucleic acids from the extracellular space, over cell- and organelle-membranes, into the cytosol. Both pharmaceutical, biological, physical and engineering aspects of our work will be highlighted. A special emphasis will be on the delivery of nucleic acids to targets in the eye.

POSTER OVERVIEW

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No. 8	**The combination of 2D (graphene oxide) immunomodulators and fibrin hydrogels as a neoadjuvant for post-resection glioblastoma** Despoina Despotopoulou / Catalan Institute of Nanoscience and Nanotechnology, Barcelona, Spain
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Poster

No. 1

The water-soluble fullerenes for pancreatic cancer treatment

Maciej Serda[a], Katarzyna Malarz[b,c], Julia Korzuch[a], Magdalena Szubka[b], Anna Mrozek-Wilczkiewicz[b,c], Patryk Rurka[b], Dominik Dreszer[a], Magdalena Rost-Roszkowska[d] and Robert Musioł[a]

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Pancreatic ductal adenocarcinoma (PDAC) is an extremely aggressive type of cancer with a poor prognosis and a five-year survival rate of less than 5%. Unfortunately, traditional chemotherapy, which is usually based on gemcitabine is not effective. Although new therapies are vigorously sought after in this field, no efficient remedy is available as yet, including novel drug design strategies, such as nanomedicine, immuno- or gene related therapies. The main goal of our studies was to develop a fullerene-based nanoplatforms for treatment and diagnosis of pancreatic tumor. The first line of chemotherapeutic drugs that were modified in that study were gemcitabine and EGFR inhibitor Erlotinib.

Here we used direct amination strategy to add aliphatic amines to fullerene core (C60, C70 and Gd@C82) and then conjugate it with modified EGFR inhibitor Erlotinib. The developed synthetic protocol was able to produce a highly water-soluble[60]fullerene nanoconjugate with Erlotinib in good yields. The physio-chemical characteristics of the fullerene nanomaterial confirms that two Erlotinib units are attached to the [60]fullerene scaffold via formation of amide bonds. We characterized modified buckyballs using NMR, FT-IR, UV-VIS and XPS spectroscopy. The size and morphology of synthesized fullerene nanomaterials were analyzed using dynamic light scattering methodology followed by TEM analysis, confirming formation of fullerene aggregates. For gadolinium containing fullerene derivative Gd@C82, both relaxation times T1 and T2 were measured, confirming its high potential for using as MRI-contrast agent.

All the synthesized fullerene nanomaterials were tested on pancreatic cancer cell lines. C60BUT exhibited the highest anticancer activity on PANC-1, PAN02 and AsPC-1 (IC50 ranging from 16.88 to 45.46 uM). Further detailed studies focused on the molecular mechanism of action revealed that the tested fullerenes were able to cell cycle arrest in G0/G1 phase. This effect was confirmed by downregulation of cyclin E1 and upregulation of p27 protein. Interestingly, C60BUT and C70BUT-ERL triggered autophagic cell death by inhibiting mTOR, while Gd@C82EDA-ERL induced apoptosis in PAN02 and AsPC-1 cells. Moreover, the effects of the tested fullerenes on the regulation of EGFR signaling were determined. Our analyses showed that C60BUT and C70BUT-ERL effectively inhibited the expression of p-EGFR, p-Akt, PI3K proteins. Moreover, in the midgut epithelium of D. melanogaster individuals (both males and females), no changes in cell ultrastructure were observed compared to the control group.

Poster No. 2	**Fish collagen-based nanocomposite polymeric films: a design approach for antibacterial and hemostatic activity in tissue regeneration**
	Jayabal Prakash, Santosh S. Mathapati
	Biomaterials Laboratory, Translational Health Science and Technology Institute, Faridabad, India

A novel class of wound dressing material was developed, characterized, and tested for accelerated healing and healthy recovery of cells/tissues, derived from fish waste and modified with a polymer nanocomposite. The initiative aimed to contribute to waste management strategies by understanding waste generation volume and potential valorization. Highly pure collagen extraction from fish waste was achieved through a hybrid approach of enzymatic and chemical extraction techniques. The project main novelty involved the development of novel Fish Collagen (FC) films modified with Mxene/ZrO_2-Cu polymer nanocomposite using an eco-friendly Gel casting technique. The as-developed patches underwent comprehensive characterization for their physio-chemical, mechanical, photo, and barrier properties. In vitro studies were conducted on the patches to heal human fibroblast cell lines, providing insights into performance and biocompatibility. Additionally, the realistic capacity of the wound dressing, including durability and antibacterial ability, was investigated in vivo using mice as a model for wound healing.

Poster No. 3	***In vivo in situ* engineered BMSCs promote bone healing through gene silencing**
	Wu Yang
	Faculty of Science and Engineering, Åbo Akademi University, Turku, Finland Department of Orthopaedics, Shanghai Jiao Tong University School of Medicine, China Turku Bioscience Centre, University of Turku and Åbo Akademi University, Turku, Finland

Background: Most fractures heal with treatment, but delayed fracture healing and nonunion (bone nonunion) still occur in about 5–10% of patients. BMSCs in bone nonunion show decreased numbers and altered gene expression. For example, the expression of long-stranded noncoding RNA maternal expressed gene 3 (lncRNA MEG3) is upregulated in bone tissue from bone nonunion. Therefore, bone seeding incorporating RNA interference (RNAi) may be a promising strategy for promoting bone healing.

Methods: An siRNA for MEG3 (siMEG3) was screened and constructed. Calcium phosphate (BCP) nanoparticles functionalized with BSA and alendronate were used to encapsulate siMEG3 as a gene carrier (siMEG3@BCP), loaded into hydrogel microspheres as a secondary structure (siMEG3@BCP@MS), and then modified hydrogel microspheres with SDF-1α (siMEG3@BCP@MS/SDF). The ability of siMEG3@BCP@MS/SDF to recruit BMSCs as bone seeds was verified *in vivo* and in vitro. The role of siMEG3@BCP@MS/SDF for osteogenic differentiation of BMSCs was tested. The role of siMEG3@BCP@MS/SDF in repairing cranial defects was characterized in rats.
Results: siMEG3@BCP@MS/SDF was successfully constructed. As a gene silencing-based bone seed, siMEG3@BCP@MS/SDF could recruit BMSCs *in vivo* and *in vitro*. furthermore, siMEG3@BCP@MS/SDF could promote bone repair by promoting the osteogenic differentiation of BMSCs.
Conclusion: siMEG3@BCP@MS/SDF exemplifies bone repair potential in vitro and in vivo by recruiting and modifying BMSCs.

