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nanoBioMed2023 Foreword

On behalf of the Organising Committee, we take great pleasure in welcoming you to Barcelona (Spain) for the nanoBio&Med2023 International Conference.

This event, after successful editions organised within ImagineNano in Bilbao 2011 & 2013, and in Barcelona in 2014, 2015, 2016, 2017, 2018, 2019 & 2022 is going to present again in-person the most recent international developments in the field of Nanobiotechnology and Nanomedicine and will provide a platform for multidisciplinary communication, new cooperations and projects to participants from both science and industry. Emerging and future trends of the converging fields of Nanotechnology, Biotechnology and Medicine will be discussed among industry, academia, governmental and non-governmental institutions.

nanoBio&Med2023 will be the perfect place to get a complete overview into the state of the art in those fields and also to learn about the research carried out and the latest results. The discussion in recent advances, difficulties and breakthroughs will be at his higher level.

We are indebted to the following Scientific Institutions and initiatives for their support: Institute for Bioengineering of Catalonia (IBEC) and NanomedSpain.

In addition, thanks must be given to all speakers and participants that join us in-person this year and to the staff of all the organising institutions whose hard work has helped planning this conference.

Hope to see you again in the next edition of nanoBio&Med in Barcelona.

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SENTIATECH Technological Platform Workshop
November 22, 2023: 14:30 - 16:30 (Room 3TI)
"Retos actuales y futuros en tecnologías biosensor, nanomedicina y diagnostico"

nanoBioMed2023 Speakers list

Nanotechnology Enables Advanced and Precision Therapies

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Advanced and precision therapies that utilize proteins and RNA-based polynucleotides, are taking an increasing space in the industry pipelines. Despite their potency, the adequate exploitation of these macromolecules has been restrained by their difficulties for overcoming biological barriers and reach the intracellular targets.

Fortunately, improved understanding of the biological barriers, as well as advances in chemical biology and the synthesis of functional biomaterials is paving the way for a more comprehensive and rational design of advanced nanomedicines. Our laboratory, with a long-track record in formulating biological drugs using biomaterials, has significantly contributed to this field. As an example, in the 90's we were the first to report that nanoparticles made of biopolymers and/or lipids were efficient vehicles for the transmucosal delivery of proteins, antigens and polynucleotides. In the last few decades, we have made significant advances in terms of enabling the intracellular delivery of monoclonal antibodies and RNA molecules. These efforts have resulted in an array of nanotechnologies that can be used to develop advanced therapies and vaccines as well as personalized treatments.

In my presentation, I will focus on the design of carriers for proteins and RNA molecules that could be used in two major therapeutic areas: (i) nanovaccines, i.e. HIV and COVID vaccines (ii) oncological personalized therapies based mAb and siRNA targeted to intracellular onco-proteins.

Overall, our experience in this field has benefited from integrative approaches adopted by specifically designed consortia. Hopefully, the results of these cooperative efforts will help to accelerate the progress of a rational design of protein-based nanomedicines.

More information about these projects and associated publications can be found at:

http://www.usc.es/grupos/mjalonsolab/

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Researchers contributing to these projects include:

Anticancer drug delivery: Desirée Teijeiro, Dolores Torres, Ana López, Philipp Lapuhs, Spain.

Vaccine delivery: Mireya Borrajo, Shubaash Anthiya, Gustavo Lou, Jose Crecente, Tamara Gómez from the USC and Ma Luo from University of Manitoba, Canada.

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Smart paper-based electrochemical (bio)sensors for sustainable detection of biomarkers

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As reported in my recent review entitled "Electrochemical paper-based devices: When the simple replacement of the support to print ecodesigned electrodes radically improves the features of the electrochemical devices" published in Current Opinion in Electrochemistry SI: Emerging Opinions (2022) [1]: "*Paper-based electrochemical (bio)sensors have emerged as highly attractive analytical devices for their superior sustainable features, such as avoiding the use of polyester as support and the reduction of waste, being incinerated after use. However, paper-based electrochemical (bio)sensors have recently demonstrated further advantages, including the simple combination with vertical microfluidics and their use as a reservoir to deliver smart electrochemical (bio)sensors able to i) contain the reagents, ii) preconcentrate the target analyte, and iii) synthesize the nanomaterials inside the paper network. Furthermore, these devices have demonstrated their ability to overcome the limitations of the other printed electrochemical sensors in the measurement of entirely liquid samples by detecting the target analyte in the aerosol phase or solid sample, without the additional sampling system. These achievements highlight their valuable and varied advantages in the sensing sector*". During my presentation, I will report on the roadmap research activity carried out in the last 8 years related to the development of paper-based electrochemical devices as smart and sustainable point-of-care devices [2-6].

Acknowledgements
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Targeting Macromolecular Transport for Alzheimer's Therapy

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The intricate regulation of the blood-brain barrier (BBB) significantly impacts neurological processes, often playing a pivotal role in the pathogenesis of dementia and Alzheimer's disease (AD). Recent research has highlighted the key involvement of low-density lipoprotein receptor-related protein 1 (LRP1) in the clearance of amyloid-β (Aβ) and phosphorylated tau (p-tau) [1]. Dysfunctions in these processes, compounded by disruptions in the transcription factor NRF2, lead to chronic inflammation, synaptic dysfunction, and cognitive impairment in AD [2]. Building on these insights, we are exploring the intricate relationship between BBB dysfunction and AD, particularly focusing on the involvement of LRP1/syndapin-2 transcytosis in inflammation and cellular stress [3,4].