Poster	***In situ* generating CO gas for destroying bacterial biofilms**
No. 4	**Pengzhen Zhuang,** Wu Yang, Yu Zhang, Yu Chen, Hongbo Zhang, Wenguo Cui
	Faculty of Science and Engineering, Åbo Akademi University, Turku, Finland

The resistance and impermeability of bacterial biofilms lead to incurable infections. Interference with bacterial respiration is the key to the eradication of bacterial biofilm, but breaking the deep-tissue biofilm barrier to disrupt bacterial respiration still lacks effective means. Here, we report a hydrogel microsphere that disrupts bacterial respiration, supports in situ production of carbon monoxide gas (CO) to enhance the oxygen-depleted environment of biofilms and disrupts the bacterial respiratory chain, eliminating the bacterial biofilm ecotone (BRDMs). Under the specific interaction of α-helical structure and bacterial biofilm, BRDMs rapidly anchored and accumulated on the surface of bacterial biofilm within 8 h. Meanwhile, 8.64 μM CO gas was released in situ under an oxidative stress environment to deeply penetrate the biofilm and continuously destroy bacterial terminal oxidase, block bacterial respiration and finally disintegrate the biofilm. In a model of osteomyelitis, BRDMs disrupt the ecotopic colonization of MRSA biofilms in deep tissues, reduce inflammation, restore internal environmental homeostasis and accelerate tissue regeneration. BRDMs could be designed to remove drug-resistant biofilms from a wide range of deep tissues.

Poster

No. 5

Phase-transforming nanodroplets for acoustically enhanced mRNA delivery

Haikun Liu[a,b], Mark Louis P. Vidallon[a,b,c,d], Henry Gordon[a,b], Aidan P.G. Walsh[a,b,d], Yuyang Song[a,b], Mitchell J. Moon[e], Karlheinz Peter[b,d,e,f] and Xiaowei Wang[a,b,d,f]

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Since the emergence of the COVID-19 pandemic, mRNA therapeutics have captured global attention, accelerating research and development in this field. However, the progress of mRNA therapy for cardiovascular diseases (CVDs) has been hindered by the lack of advanced delivery systems tailored to the unique demands, such as low immunogenicity and targeted delivery. In this study, we developed phase-change perfluorocarbon (PFC) nanodroplets with potential mRNA theranostic applications: multimodal imaging capabilities using either ultrasound or MRI with acoustically triggered mRNA release for localised and enhanced mRNA transfections. Lipid-coated PFC nanodroplets (NDs) were engineered using three different PFCs—perfluoro-15-crown-5-ether (PFCE), perfluorohexane (PFH), and perfluoropentane (PFP)—through a cutting-edge high-pressure homogenization technique. Resulting emulsion droplets had diameters between 200–300 nm and possessed positive surface charge, rendering them ideal for mRNA delivery. All these three NDs exhibited strong ultrasound contrast and PFH NDs significantly improved ^{19}F MRI contrast compared with the control group. In a live mice imaging model, injections of all three PFC NDs at 3 mg mL^{-1} demonstrated significant ultrasound contrast enhancement within the lumen of the inferior vena cava. Contrast enhancement observed via *in vitro* and *in vivo* diagnostic ultrasound and ^{19}F MRI imaging demonstrates the trackability of these nanodroplets, positioning them as potential theranostic materials for on-demand localised release mRNA therapeutics. To test the transfection efficiency of nanodroplets, we used the mRNA that codes for the enhanced green fluorescent protein (eGFP mRNA) to directly visualize the translated fluorescent protein after introduction into Chinese hamster ovarian (CHO) cells. Following a direct transfection, we found PFH and PFP nano droplets provided significantly higher GFP expression, as compared to PFCE (47%, 43% vs 9%, $p<0.0001$) via fluorescence microscopy and flow cytometry. Using ultrasound stimulus for controlled release studies, we observed that ultrasound bursts increase the transfection efficiency of PFH and PFP ND by about 20%, compared to the control and all ND treatments without ultrasound burst. In contrast, PFCE nanodroplets exhibited marginal eGFP mRNA transfection efficiency with and without ultrasound treatment. All three

formulations are highly bio/hemo-compatible in blood and show no toxicity to cells *in vitro*, hence these fabricated materials are prospective candidates for further development and application *in vivo* (work in progress). In summary, PFC nanodroplets are potential nanomaterials for an ultrasound imaging- or MRI-guided theranostic system with ultrasound-stimulated release capabilities for localised mRNA transfection. With these capabilities, these nanodroplet systems represent highly promising mRNA delivery systems for the treatment of CVDs.

Poster No. 6	**Platelet-membrane bubbles: Dual functionality ultrasound contrast and precision clot-targeting agents**
	Mark Louis P. Vidallon[a,b,c,d], Mitchell J. Moon[a,b], Haikun Liu[a,b], Yuyang Song[a,b], Simon Crawford[c], Boon Mian Teo[c], James D. McFadyen[a,b,c,d,e] Alexis I. Bishop[c], Rico F. Tabor[c], Karlheinz Peter[a,b,c,d,e], Xiaowei Wang[a,b,c,d] [a]Baker Heart and Diabetes Institute, Melbourne, Australia [b]University of Melbourne, Parkville VIC 3010, Australia [c]Monash University, Clayton, Australia [d]La Trobe University, Bundoora, Australia [e]The Alfred Hospital, Melbourne, Australia

Thrombosis-related cardiovascular diseases remain the leading global cause of mortality and morbidity. Detection and treatment of these diseases necessitate the use of advanced materials with thrombus-specific targeting capabilities. In this study, we present a pioneering approach in the field of biointerfacing and colloid science, with a focus on clinical translation, aimed at advancing early diagnosis and enhancing treatment options for thrombotic disorders. We introduce the fabrication of platelet membrane-derived bubbles (PMBs), which exhibit distinctive advantageous characteristics for diagnostics and theranostics, as compared to conventional nanoparticles.

PMBs were fabricated via our patented rapid sonication technique [1,2]. Fabricated PMBs possess an average diameter of 700 nm and a negative ζ-potential, mirroring the attributes of parent platelet membranes. Utilizing diagnostic ultrasound imaging, we demonstrated the ability to visualize PMBs as hyperechogenic entities in agarose phantoms *in vitro* (100- to 400-fold increase in intensity vs buffer control, $p < 0.0001$). We also visualised and quantified an increase in signal intensity *in vivo*, in the vessel of mice after intravenous injection (two- to three-fold increase in intensity vs buffer control, $p < 0.0001$). Furthermore, through confocal laser microscopy, we verified the retention of crucial transmembrane proteins, such as CD41 (GPIIb) and CD42 (GPIb), pivotal in conferring platelet-specific targeting functions. Importantly, our platelet aggregation studies confirmed that PMBs do not induce platelet aggregation, as compared to the platelet agonist thrombin receptor-activating peptide (TRAP, 30 µM) ($p < 0.0001$). Interestingly, we demonstrated the binding ability of PMBs and their adherence to preformed platelet-rich *in vitro* thrombi. Furthermore, PMBs are highly

compatible with blood and showed no toxicity to cells *in vitro*, hence these fabricated materials are prospective candidates for further development and application *in vivo*. Overall, our work showcases the safe and precise utilization of PMBs to directly target acute thrombosis induced by laser injury in murine mesenteric veins *in vivo*, as visualized through intravital microscopy.

In conclusion, we have successfully developed a rapid method for generating PMBs with unique ultrasound-directed and thrombus-targeting properties. These exceptional attributes of PMBs hold significant promise for advancing the field of ultrasound diagnostic thrombus imaging and clot-targeted therapy in the clinical context.