We have successfully manipulated BBB transport using LRP1-targeting polymersomes (POs). In an Alzheimer's mouse model, the administration of these POs led to a significant reduction in brain Aβ levels. Notably, the POs not only reinitiated the LRP1-mediated transport of misfolded proteins but also influenced several BBB markers, effectively counteracting the negative trends associated with Alzheimer's disease. The consequential impact on the cognitive decline of APP/PS1 animals was substantial. These results underscore the potential of leveraging multivalent nanoparticles to elevate LRP1 levels and proficiently eliminate Aβ from the brain. This approach demonstrates the scalability of drug design through the integration of multiple ligands into multivalent scaffolds. Additionally, the supramolecular nature of these structures introduces an additional dimension, enabling further functionalities. We have engineered biodegradable scaffolds derived from Krebs' cycle diacids, capable of transforming into bioactive compounds. These materials introduce an added therapeutic dimension, exhibiting promise as potent anti-inflammatory agents by enhancing antioxidative gene transcription and influencing the NRF2 pathway.

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Patient-specific nanovectors against glioblastoma multiforme

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The current advances in nanotechnology allows for a wide spectrum of possible applications in different fields, in particular in medicine. Nanoparticles can be exploited as efficient drug delivery systems thanks to their capacity to encapsulate high payloads of drugs that are otherwise poorly soluble in the biological milieu, increasing their bioavailability and biocompatibility. In other cases, nanoparticles can act themselves as a therapeutic or diagnostic agent (*e.g.*, superparamagnetic iron oxide nanoparticles, -SPIONs-). Nanostructured lipid carriers (NLCs) offer several advantages as compared to other systems, such as a relatively easy, green, low-cost, and scalable preparation protocol, biocompatibility / biodegradability ensured by the lipid constituents, a high drug payload, and physicochemical stability in bodily fluids.

We demonstrated that NLCs loaded with SPIONs and a chemotherapeutic agent are able to selectively induce apoptosis in glioblastoma multiforme cells [1, 2]. Moreover, NLCs functionalized with the peptide angiopep-2 and encapsulating temozolomide have been efficiently exploited in an *in vivo* approach to promote suppression of human glioblastoma by the synergistic action of the chemotherapeutic drug loaded into the nanocarrier ([NLCs]=24 mg/[TMZ]=0.98mg /kg weight mice; injection volume= 3 µL) and the cell sensibilization in response to the local heating ($H \times f$ = $4.2 \cdot 10^9$ A/ms, t = 30 min, 3 consecutive days, 24 h after NLC intratumoral administration). Obtained data on orthotopic U87MG human glioblastoma tumor-bearing nude mice evidenced an effective suppression of the tumor growth, and a significantly improved medium survival time after the treatment (75% of the subjects still alive at the end of the study), suggesting the suitability of the proposed nanoplatform for the GBM treatment [3].

In the aim of reaching full targeting potential and provide a patient-personalized treatment, we are now working on developing new nanocarriers based on NLCs and coated with extract of glioblastoma cell membranes derived from patients' samples. Cancer cell membrane coating confers extraordinary targeting abilities to the nanovectors, increasing the accumulation of therapeutics in diseased tissues and significantly reducing side effects [4]. These nanovectors co-deliver both SPIONs, for hyperthermia treatment, and chemotherapeutic drugs. The targeting efficiency, the ability of crossing the blood-brain barrier, and the selective anticancer activity of the nanovectors are studied by means of state-of-the-art fluidic systems to closely mimic the complex tumor microenvironment *in vitro* [5].
This work will be of great importance in the development of new technologies for precision medicine and for theranostic applications, thanks to abilities of SPIONs to act both as a therapeutic tool and as a magnetic resonance imaging -MRI- contrast agent, depending on the magnetic field used. Moreover, thanks to the versatility of the formulation and testing tools, this new approach could be easily remodeled to be applied for the treatment of other oncological pathologies.

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Carbon nanomaterials based biosensors for electrochemical detection of biointeractions

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Abstract

Biosensors provide a sensitive and selective detection of nucleic acids, drugs, proteins, toxins etc. Advanced biosensors based on nanomaterials could be significantly applied to the areas of genomics, proteomics, biomedical diagnostics and drug discovery due to the advantages of different nanomaterials having unique electronic, optical, mechanical, and catalytic properties. Carbon based nanomaterials such as, carbon nanotubes, carbon nanofibers etc. have different applications in drug delivery, tissue engineering, cancer therapy and diagnosis including biosensors.

Electrochemical biosensors have an essential specificity based on biorecognition reactions resulting with the high sensitivity on the detection of target analytes; such as, nucleic acids, drugs, proteins. This study is overviewed the recent progress on electrochemical biosensors based on carbon nanomaterials, and discussed with the further applications.

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Tuning the nanotexture of bioinspired bone substitutes: interaction with proteins and *in vivo* bone formation

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Abstract

The recent advances in 3D printing technologies have opened the door to the design of customized bone substitutes, mainly based on calcium phosphates. In this context, we have recently developed self-hardening calcium phosphate inks based on the combination of reactive ceramic particle suspensions with hydrogel binders, which harden at low temperature through a dissolution-precipitation process. This approach has several advantages. On the one hand, it avoids the shrinkage associated with high-temperature sintering processes and, on the other hand, the final product is a nanostructured biomimetic apatite, very close to the mineral phase of bone and more reactive than calcium phosphate ceramics.

The reaction kinetics of these self-hardening inks can be adjusted by modifying the reaction conditions, using biomimetic or hydrothermal methods. In addition to influencing the reaction duration, this allows for tailoring the crystalline morphogenesis in the hydrolysis process, resulting in changes in the physicochemical and textural properties of the final product [1,2]. Thus, in addition to the external shape of the implant, it is possible to control the internal pore architecture of the scaffold.

This presentation will focus on the effect of the nanoscale textural properties of 3D printed calcium phosphate scaffolds on their biological performance, highlighting the extent to which differences in nanostructure, nanoporosity and nanopore size are key to both the interaction with proteins in solution [3] and the osteogenic potential of the materials *in vivo*.

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Figures

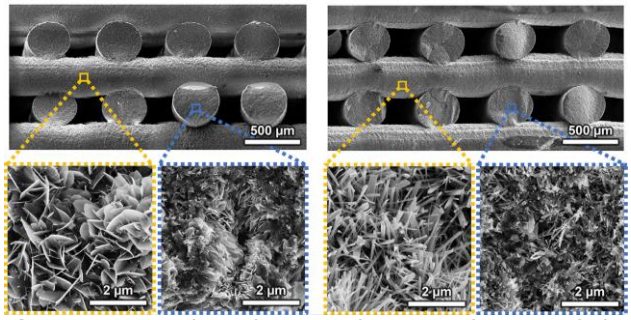

Figure 1. Scanning Electron Microscopy images of the architecture and nanostructure of 3D-printed biomimetic apatite bone grafts.

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Micromaterials for reinforcing living implants for application in wound healing and regenerative medicine

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Abstract

Hydrogel-based biomaterials have been developed and reinforced with micromaterials to alleviate anoxic stress, stimulate vascularization, and improve the engraftment of cellularized implants[1-4]. However, the effects of self-oxygenation materials on different aspects of regenerative medicine such as healing of the myocardium after myocardial infarction (MI) or stem cell fate in cellularized hydrogels have not yet been studied.

In my talk, I will shine some light on oxygenating biomaterials that were mixed with different tissue-adhesive hydrogels for two different applications: 1.) Better tissue regeneration in MI and 2.) Stem cell fate commitment for regenerative medicine such as bone regeneration (**Figure 1**). Calcium peroxide as the source of oxygen was encapsulated in polycaprolactone to produce oxygenating microparticles with prolonged oxygen release profiles[1]. These oxygen-generating microparticles were incorporated into bioadhesive silk-alginate or gelatin methacryloyl (GelMA) based hydrogels with or without mesenchymal stem cells (MSCs), for MI and osteochondral differentiation, respectively. For MI, these hydrogels were conjugated with stromal cell derived factor (SDF) to orchestrate chemotaxis and angiogenesis and generate oxygen via oxygenating microparticles (OMPs) to alleviate the cytotoxic anoxic environment. Silk fibroin (SF) was explored to endow elasticity and resilience to the injectable hydrogel. Additionally, tyramine (TA) was conjugated to alginate (TA-Alg) and SF, producing a mechanically robust and tissue adhesive hybrid hydrogel (TSF) that encourages tissue adhesion and enhances the injectability of the hydrogels.

For stem cell fate commitment for bone tissue engineering applications, oxygen-generating microparticles were incorporated into GelMA hydrogels in the presence/absence of osteoinductive silicate nanoparticles (SNPs). A comparative study revealed the osteogenic fate of hMSCs in the designed hydrogels under normoxic (the gold standard for in vitro cultures) and anoxic (common in large bone defects; <0.1% oxygen) conditions.

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Figures

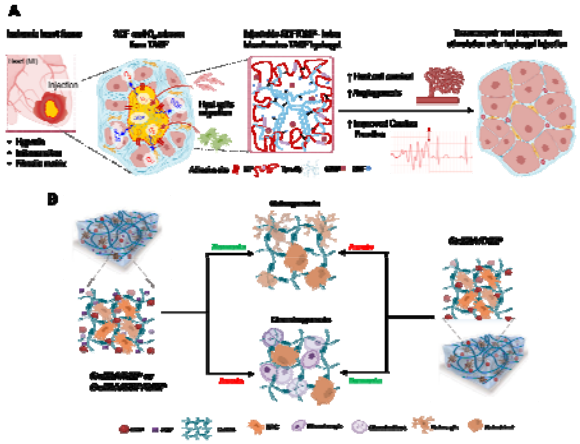

Figure 1. A). Schematics showing for myocardial infarction (MI) healing process by oxygenating and cardioprotective tissue adhesive tyramine conjugated alginate and silk fibroin (TSF) hydrogels encapsulated with oxygen releasing microparticles (OMPs) and conjugated stromal differentiation factor (SDF) applied at the ischemic site and the corresponding vessel regeneration and improved contractile function due to host cells migration and then their survival and maturation at the injured area. B). Depiction of the effect of OMP, SNP, and OMP+SNP on the differentiation fate of hMSCs toward osteogenesis or chondrogenesis under normoxia and anoxia, respectively.