Representative optical (*top left*) and transmission electron micrographs (*top right*) of platelet membrane-derived bubbles (PMBs). Inset shows the dynamic light scattering size distribution of PMBs. Representative sum projections of thrombi formed in intravital laser-induced model of thrombosis before (*top middle*) and after (*top right*) intravenous injection of $DiIC_{12}$–labelled PMBs. Platelet GPIb shown in purple and PMBs in red. Representative ultrasonograms of mouse inferior vena cava (*bottom middle*) before and (*bottom right*) after injection of PMBs.

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Poster	**Composite functional vesicles induce "find-me/eat-me" in macrophages to regulate RA disease**
No. 7	**Yu Zhang**[a], Yawei Du[b], Yu Chen[a], Pengzhen Zhuang[a], Wu Yang[a], Wenguo Cui[b], Hongbo Zhang[a] [a]Pharmaceutical Sciences Laboratory, Åbo Akademi University, Turku, Finland. [b]Ruijin Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai, China.

Introduction

Patients with rheumatoid arthritis present with synovial hyperplasia and small joint swelling, which is mainly due to the destruction of the patient's immune homeostasis. M1 type pro-inflammatory macrophages continue to increase in the synovium, thereby breaking the M1/M2 balance and promoting the inflammatory process of RA[1]. The application of functional nanoparticles provides the possibility to regulate macrophage phenotypes[2]. Artemisinin plays an important role in anti-inflammatory aspects. Its derivative (artesunate,

ART) is a good precursor for lipid synthesis and can well modify apoptotic vesicles. Its modified complex plays a key role in immune regulation and anti-inflammation in RA.

Materials and Methods

Artemisinin has anti-inflammatory and phagocyte regulation effects. As its derived lipid, artesunate is relatively stable and has a long circulation cycle in the body and is used to prepare composite nanovesicles. Synovial fibroblast drugs stimulate cell apoptosis, and apoptotic membrane extraction kits isolate apoptotic membranes. Labeling of prepared liposomes and apoptotic membrane vesicles with PKH67 reagent. Artesunate lipids and apoptotic membranes were ultrasonically oscillated for 30 minutes at an ultrasonic amplitude of 30% with an interval of 3 seconds. Subsequently, the composite vesicles were co-cultured with macrophages. After 12 hours, rhodamine and DAPI dyes labelled macrophage nuclei and cytoskeleton respectively. And the ability of macrophages to phagocytose the composite vesicles was detected by confocal microscopy.

Results and Discussion

After incubation of apoptotic vesicles with macrophages for 12 hours, PKH67-labeled normal liposomes (control group) and non-apoptotic cell vesicles (Nms) were rarely phagocytosed by macrophages, while PKH67-labeled artesunate the amount of non-apoptotic vesicles (ART&Nms), artesunate liposomes (ARTs) and apoptotic vesicles (Ams) phagocytosed by macrophages was increased. However, PKH67-labeled artesunate & apoptotic cell vesicles (ART&Ams) were significantly phagocytosed by macrophages (Figure 1). These phenomena showed that macrophages recognize and phagocytose artesunate-modified apoptotic complex vesicles.

Conclusion

It could be shown that the apoptotic composite membrane system (ART&Ams) significantly increases the phagocytic capacity of macrophages.

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Poster	**The combination of 2D (graphene oxide) immunomodulators and fibrin hydrogels as a neoadjuvant for post-resection glioblastoma**
No. 8	**Despoina Despotopoulou**[a], Maria Stylianou[b], Lorena Fernandes[b], Thomas Kisby[b], Neus Lozano[a], Kostas Kostarelos[a,b]
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Glioblastoma (GBM), a grade IV primary brain tumor, is one of the most lethal forms of cancer due to high recurrence rates after surgical resection, radiotherapy and systemic chemotherapy [1]. To enhance post-resection treatment efficacy, we explored novel 2D nanoscale-based platforms and hydrogels for improving local therapies. Medical-grade graphene oxide (GO) stands out for its large surface area offering enhanced loading of biologically-active molecules and its facile biodegradability [2,3]. First, GO was noncovalently complexed with the immunomodulator R848, a synthetic TLR7/8 agonist, yielding stable flat-shaped nanoconstructs. Physicochemical characterization confirmed successful complexation, and in vitro studies demonstrated immunomodulatory activity [4].

In vivo investigations demonstrated the GO:R848 complex ability to alter the immunosuppressive landscape of aggressive cancer types through macrophage reprogramming, using a syngeneic GBM model [5]. Then, we leveraged biocompatible FDA-approved fibrin hydrogels to explore two alternative neoadjuvant strategies for local post-surgery treatment. Our optimized hydrogels exhibit rapid gelation and low mechanical stiffness, crucial for brain tissue applications. *In vivo* studies evaluated the hydrogel capacity to either ´seal´ pre-deposited GO nanosheets in the post-resection cavity or release hydrogel-incorporated GO in a controlled dosage design (Fig. 1). These findings suggest that GO can be decorated with immune-activating molecules, paving the way for advanced cancer immunotherapeutics. In parallel, the use of fibrin hydrogels as either sealants or carriers shows potential to enhance local therapies in post-resected GBM.

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Poster

No. 9

Enhancing the relaxivity properties of magnetic nanoparticles by rational control of the local magnetic environment

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Cancer is a major problem of the public health, a study published by the EC in 2024 showing that the main cause of death of people under 65 in 2021 was cancer. Apparently, it is an unbalanced but not hopeless battle due to the efforts made in cancer-oriented R&I globally. To reduce inconsistencies between countries contribution and pushing the balance towards decreasing cancer death rate, setting up new laboratories in cancer research is needed. Since January 2021, a new laboratory, the Nanotechnology Laboratory, started to be shaped at the Regional Institute of Oncology of Iasi, Romania. It's establishment is funded by two projects, a H2020-ERA Chairs grant and a national project. Although it has just opened its doors, the team is ready to align itself with international efforts against cancer and is committed to providing valuable results needed to advance the knowledge of applied nanomedicine for diagnosis and therapy, while forming new specialists. One of the topics addressed deals with the development of iron-based nanoparticles for two types of cancer that are themselves at the extremes of clinical translation, namely glioblastoma and breast cancer. Magnetite and metal-doped magnetite were synthesized by coprecipitation using chlorides as metal precursors and Pluronic F127 as dispersant. They were characterized by ICP, XRD, XPS, FTIR, TEM, DLS, Zeta potential, VSM to assess the real concentration of metals in the samples and their surface/bulk distribution, crystalline structure, morphology and (hydrodynamic) size and size distribution, surface charge and magnetic properties. The viability tests performed on three tumors lines of GBM and three tumors lines of breast cancer and corresponding normal cell lines confirmed their safety. They proved promising relaxometric properties measured on agarose phantoms with r_2 values at 1.0 T above those of commercially available relaxation agents. Conjugation with anticancer drugs is currently ongoing in the group.

Poster

No. 10

Physiology-inspired multilayer nanofibrous membranes modulating endogenous stem cell recruitment and osteo-differentiation for staged bone regeneration

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Introduction

Plentiful studies concerning bone defect repair with tissue engineered scaffolds have reported on bone regeneration strategies in a unifactorial fashion focusing on single stimuli for biomaterial design, such as the effects of biochemical, structural and chemical properties, or externally applied mechanical stimulation.[1] However, these scaffolds seemed hard to emulate the highly intricate bone regeneration post-injury due to the multifactorial cues

associated with the cooperative regulation of bone niche.[2] For this reason, cognition of a set of biological cascade phenomena throughout bone physiological and regenerative mechanisms could provide valuable enlightenment for the innovation of regeneration-driven biomaterials.[3]

Aim

To prepare the multilayer nanofibrous membranes that mediate early-phase stem cell recruitment and late-phase osteo-differentiation for achieving bone repair.

Method

Through micro-sol electrospinning and manual multi-layer stacking, the time- and dose-programmed release behaviors of initial rapid release of SDF-1α (a stem cell recruitment factor) and long-term sustained release of MT01 (an osteogenesis-inducing oligodeoxynucleotide) were achieved by the construction of nanofibrous membranes with the sandwich structure of MT01-encapsulated micro-sol electrospun nanofibrous membrane as the intima and SDF-1α-anchored electrospun nanofibrous membrane as the adventitia.

Results

The outer layers of the multilayer nanofibrous membranes could rapidly release the SDF-1α to amplify the SDF-1α/CXCR4 axis for promoting stem cell migration at the early stage, whilst the long-term sustained release of MT01 from the inner layer activated the MAPK pathway to induce stem cell osteo-differentiation. Further animal experiments verified the synergetic effects of multilayer nanofibrous membranes on bone regeneration and vascularization, which were attributed to the dual-delivery of SDF-1α and MT01 for sequentially emulating the process of endogenous stem cell migration and osteogenesis *in vivo*.

Conclusion

The multilayer nanofibrous membranes that mimic the endogenous cascades of bone regeneration could enrich the physiology-mimetic staged regenerative strategy and represent a promising tissue-engineered scaffold for the bone defect.

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Poster	**Bio-inspired porous microneedles dwelled stem cells for diabetic wound treatment**
No. 11	**Lu Fan**[a,b], Xiaoxuan Zhang[c], Li Wang[a,b], Yizuo Song[a], Kexin Yi[b], Xiaoju Wang[b], Hongbo Zhang[a,b,d], Ling Li[e], Yuanjin Zhao[a,c]
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Introduction
Diabetic wound healing is a serious, complex, and chronic process; one current promising and focusing technology in this area is stem cell treatment. However, most of hydrogels loaded stem cell exist complex chemical reactions and cannot provide a pleasant micro-ecological environment. In addition, during the wound healing process, the formation of scab or epithelialization results in skin hardening. Thus, new strategies to enhance stem cell therapy for wound healing are yet to be explored.
Aim
To generate biomimicking porous microneedles (MN) arrays with hydrogel-encapsulated human adipose-derived stem cells (ADSCs) post-perfusion for diabetic wound treatment
Method
We fabricated the desired porous MN arrays with ADSCs hydrogel delivery by a combined template filling. The porous MN arrays were fabricated by simply using UV-curable gelatin methacryloyl (GelMA) and poly(ethylene glycol) diacrylate (PEGDA) hybrid mixed with glass microspheres to fill negative molds, followed by overnight etching. The stem cells were loaded by perfusing ADSCs-encapsulated Matrigel into the porous MN arrays. Through in vitro and *in vivo* experiments, therapeutic ability of porous microneedle scaffolds were further evaluated.
Results
The biocompatible Matrigel offers a bionic microenvironment of stem cell nest suitable for growth. Benefiting from the numerous pore structures of MNs, the loaded ADSCs have enough space to fully absorb nutrients, proliferate greatly and exhibit prompted function. In addition, MN arrays have enough mechanical strength to penetrate the skin, allowing the ADSCs to get into the deep wound areas. It was demonstrated the performance of the resultant MN arrays in promoting tissue regeneration, collagen deposition and angiogenesis in diabetes wounds of rat models.
Conclusion
ADSCs dwelled on porous MNs have enough space to fully absorb nutrients, proliferate greatly and exhibit prompting functions to secrete factor, as well as exhibit significant treatment for diabetic wound. we are convinced that the bioinspired porous MNs can serve as excellent stem cell scaffolds and become a promising therapy for clinic wound healing

Poster

No. 12

Self-healing dynamic hydrogel microparticles with structural color for wound management

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Introduction

Chronic diabetic wounds confront a significant medical challenge because of increasing prevalence and difficult-healing circumstances. It is vital to develop multifunctional hydrogel dressings, with well-designed morphology and structure to enhance flexibility and effectiveness in wound management.[1]

Aim

To develop an intelligent wound dressing with monitoring function, which could efficient deliver the active substances to promote tissue regeneration at the wound.

Method

Silica photonic crystal microspheres are used as sacrificial templates for inverse opal framework. The microsphere comprised a photothermal-responsive inverse opal framework, which was constructed by hyaluronic acid methacryloyl (HAMA), silk fibroin methacryloyl (SFMA) and black phosphorus quantum dots (BPQDs), and was further re-filled with a dynamic hydrogel. The dynamic hydrogel filler was formed by Knoevenagel condensation reaction between cyanoacetate and benzaldehyde-functionalized dextran (DEX-CA and DEX-BA).

Results

The composite microspheres can be applied arbitrarily, and they can adhere together upon near infrared (NIR) irradiation by leveraging the BPQDs-mediated photothermal effect and the thermoreversible stiffness change of dynamic hydrogel. Additionally, eumenitin and vascular endothelial growth factor were co-loaded in the microspheres and their release behavior can be regulated by the same mechanism. Moreover, effective monitoring of the drug release process can be achieved through visual color variations.

Conclusion

The microsphere system has demonstrated desired capabilities of controllable drug release and efficient wound management. These characteristics suggest broad prospects for the proposed composite microspheres in clinical applications.

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Poster

No. 13

Magnetic iron oxide nanoparticles for antimycobacterial application

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Metallic nanomaterials are easy to synthesize and may be used as nano-vehicles for therapeutic agents. This study focused on investigating the antimicrobial effect of magnetic iron oxide nanoparticles (MION) stabilized with one or more metals against *Mycobacterium smegmatis* mc2155 (*M. smeg*).

MION was synthesized via the chemical co-precipitation of FeSO4.7H2O and FeCl.6H2O and surface-coated with secondary metals producing bimetallic nanoparticles (NP): Ag/MION, Cu/MION, Ni/MION, and Au/MION. The individual and combined antimicrobial effects of these bimetallic NP against *M. smeg* were evaluated using a time-kill assay. Additionally, ternary nanoparticles (Ag/Cu/MION and Ag/Ni/MION) formed from active synergistic combinations were tested for their antimicrobial properties, and their impact on cell morphology was examined. The uptake of nanoparticles by bacterial cells was quantified using ICP-MS, and their cytotoxicity was assessed on murine RAW 264.7 macrophage cells.