Nanophotonics Biosensors for the clinical management of bacterial infectious diseases

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Infections by pathogenic bacteria and their multidrug resistance have become a major healthcare issue in the XXI Century. Bacterial Infections result in millions of cases every year and in an increasing incidence of deaths. In Europe 5-10% of all hospitalizations results in nosocomial infections, especially in surgical and intensive care units (ICU). This is 4.1 million patients, of which 37,000 die. These infections contribute to serious events including long stays at hospitals, additional antibiotic treatment and susceptibility to further infections, risk of developing sepsis, and the need of advanced medical intervention, resulting in a strong economic and heath impact. The early detection of bacterial infections increases dramatically the chances of survival. The alarming rise of antibiotic-resistant bacteria aggravates this global health concern. The massive use of antibiotics has promoted intense selective pressure on bacteria, contributing to the emergence and spread of the resistant mutants. Common illnesses as pneumonia, postoperative infections, or tuberculosis are becoming increasingly untreatable. According to the Review on Antimicrobial Resistance (AMR-review.org), at least 50,000 lives are lost each year in Europe and US due to infections caused by antimicrobial-resistant bacteria. Moreover, this number is increasing alarmingly year by year and it could become the first cause of death in which 10 million victims per year are envisioned at the horizon 2050.

A critical barrier for managing infections and antibiotic resistance is the lack of rapid diagnostics, resulting in either the use of unnecessarily broad first-line antibiotics, or long delay in administering the appropriate one. TWO pages abstract format: including figures and references.

The use of point-of-care nanophotonics biosensors as rapid diagnostic platforms can fill in the gaps in the problematic situation of the rapid and accurate diagnostics of infections by providing sensitive, reliable, quantifiable and selective analysis, while reducing test and therapeutic turnaround times, decreasing and/or eliminating sample transport, and using low sample volume.

Within the PHITBAC project (www.phitbac.es) we aim to introduce such new, disruptive, and versatile point-of-care nanobiosensor technology for the whole diagnosis and clinical management of bacterial infectious diseases. The groundbreaking diagnostic device will prove rapid detection of most relevant pathogenic bacteria, including an on-site identification of antibiotic resistance, and a personalized monitoring of antimicrobial therapy effectivity.

We have already demonstrated the detection of E. coli at extremely low concentrations (LOD~4 CFU/mL) in human ascitic fluid in a direct format for spontaneous bacterial peritonitis detection, with a time to result of only 25 min and no need of any sample purification. Moreover, we have detected P. aeruginosa (LOD~30 CFU/ML) and methicillinresistant S. aureus (MRSA), both being considered two of the most prevalent bacteria associated with nosocomial infections. Our approach enables the specific identification of the resistant pathogen (MRSA) and its differentiation from methicillin-susceptible S. aureus (MSSA) [1]. Moreover, we have developed an ultrasensitive methodology for the detection of genes associated with the multidrugresistance found in Gram-negative bacteria as E. coli without using any PCR amplification step [2]. We have detected genes encoding several β-lactamase enzymes able to hydrolyze a broad spectrum of beta-lactams. As a proof-of-concept, we detected two genes of the unamplified genomic DNA directly extracted from bacteria commonly found in samples of patients attended at Vall d'Hebron Hospital. All the steps took 30 min, achieving an estimated LOD of only 28 aM (~10e5 copies) [22], one of the most sensitive DNA detection without amplification or labelling steps reported up to date.

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Mesoporous Silica Nanoparticles for Nanomedicine

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In recent years, nanomedicine has emerged at the forefront of nanotechnology, generating great expectations in the biomedical field. Researchers are developing novel nanoparticles for both diagnostic applications using imaging technology and treatment purposes through drug delivery technologies. Among all the available nanoparticles, inorganic mesoporous silica nanoparticles (MSNs) are the newcomers to the field, contributing with their unique and superlative properties. MSNs present well-defined and tunable physicochemical properties, including particle size, pore size, pore volume, surface area, volume area, pore structure, and surface functionality. The porous structure of MSMs provides cavities that can host and release a great variety of biomolecules and therapeutic agents. In fact, the versatility of MSNs in size, morphology, and texture has fuelled their application as controlled drug delivery nanocarriers. MSNs can provide a novel therapeutic armamentarium capable of addressing some of the main pitfalls of conventional medicine, such as the lack of drug specificity, the narrow window of efficacy of some medicines, the possible low drug solubility and/or stability, adverse pharmacokinetic profiles, and some possible side effects.
During this talk, I would try to give an overview of the work that we do regarding the use of Mesoporous Silica Nanoparticles as Nanomedicines, and their potential application for treating certain diseases.