The NP had an average diameter of less than 80 nm. Among these, the Ag/MION and Cu/MION demonstrated antimicrobial potential, with minimum inhibitory concentrations (MIC) of 3.9 µg/mL and 62.5 µg/mL, respectively. Synergized bimetallic NP exhibited varying levels of antimycobacterial activity in the following order: Ag/MION+Cu/MION (0.5 µg/mL + 1 µg/mL) > Ag/MION+Ni/MION (1.0 µg/mL + 7.8 µg/mL) > Ag/MION+Au/MION (4 µg/mL + 31.25 µg/mL). Similarly, ternary nanoparticles followed the trend of Ag/Cu/MION > Ag/Ni/MION, with MIC of 1.95 µg/mL and 3.9 µg/mL, respectively. The nanoparticles induced morphological changes in treated cells and were non-cytotoxic at the tested concentrations. An ICP-MS study revealed the cellular uptake of nanoparticles.

Conclusively, metal-coated MION demonstrated a potent antimycobacterial effect against *M.smeg*. Future studies will explore the impact of these nanoparticles at the genomic level.

Poster

No. 14

Self-assembled gold nanoclusters for bio-photonic applications

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Gold Quantum Clusters (GQCs) are a class of ultra-small particles presenting tunable photoluminescence (PL) in the first (NIR-I) and second (NIR-II/SWIR) near-infrared windows, making them appealing as tracers for high-resolution imaging of blood vasculature in mice [1-3]. Interestingly, we also noticed a remarkable change in their optical properties with a boost of their absorbance and PL in the NIRs windows when GQCs self-assemble. The main objectives of the SEQUOIA project are now to make the proof of concept that such self-assembly can be obtained and controlled by *in vivo* bio-orthogonal click chemistry after successive intravenous administrations of biocompatible polymer chains and GQCs bearing complementary functions such as strained cyclooctynes (DBCO) and azides. After in situ generation, the nano-assemblies will present enhanced biophotonic properties. Thanks to the presence of cRGD-peptide targeting ligands on the polymer, non-invasive diagnostic applications will lead to the sensitive detection of angiogenesis and of a specific biomarker expressed on activated neo-endothelial cells (integrin αvß3).
Controlled formation and the properties of the nano-assemblies will be studied in increasingly complex environments: serum-containing solutions, microfluidic devices, 2D/3D cell culture and then in mice carrying proangiogenic cellulose sponges implanted subcutaneously. All methods are currently available in our laboratories. The versatility and new optical properties of this platform could be exploited for the definition of the next generation of theranostic agents, but also eventually biophotonic sensors and optoelectronic devices.

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Poster

No. 15

Biodegradable polyphosphoester micelles act as both background-free ^{31}P magnetic resonance imaging agents and drug nanocarriers

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Heteronuclear, “hotspot” Magnetic Resonance Imaging (MRI) is a versatile tool for the monitoring of novel therapies, especially nanomedicines and immunotherapies for treatment of severe diseases, ranging from cardiovascular disease to cancer.[1-2] However, current hotspot agents are usually based on liquid per-and polyfluoroalkyl substances (PFAS) for the detection of fluorine-19 in ^{19}F MRI. PFAS are recognized as major environmental pollutants. Additionally, PFAS can accumulate in organs for long time in a range of month or longer, hampering for example imaging the repeated injections which are crucial for monitoring of therapeutics. We present biocompatible, biodegradable micelles, based on phosphorus-based polymers, polyphosphonates that are uniquely suitable for ^{31}P MRI and drug delivery.[3-4] We solved the fundamental challenges that have so far hampered the MRI of ^{31}P, namely the low sensitivity and intrinsic background by tuning the properties of polyphosphonates. We used anionic ring-opening polymerization to tailor the chemical shift and the microstructure of polymers chains, ensuring sufficient mobility after self-assembly to micelles (Figure 1A). The structural properties of were characterized by Small Angle Neutron Scattering. The gradient microstructure resulted in MRI relaxation times suitable for rapid imaging sequences, such as spin-echo. The micelles were imaged with MRI *in vivo* and are biodegradable (Figure 1B). Moreover, we have shown that the micelles can be imaged with MRI *in vivo* and are biodegradable. Moreover, due to amphiphilic character of gradient copolymers, the micelles can stabilize hydrophobic payload, simultaneously acting as drug delivery agents. Here, we encapsulated a potent anti-cancer, protein-degrading drug PROTAC (ARV-825), and studied the effect of polymer properties on drug encapsulation and release. In summary, we developed unique polyphosphonate gradient copolymer micelles that enable ^{31}P MRI and delivery of therapeutics.

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Poster No. 16	**Chitosan functionalized Mn_3O_4 nanoparticles counteract ulcerative colitis in mice through modulation of cellular redox state**
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Ulcerative colitis (UC) is a class of inflammatory bowel disease (IBD) that causes irreversible and chronic damage to the structure and function of colon mucosa. Recent findings suggest a key role for reactive oxygen species (ROS) in the pathogenesis and progression of ulcerative colitis (UC). To date, no effective and cost-efficient therapeutic strategy has been developed. Here we show that synthesized nanoparticles consisting of chitosan functionalized tri-manganese tetroxide (Mn3O4) can restore redox balance in a mouse model of UC induced by dextran sulfate sodium. Our in-vitro characterization of the developed nanoparticle confirms critical electronic transitions in the nanoparticle to be important for the redox buffering activity in the animal model. Its efficacy as a redox buffer has been evaluated in a cancer cell line (i.e., A549). Further, its therapeutic efficacy was evaluated in a preclinical mice model (BLAB/c) of UC regarding changes in the immunological marker (e.g., IL-1β, IL-12) and histopathology. Expression of several proteins relevant to pyroptosis (like NF-κβ, Caspase I) and mitochondrial redox parameters were estimated to get a glimpse of the molecular mechanism of action.
Oral treatment of Ch-Mn_3O_4 NPs (~3 nm, spherical) efficiently reverses the damage of the colon mucosa with improvements in all immunological and biochemical parameters studied. The histopathological analysis supports the same. A careful administration of the developed nanoparticle reduces inflammatory markers in the animals and the mortality rate from the induced disease. This study presents the potential of Ch-Mn_3O_4 NPs as an inorganic, orally administrable biocompatible nanoparticle with synergistic anti-inflammatory and redox buffering capacity to prevent and treat ulcerative colitis.

Poster No. 17	**Green synthesis and characterization of hyaluronic acid-modified selenium nanoparticles for melanoma therapy**
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Introduction

Melanoma is one of the most aggressive cancers worldwide causing most skin cancer deaths[1]. Resectable lesions are removed by surgery, followed by adjuvant therapy in severe cases[2,3]. Data show that severe forms are associated with activating mutations of BRAF[4,5], which is currently a therapeutic target. However, most patients develop resistance to the existing treatments experiencing recurrences over time. Therefore, overcoming the above-mentioned resistance is still a challenge in melanoma care[6].

Selenium nanoparticles (SeNPs) have proven effective in cancer treatment. Moreover, they have displayed a synergistic effect when loaded with oligonucleotides for cancer gene therapy[7]. Here, hyaluronic acid (HA) modified SeNPs are proposed as targeted platform to deliver siRNA to melanoma cells.