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Chemical Communication Principles and Applications

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Engineering chemical communication (communication through the exchange of chemical messengers) between micro/nanosystems is receiving increasing attention from scientists. Although a number of micro and nanodevices (e.g., drug carriers, sensors, motile systems, nanoreactors) have been developed in the last decades, engineering communication at the micro/nanoscale is a recent emergent topic. In fact, most of the studies in this research area have been published within the last 5 years. The importance of the topic relies not only on its novelty and interdisciplinarity, but it is also expected to provide breakthroughs in many areas including nanotechnology, biomedicine, biotechnology and ICT.

In communication theory terms, communication occurs upon the exchange of information between two entities. Thus, there is a sender that receives a stimulus and converts the information into a code. The message is transmitted from the sender to the receiver through a certain medium. The receiver finally decodes the message and produces a certain action. In this context, chemical communication offers certain advantages over traditional telecommunications, such as the small dimensions of molecular components, low energy requirements and the possibility to actuate in aqueous environments and biological contexts.

Inspired by nature – where information is exchanged by means of molecules – the development of chemical communication strategies holds wide implications from different points of views. Published examples rely on nanotechnology and synthetic biology for the creation of micro and nanodevices that can communicate. Communication enables to construct new complex systems, capable of performing advanced coordinated tasks that go beyond those carried out by individuals, which is useful in many different fields already employing synthetic micro/nanodevices. In addition, the possibility to communicate synthetic and living systems can further advance in our understanding of biochemical processes, provide completely new tailored therapeutic and diagnostic strategies, ways to tune cellular behavior and develop new biotechnological tools.

This presentation, we will summarize advances by our laboratory in the design of modes of chemical communication. These models embrace from simple linear communication (transmission of information between two points) to more complex pathways such as interactive communication (feedback) and multicomponent communication (involving several entities). Using illustrative experimental designs, we will demonstrate the realization of these models which involve not only communication between engineered particles, but also between particles and living systems. [1-5]

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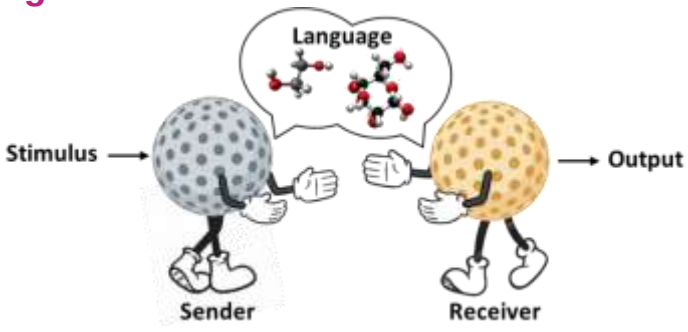

Figure 1. Schematic illustration of the concept of chemical communication between micro/nanoparticles.

Biological Fate and In vivo Degradation Studies of Hybrid Nanomaterials for Drug delivery

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Abstract

Nanoformulations offer multiple advantages over conventional drug delivery, enhancing solubility, biocompatibility, and bioavailability of drugs.[1] Following systemic delivery nanocarriers must deliver encapsulated drugs, usually through nanocarrier degradation. A premature degradation or the loss of the nanocarrier coating may prevent the delivery of drugs to the targeted tissue. Despite their importance, stability and degradation of nanocarriers in biological environments are seldom studied in literature. Understanding fate and how nanomaterials change in biological matrixes is also fundamental for their toxicological evaluation as changes in nanoparticles surface or release of ions or molecules can induce toxicological endpoints. One of the main areas of research in our group in the last years has been the study of the fate of nanomaterials, aiming to understand how their properties change in biological environments-.In this presentation issues related to the biological fate and stability of nanocarriers in biological matrixes will be discussed: the interaction of the nanocarriers with proteins, the biodistribution of the nanocarriers, their biological fate, the kinetics of drug release in vitro/in vivo and the stability of the core and surface coating of the nanocarriers. Different types of nanomaterials will be discussed: poly lactic co glycolic nanoparticles[2], polymersomes[3], polyplexes for siRNA delivery[4], and mesoporous silica nanoparticles[5]. In vitro, we will make use of Fluorescence Correlation Spectroscopy for studying nanocarriers stability, the fate of protein corona after translocation and the relation between surface chemistry, protein corona formation and the aggregation of nanocarriers intracellularly. In vivo, we will apply Positron Emission Tomography and Single Photon Emission Tomography to study the biodistribution of nanocarriers, the stability of surface coatings and nanocarrier dissolution, making use of advanced radiolabeling strategies.

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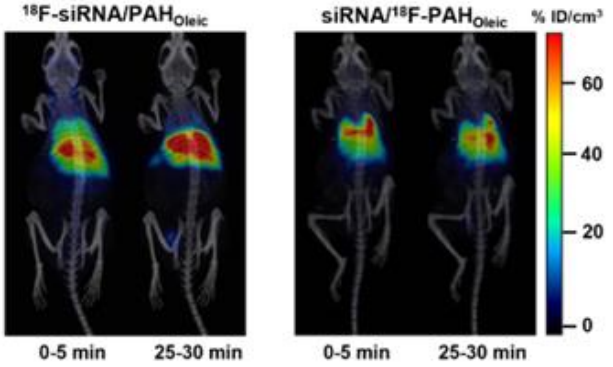

Figure 1. PET images of the biodistribution ^{18}F-siRNA/polyamine and siRNA/^{18}F-polyamine polyplexes. From the comparison of the two images the stability of the polyplexes is assesed.

Is the future of antioxidants mineral? Nanozymes and other nanotechnology solutions

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The role of antioxidants in biology became popular in the second half of the 20th century at times when Linus Pauling (1954 and 1962 Nobel laureate) worked on the so called orthomolecular medicine, based on nutritional supplementation and high doses of ascorbic acid. It bumped again in the 90s, as consequence of a large human study suggesting that vitamin E supplements could be associated with a reduced risk of heart diseases. During these years, other works, basically pre-clinical and epidemiological, also reported beneficial effects of antioxidant substances in chronic inflammation, neurodegeneration, and cancer. As a consequence of that, antioxidant therapies were evaluated in placebo-controlled trials involving tens of thousands of patients and, despite pathophysiologic, epidemiologic, and mechanistic compelling evidence, these clinical trials have been, to date, mostly negative. This has given rise to a pessimistic view on antioxidant therapies. This has been attributed to the non-drug-likeness of available antioxidant compounds. These compounds have high unspecific uncontrolled reactivity, poor solubility, and hence limited absorption profiles, low bioavailability and low concentrations at the target site. During this time, nanomaterials has been proposed for use in treating human diseases, primarily as drug delivery agents, showing potential benefits in terms of pharmaceutical flexibility, selectivity, dose reduction, and minimization of adverse effects. Thus, efforts have been made towards loading antioxidant molecules such us coenzyme Q10, vitamin E and vitamin A, resveratrol and polyphenols, curcumin, lycopene, silymarin, and superoxide dismutase in nanocarriers such as liposomes, polymeric NPs, lipid NPs, and self-emulsifying systems. More recently, nanotechnology has shown us how rare earth mineral antioxidant NPs, especially cerium oxide NPs, nanoceria[1], are powerful antioxidant and consequent anti-inflammatory agents that can treat many inflammation-related diseases. This is a new paradigm, where the nanoparticle itself, thanks to its nanometric form and high concentration of oxygen vacancies at its surface, is the active principle, not a vehicle. Interestingly, nanoceria is safe, xenobiotic, and highly traceable material.

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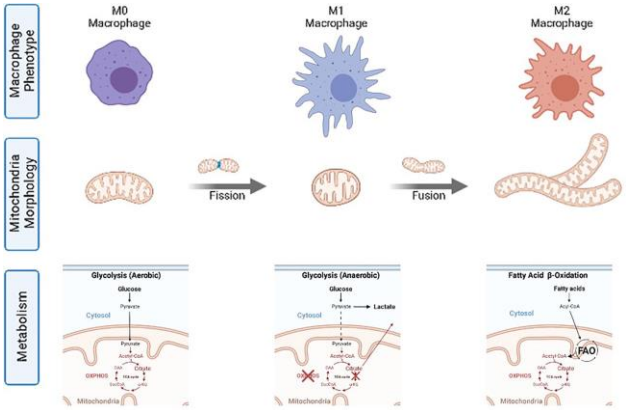

Figure 1. Macrophage phenotypes, mitochondria morphology and corresponding catabolic pathways