Methods

siRNA-HA@SeNPs were obtained by chemical reduction of Se in sodium selenite to elemental Se. The reaction was carried out using ascorbic acid in aqueous solvent. A complete characterization was carried out by performing Fourier-transform infrared spectroscopy (FTIR), dynamic light scattering (DLS), zeta potential (ZP) and Transmission electron microscopy (TEM) analyses. Their stability over time and in different buffers was also investigated. The *in vitro* cytotoxic activity of the prepared SeNPs was assessed in human melanoma cell lines and in non-neoplastic controls.

Results

The siRNA-HA@SeNPs were successfully prepared through an aqueous chemical synthesis. They showed a rounded, regular shape, a size around 200 nm and a PDI < 0.2. The FTIR and TEM confirmed the surface modification of the SeNPs (Figure 1).

Conclusions

New siRNA-HA@SeNPs for targeted gene therapy in melanoma were successfully obtained, using an eco-friendly and easily scalable strategy.

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Poster

No. 18

Active targeting of molecularly imprinted polymers to MCF7 breast cancer cells, dual drug delivery and biosensing

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The interaction between the proteins within the cells is important for most of the biological processes such as intercellular communication, program cell death and therapeutic targeting. Therapeutic antibodies demonstrate the potential of such targets, and their market value is $125 billion USD. However, these antibodies are difficult to manufacture, have high production costs, poor oral bioavailability, and cell membrane permeability. Cancer cell surfaces have several receptors that are antigens and can be utilized for targeting. In this study, we evaluate molecularly imprinted polymer nanoparticles (nanoMIPs) as an alternative to antibodies to target the breast cancer cell receptors (HER3) for the purpose of dual drug delivery and biosensing. HER3 are targeted due to their overexpression in breast cancer cells, additionly, HER3 when combine with HER2 and/or EGFR receptors, form heterodimers resulting in increase of transphosphorylation and subsequently activates downstream pathways that promote cancer cell growth. Preclinical data shows that overexpression of HER3 worsen overall cancer patient survival and contributes in resistance to antibiotics, targeted therapy and chemotherapy. Moreover, in this study we have also addressed the issue of breast cancer cell killing and imaging at an early stage and accelerate apoptosis by simultaneously adding bioactive substances curcumin and doxorubicin to the nanoMIPs, resulting in dual functional MIPs. We are using bioactive molecules with commercial drugs since they have a low impact on normal cell lines, can reverse multi drug resistance and can be used in the intial stages of breast cancer. The results showed that the nanoMIPs exhibited strong cytotoxicity and antimigration effects against the MCF7 cancer cell line and notably enhance the apoptosis, ROS production, nucleus degeneration, and tumour inhibition in vitro. The rhodamineB attached to the nanoMIPs is used for point of care detection. The dual imprinted MIP is designed to specifically detect the HER3 marker molecule on the surface of breast cancer cells and emit fluorescence.

Poster
No. 19

Refining iron supplementation: preparation, optimization & characterization of liposomal delivery systems

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Introduction: Iron-deficiency anemia (IDA) remains a global health issue with high prevalence, despite the continuous efforts to manage this condition. Oral iron supplementation is a mainstay of treatment choices due to its practicality, accessibility and affordability; however, low patient adherence due to gastrointestinal side effects and poor iron bioavailability are limitations that should not be overlooked. In order to utilize the advantages of oral iron preparations while simultaneously overbridging their hindrances, the aim of our work was to develop and optimize a simple liposomal formulation containing ferrous sulphate.
Methodology: The thin lipid film hydration method was used to produce the liposomes, while central composite Design of Experiments (DoE) was employed to optimize their loading efficiency. Particle size analysis, UV/VIS spectroscopy, FTIR, and Raman spectroscopy were used for physico-chemical characterization. The compatibility of the optimized formulations was determined using a cytotoxicity assay on CaCo-2 cell lines.
Results and Discussion: All optimal iron-loaded liposomes exhibited high encapsulation efficiency (~99%), determined using a spectrophotometric method. Their size ranged between 170 and 380 nm. FTIR and Raman spectra provided insight into structural changes, including iron interactions with the lipid components. Cytotoxicity studies revealed dose-dependent cytotoxicity potential, with no significant cell viability reduction ($p<0.05$) up to the highest concentration of iron tested (200 µg/ml).
Conclusion: This study showed conclusive evidence that liposomal formulations could be used as an alternative to conventional oral iron supplementation and contributes to the development of more efficient and safer therapy approaches for IDA.

Poster
No. 20

Heterodimeric peptide-based building blocks for nanoparticle functionalization

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Currently, twelve nanoparticles have been clinically approved in oncology, either as drug delivery systems or imaging agents. However, it has been demonstrated that only 0.7% of the

injected dose effectively reaches tumor cells upon systemic administration. As an alternative solution, the development of targeted nanoparticle systems, akin to what has been achieved with antibody-drug conjugates, is gaining momentum. In this study, we present a novel strategy for functionalizing nanoparticles using a modular approach, allowing for precise biofunctionalization with tumor-associated antigens and immune-cell-associated antigens. This strategy aims to overcome the challenges associated with site-specific conjugation processes, maintaining controlled ratios of nanoparticles to antibodies, and ensuring batch-to-batch replicates with limited variations.
In this proof-of-concept study, we validated the in vitro targeting specificity of five different nanoparticles (liposomes, PLGA nanoparticles, Gd-based/Tb-based/Eu-based ultrasmall nanoparticles) designed to target HER2, CD38, and NKG2D antigens. Furthermore, in healthy mice, our findings reaffirmed the outcomes of our previously published meta-analysis study, confirming that the pharmacokinetic and pharmacodynamic properties of functionalized nanoparticles are primarily influenced by the nanoparticle itself. Finally, using a HER2+ mouse cancer model, we demonstrated that targeted nanoparticles improved tumor uptake from approximately 4% of the injected dose per gram of tumor when using liposomes alone to as high as 8% when employing HER2-targeting moieties. Additionally, these targeted nanoparticles exhibited enhanced tumor retention over time.
This study underscores the potential of our platform to generate easily implementable targeted nanoparticles capable of addressing a wide range of targets, including both immune-based and tumor antigens. This versatility opens up a broad spectrum of theranostic applications.

Poster

No. 21

Protein corona mitigates the cytotoxic effects of nanoplastics on SK-BR-3 cells

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The pervasive presence of micro- and nanoplastics (MNPs) in the environment and their potential impact on human health is a growing concern. MNPs can circulate through human blood and have been found in various organs, including the breast. The adverse biological effects of pristine MNPs, such as oxidative stress, inflammation, and cytotoxicity, are well-

documented. However, the role of protein corona on the surface of MNPs upon exposure to biological fluids in modulating these effects remains unclear. This study investigates the interactions between pristine and human plasma pre-coated polystyrene (PS) nanoparticles (NPs) with cultured breast cancer cells (SK-BR-3). Pristine cationic PS-NH_2 NPs were found to adhere to the cell membrane, inhibiting HER-2-driven signaling pathways, including the PI3K/AKT and MAPK/ERK pathways, which are crucial for cancer cell survival and growth. Conversely, the formation of protein corona around PS-NH_2 NPs facilitated their uptake by endocytic vesicles, leading to sequestration within lysosomes and a mitigation of the biochemical alterations of phosphorylated AKT and ERK levels. The protein corona was extensively characterized using dynamic light scattering, SDS-PAGE, FT-IR measuments, and mass spectrometry. These analyses revealed significant differences in the size, charge, and protein composition of the coronated NPs compared to their pristine counterparts. These findings indicate that the protein corona not only enhances the cellular uptake of NPs but also influences their intracellular fate, directing them towards lysosomal pathways and thereby reducing their cytotoxic effects. This study highlights the critical role of protein corona in modulating the intracellular fate and biological impact of MNPs, underscoring the importance of considering this factor in health risk assessments.