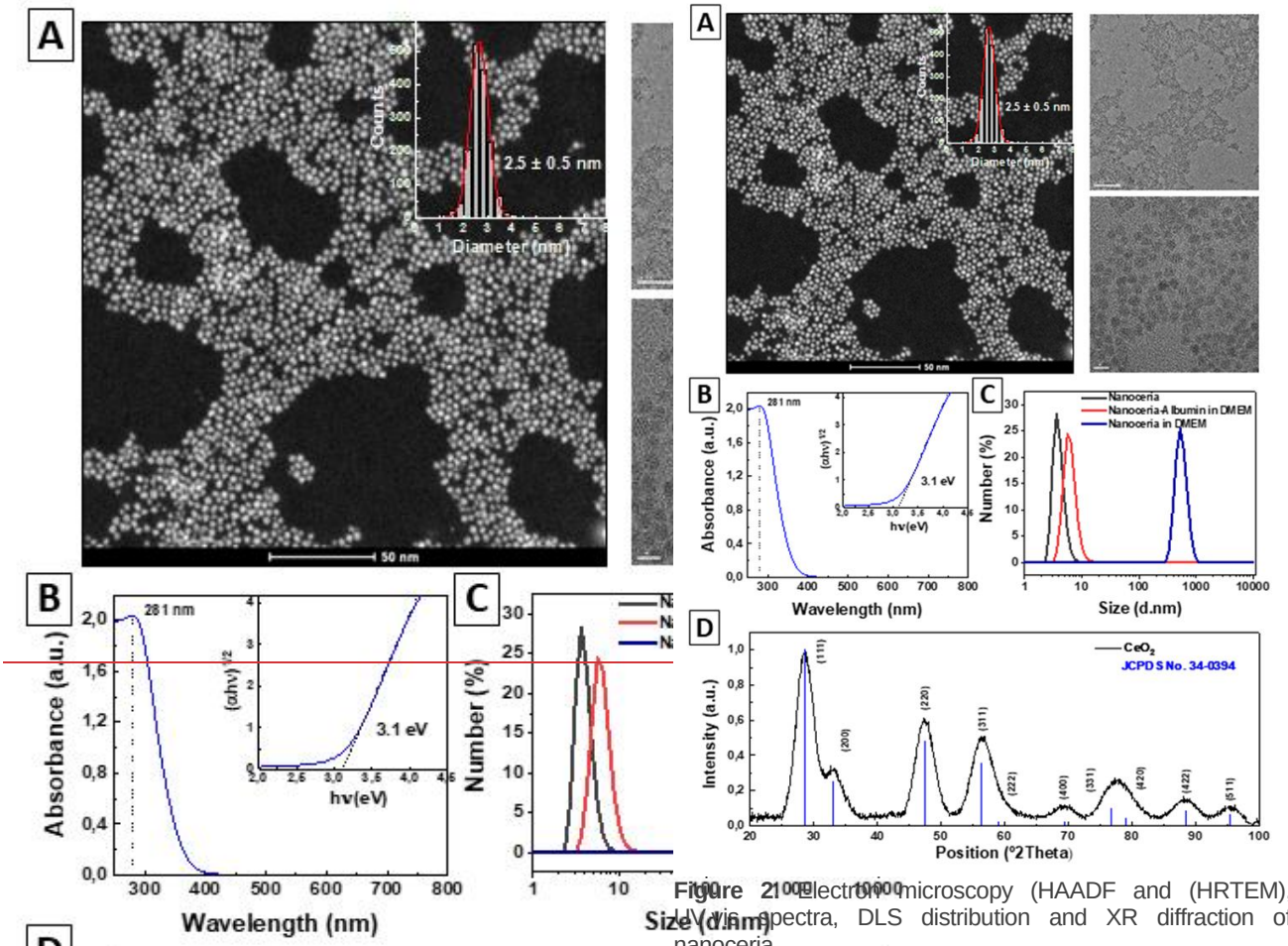

Figure 21 Electron microscopy (HAADF and (HRTEM), UV-vis spectra, DLS distribution and XR diffraction of nanoceria.

Synthetic DNA-based devices, switches and genes for clinical applications

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DNA nanotechnology uses synthetic DNA (or nucleic acids) as a versatile material to rationally engineer tools and molecular devices that can find a multitude of different applications (e.g., in-vivo and in-vitro diagnostics, drug delivery, genetic circuits etc.).

During this presentation I will introduce the field of DNA nanotechnology and I will show how to exploit the “designability” of DNA to fabricate DNA-based nanoswitches, nanodevices and synthetic genes that are specifically designed to respond to different targets and generate a measurable output or release a molecular cargo.

I will demonstrate how to characterize and recreate in-vitro several mechanisms to control the response of such DNA-based systems and how to regulate their activity with different chemical and environmental stimuli including pH, antibodies, enzymes, small molecules and redox inputs.

Using Nature's engineering principles to design biointerfaces and synthetic cells for nanomedicine

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Nature achieves unmatched functionality by the self-assembly of (macro)molecular building blocks in a hierarchical manner. All information necessary for the function is encoded at the molecular level. Unraveling such blueprints serves as a powerful paradigm in the bio-inspired synthesis of materials that can seamlessly interface with living matter or perform non-natural functions. In this talk, I will present three examples. Firstly, I will present nanoscale coatings for blood contacting medical devices that not only do not activate coagulation but that can even direct blood to digest deadly thrombi. Such coatings find applications in membrane of oxygenators, hemodialysis, and artificial hearts.[1] Secondly, I will present our concept of Kill&Repel coatings for wound dressings.[2] These coatings combine the synergistic action of in situ assembled polymer brushes with a killing mechanism that is orthogonal to eukaryotic cells. When applied to wound dressings they were able to prevent the colonization from various pathogens. The last part of the talk will focus on the development of "quasi-living therapeutics" which are synthetic cells that exert a therapeutic action by recapitulating some biological function.[3] I will illustrate this with Phagocytic Synthetic Cells (PSC) which engulf and kill bacteria and viruses. The dual mode of action is inspired by phagocytosis. The PSCs have the potential to revolutionize the way we fight infectious diseases caused by antibiotic-resistant germs, which is one of the biggest global threats to our welfare.

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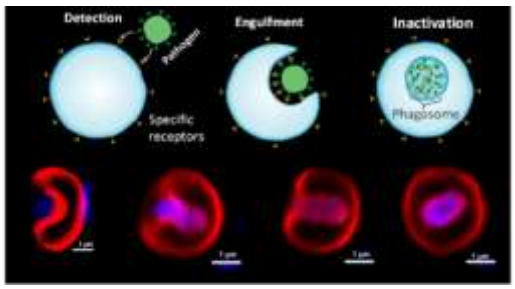

Figure 1. Phagocytic synthetic cells engulfing a living *E. coli* by simple physical interactions.