Poster

No. 22

Designing stealth bio-nano-architectures: Structuring lipid nanoparticles, DNA, and protein corona for *in vivo* gene delivery

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Lipid nanoparticles (LNPs) play a crucial role in addressing genetic disorders, cancer, and pandemics like COVID-19 and its variants. Despite significant achievements in siRNA and mRNA delivery, LNPs still face challenges in effectively encapsulating large-size DNA molecules. This limitation is significant because the successful delivery of large-size DNA holds immense potential for gene therapy, offering transformative opportunities for treating a wide range of genetic diseases. To address this gap, the present study focuses on designing PEGylated LNPs that incorporate large-sized DNA and cationic lipids, deviating from traditional RNA and ionizable lipids. The resulting LNPs exhibit a unique particle morphology characterized by distinct layered subunits composed of alternating lipid bilayers and pDNA monolayers. Inspired by pDNA's ability to neutralize cationic lipids and promote the formation of an opsonin-deficient protein corona, these LNPs were further engineered after initial assembling with a pDNA coating and plasma proteins. This novel multicomponent bionanoconstruct demonstrates enhanced transfection efficiency and safety in controlled laboratory settings and improved immune system evasion in *in vivo* tests. This capacity is attributed to its superior ability to evade lysosomal degradation and immune cell capture, a phenomenon mediated by a complex interplay among PEGylation, the protein corona, and pDNA within the structure. These findings provide valuable insights for the design and development of bionanoarchitectures for large-size DNA delivery, opening new avenues for transformative gene therapies.

Poster No. 23	**DNA condensing agent enhances CAR-T cell transfection efficiency of DNA-loaded lipid nanoparticles**
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Developing efficient and durable CAR-T therapies remains a significant challenge in cancer treatment. To address this, lipid nanoparticles (LNPs) synthetized through the state-of-art microfluidic platform have been utilized to encapsulate plasmid DNA (pDNA) encoding CAR receptors, enhancing transfection efficiency (TE) and stability without the need for a viral vector. To this end, we refined a transfection protocol involving multiple administrations, but we aimed to further enhance its efficiency due to the intrinsic challenges of pDNA encapsulation, particularly the requirement for nuclear internalization. One promising approach to overcome this issue involves using condensing agents before pDNA complexation with the lipid mixt. To this end, we conducted a preliminary investigation to characterize LNPs with and without the commercial condensing agent P3000-reagentTM (PR), focusing on their

physicochemical properties and scrutinizing the biological outcomes primarily in the HEK293 cell line. The first results demonstrated that pre-condensation of the pDNA with PR significantly increased TE of one of the tested formulations (i.e., LNP2), while confocal microscope analysis has shown reduced lysosomal colocalization and major nuclear localization. Consequently, PR - LNP2 was selected as the optimal system for further evaluation in immortalized T-lymphocytes Jurkat cell line, using both luciferase reporter gene and functional CAR-encoding plasmid by multiple administration transfection. The success of this strategy for our formulation with pre-condensed pDNA has been witnessed through flow cytometry assays. Overall, these findings underscore the effectiveness of multi-administration transfection protocols and present the potentiality of this strategy based on the use of condensing agent to enhance the efficiency of LNP systems for pDNA delivery. Finally, this work provides valuable insights into optimizing pDNA-loaded LNPs for more effective gene therapy applications.

Poster	**Advanced mixing technology for the synthesis of small and stable mRNA-loaded lipid nanoparticles**
No. 24	**Annika Brehmer**[a], Niklas Hubert[a], Linus Aulich[a], Gregor Dürre[a], Christoph Hein[a], Bernhard Bobusch[b] [a]Fraunhofer Institute for Production Systems and Design Technology IPK, Berlin, Germany [b]FDX Fluid Dynamix GmbH, Berlin, Germany

Lipid nanoparticles (LNPs) have gained considerable prominence in recent years, particularly in the context of COVID-19 vaccine development. These nanoparticles provide an efficient delivery system for mRNA therapeutics, allowing precise targeting of specific cells. In addition to vaccines, LNPs have significant potential for cancer therapy, mRNA and other nucleic acid-based vaccines for other diseases, and CAR-T cell therapies. Traditional methods for producing LNPs often face challenges related to stability and scalability. In this study, we compared a new mixing technology, the FDmix platform, with conventional impingement jet mixers. FDmix is based on an oscillating nozzle that provides short mixing times and can be applied to a wide range of flow rates. Encapsulation of the Comirnaty® lipid mixture with mRNA using the FDmix showed significantly smaller and more homogeneous particles with particle sizes of 58-65 nm and a polydispersity index (PDI) of 0.01-0.05 at different volume flows between 10-100 mL/min compared to experiments with impingement jet mixers (particle sizes 58-129 nm, PDI 0.04-0.09). In addition, in stability tests using different concentrations of sucrose as a cryoprotectant, concentrations of 15% sucrose administered by dialysis rather than added prior to freezing gave the most stable results. Using this cryoprotectant, the size and PDI of particles produced by FDmix showed significantly less particle growth after storage at 4°C and -80°C. This addition significantly improves the stability of the particles after freezing and storage, which is critical for the practical application and long-term preservation of mRNA

therapeutics. The FDmix technology represents a significant advancement in the production of small and stable mRNA-loaded lipid nanoparticles, providing a robust foundation for future therapeutic applications.

Poster	**“Cytosolic’’ nanoprobes? First replication initiative in nanobioscience**
No. 25	**Mustafa ElGharib**, Maha Said, Raphaël Lévy
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The 12th century metaphor ‘’standing on the shoulders of giants’’ implies that we see further and accomplish more thanks to the wisdom and discoveries of great minds who paved the way for our work. To know which theories are a good base to stand on, scientists have to repeat experiments. Thus, replication is a critical component of scientific research.
In nanoscience, replication is particularly challenging due to the intricate nature of the systems at the interface between disciplines. We propose to address an ongoing topic of nanoscience debate through a formal replication effort, which is, to the best of our knowledge, the first replication initiative in the field.
Our replication initiative focuses on the issue of nanoparticle endosomal escape, which is of great importance to many proposed applications, nevertheless barely discussed in the literature that report use of nanoparticles as probes for cytosolic targets. In our initiative we aim at replicating some influential articles that report intracellular sensing of analyte(s) in the cytosol such as ions, RNAs, free radicals, amino acids, or any other biomolecules.
Here, we will be presenting the key steps of this reproducibility project involving the choice of our replication articles (corpus) and the initiatives that we are taking to encourage other scientists, including the authors of those articles, to participate in this replication effort. Prior to any experimental work, we will publish pre-registration reports detailing our plans and protocols. We will encourage the community to comment on those reports and we would be expecting and welcoming as well the members of the nanobio community to review our registered reports. We adopt an open science approach and will share our data and conclusions whether they confirm or not the initial findings.
The preregistration of our first replication article has been assessed, recommended, and granted in-principle acceptance by the Peer Community In Registered Reports (PCI-RR). We have accordingly begun the experimental work and will share the first results, progress, and challenges of the article we are replicating with the community.
We hope that this initiative would enable us to clarify contested issues in the field of nanobiotechnology, to correct the scientific records and raise the standards in the field, as well as to advance the field of nanomedicine.

Poster

No. 26

Nanotechnology in medicine: promises of intracellular use vs precise localisation of nanomaterials

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The promising applications of nanotechnology are massively advocated in the scientific literature, especially for therapies and diagnostics. Claims made in this field generate a lot of hype and hope, resulting in money being invested to fund nano-related projects, patents being granted, companies being established to commercialise nanoprobes, a large number of articles published annually and many researchers using this literature as basis for their studies. The hype surrounding nanotechnology has some *side effects* in science: a large number of errors, inconsistent information and even misconduct, resulted from the *publish or perish* pressure. This is, of course, not just a problem of nanotechnology, but (unfortunately) of science as a whole. In this work we will focus on some errors encountered in the nanobiosciences field, and how we attempt to correct them. The NanoBubbles ERC Synergy Project investigates three big questions: *how, when and why science fails to correct itself.* This interdisciplinary project encompasses the humanities and social sciences (linguistics, sociology, history and philosophy of science), natural sciences, engineering (natural language processing), physics, chemistry and biology. In this work, we will introduce the postpublication peer review (PPPR) initiative, and present our methods to contribute to the correction of science by critically analysing pre-selected articles and posting reviews on PubPeer, the online journal club. Our main selection criterion for the articles commented on is to have reported the use of nanoparticles for intracellular detection of cytosolic targets, or to have claimed the ability of nanoparticles to cross the blood-brain barrier (BBB). The former generally do not address the mechanism of cellular uptake and do not prove the endosomal escape of the nanomaterial. The latter sometimes ignore the fact that only macromolecules with a molecular weight of up to 400 Da have been reported to cross the BBB, which excludes nanoparticles. The major issue flagged in our PubPeer comments is the lack of discussion on the access of nanoparticles to the cytosol. In this process, we also point out image duplications, problems with nanoparticles characterisation, problems regarding the rationale of the work, and methodology, as important missing information can boost the *replication crisis*. One of the aims is to make more readers aware of the concerns pointed out in our comments, and encourage scientists in all levels of research to engage in PPPR, as a daily practice, and as part of their work.

Poster No. 27	**Development and characterization of plumbagin-loaded thermosensitive injectable hydrogel for diabetic wound healing**
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Diabetic wound healing is a significant clinical challenge due to impaired tissue repair and increased susceptibility to infection. This study explores the development and characterization of a novel plumbagin-loaded thermosensitive hydrogel designed to enhance diabetic wound healing. Plumbagin, a naturally occurring naphthoquinone, possesses potent anti-inflammatory, antimicrobial, and antioxidant properties, making it an ideal candidate for promoting wound repair. The thermosensitive hydrogel, synthesized using biodegradable polymers (Chitosan and Pluronic F127), exhibits a sol-gel transition at physiological temperatures, ensuring ease of application and sustained release of plumbagin at the wound site. This study involves the formulation and optimization of the hydrogel, followed by extensive physicochemical characterization, including gelation temperature, rheological properties, and drug release kinetics. Fourier-transform infrared spectroscopy (FTIR) analysis was conducted to assess the compatibility between the hydrogel components.Scanning electron microscopy was used to assess the morphology of the developed thermosensitive hydrogel. Furthermore, *in vivo* studies using a diabetic wound model evaluate the hydrogel's efficacy in accelerating wound closure. The findings of this research aim to provide a comprehensive understanding of the potential of plumbagin-loaded thermosensitive hydrogel as a therapeutic strategy for diabetic wound management, highlighting its advantages over conventional treatments and paving the way for future clinical applications.

Poster No. 28	**The development of a single exposure radical cure antimalarial formulation - the artemether plus WOG19 Sting!**
	Samuel Uzondu
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Malaria is a global health challenge. The development of Antimalarial drug resistance, which has retired most antimalarial drugs including Chloroquine, Sulphadoxine/Pyrimethamine, mefloquine etc. is very worrisome. More disturbing is the discovery of artemisinin-resistant Kelch 13 P.falciparum mutants in Rwanda and Uganda(1).This poses a public health threat in Africa which accounts for over 90 percent of the global malaria burden(2).To address these burgeoning challenges, World Health Organization(WHO) is strongly advocating for the

development of a single exposure radical cure and prophylaxis(SERCAP) agents that would cure and block the transmission of malaria.
In response to the call by WHO, our research focused on developing a single exposure radical cure system comprising of an immediate/ sustained-release formulation of Artemether and WOG19 delivered as a nanosized Lipid-Polymer constructs(nLPCs) for the treatment of Resistant P.berghei .A combination of Compritol 888 ATO and PEG 4000 were used as the Lipid-PolyMalaria is a global health challenge. The development of Antimalarial drug resistance, which has retired most antimalarial drugs including Chloroquine, Sulphadoxine/Pyrimethamine, mefloquine etc. is very worrisome. More disturbing is the discovery of artemisinin-resistant Kelch 13 P.falciparum mutants have been discovered in Rwanda and Uganda(1).This poses a public health threat in Africa which accounts for over 90 percent of the global malaria burden(1).To address these burgeoning challenges, World Health Organization(WHO) is strongly advocating for the development of a single exposure radical cure and prophylaxis(SERCAP) agents that would cure and block the transmission of malariamer matrix. The formulations were characterized for particle size and polydispersity index using Nano Zetasizer, DSC, FTIR, and SEM. Also, entrapment efficiency and loading capacity were assessed. The formulations [nLPCs]) were tested for efficacy against resistant (ANKA) strains of P. berghei in mice using the curative model, with a commercial ACT and unloaded NLCs as positive and negative controls respectively.
The results showed stable nano-sized formulations with high entrapment efficiencies and moderate loading capacities. In vitro 12h release studies in Simulated Intestinal Fluid (without enzyme) at pH 6.8 showed a dual release (burst and sustained) profile of Artemether and WOG19 from the lipid matrix. *In vivo* antimalarial activity assay of the formulations using mouse models showed that the WOG19-loaded NLCs gave a 40% reduction in parasitaemia the resistant strains (Anka) of P. berghei in the curative model,. Percentage parasitaemia reduction of the formulations was comparable to the positive control (a Commercial ACT)
This is a preliminary study which has shown that the formulated nLPCs have very promising efficacy resistant strains of Plasmodium parasite, indicating that development of Single exposure radical cure and prophylaxis agent(SERCAP) against the malaria parasite as advocated for by World Health Organization(WHO) is a possibility!. The observed antimalarial activity of both WOG19 and WOG19 +Artemether nLPCs will contribute immensely in the global fight against malaria and antimalarial drug resistance, thus bringing global efforts a step closer to the actualization of the WHO Ideal goal of malaria eradication by 2030.

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