Treating bladder cancer with self-propelled nanobots

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One of the dreams in nanotechnology is to engineer small vehicles and machines, called here nanobots, which can eventually be applied *in vivo* for medical purposes. Yet, reaching that fascinating goal is not a trivial thing and several challenges need to be addressed. First, researchers need to incorporate efficient but also bio-friendly propulsion mechanisms into the nanobots. Our strategy comprises the use of biocatalysts such enzymes for converting biologically available fuels into a propulsive force. Secondly, nanoparticles' chassis should be generally recognized as safe (GRAS) material, biocompatible and/or biodegradable.
In my talk, I will present how we bioengineer hybrid nanobots combining the best from the two worlds: biology (enzymes) and (nano)technology (nano-micro-particles, Figure 1) providing swimming capabilities, biocompatibility, imaging, multifunctionality and actuation.
Besides the understanding of fundamental aspects (1), and controlling the performance of micro-nanobots (2) I will present some of the proof-of-concept applications of biocompatible nanobots such as the efficient transport of drugs into cancer cells (3) and 3D spheroids (4), sensing capabilities (5), anti-bactericidal applications (6) and the use of molecular imaging techniques like PET-CT (7) or Photoacoustic (8) for the tracking and localization of swarms of nanobots both in vitro and in vivo in confined spaces like mice bladder. Moreover, I will present our recent advances in the treatment of bladder cancer in mice using radionuclide-labelled nanobots (9).

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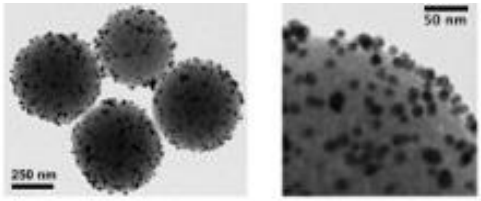
Figure 1. Mesoposous silica nanoparticles used as self-propelled nanobots



Artificial receptors and signaling cascades in synthetic cells

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Artificial, synthetic cells are a fascinating research discipline. Cellularity is the foundation of life, and mimicking the design and the functions of cells is an intriguing possibility. It is fundamentally important and also has prospects for applications in e.g. biosensing, bioproduction, and biomedicine.

One of the most characteristics of cells in nature is their responsive behaviour. It is this attribute of life that makes cells interact, allows individual cells to assemble into multicellular organisms, and enables the cells to fight for habitat. Engineering responsive behaviour into synthetic cells is a grand challenge. This is because the molecular mechanisms of responsive behaviour in nature include transmembrane proteins for signalling, and intracellular signalling cascade to propagate the signal. These proteins are embedded into the homeostasis, which makes the prospect of reconstitution of receptors and cascades into synthetic cells receptors futile.

In our work, we approach the design of responsive synthetic cells through the engineering of artificial receptors and the design of artificial signalling cascades.

For transmembrane signalling, we design artificial receptors. These are small organic molecules, not proteins. Receptor molecule features an membrane anchor and an exofacial ligand for receptor activation. The mechanism of signal transduction is based on the chemistry of self-immolative linkers. Upon receptor activation, the decomposition of the self-immolative linker leads to the release of a secondary messenger molecule. The latter is released from the lipid bilayer, moves to the interior of the synthetic cell, and therein activates the proteins that comprise the downstream signalling cascade.

The design of signalling cascades requires that we engineer the tools to activate enzymatic activity on demand. We did so using the chemistry of thiols. “Thiol switching” is a nature-inspired approach to control enzymatic activity and reversible switch it, on demand. We applied this to proteases, kinases, and polymerases, thus building up a versatile toolbox to engineer responses in synthetic cells.

Most importantly, transmembrane signalling and enzyme activation could be coupled into signalling cascades that connect extracellular receptor activation and intracellular enzymatic responses. In so doing, we engineer synthetic cells with responsive behaviour. Responses of synthetic cells can be triggered by biochemical cues, mammalian cells, pathogens, and even inorganic surfaces.

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Different Approaches to Graphene Electrochemical Biosensors

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Abstract

Graphene biosensors have attracted growing interest in recent years due to their unique physical and chemical properties stemming from graphene's 2-dimensional, hyperconjugated carbon lattice. Although there have been many types of graphene biosensors reported or suggested, e.g., based on surface plasmon-polaritons [1], on surface acoustic waves [2], on coupling nanoparticles to graphene [3], to name just a few, most of the literature is based on liquid-gate field-effect transistors (GFETs) and other electrochemical configurations. The reason is that these architectures are relatively simple to construct, effectively exploring the graphene-electrolyte interface, allowing us to interrogate and follow the rich chemistry and other electronic processes that occur at the interface with great accuracy. Functionalizing graphene surfaces with specific molecular probes and collecting the electrical signal resulting from transducing biorecognition events makes it possible to reach untold detection limits with high specificity and sensitivity in a label-free assay [4]. Electrochemical graphene sensors can be designed and operated in different modes. This communication presents results obtained with our graphene electrochemical multi-transistor array chips fabricated using chemical vapor deposited (CVD) graphene and cleanroom technology [5], operated under DC and AC stimulation. In both cases, the sensor signal is a Dirac voltage shift upon biorecognition events that, in DC, appears as a shift in the GFET transfer curve minimum, whereas, under AC sinusoidal stimulation with a DC offset to compensate for graphene non-intentional doping, it appears as distortion in the frequency-doubled output curve. These two detection strategies are illustrated in Figure 1 by plotting simulated transfer and output curves. These curves are built with the analytical model discussed in [6], which describes the conductance of single-layer graphene as a function of the carrier resonant scattering caused by adsorbates at the graphene surface [7]. The graphene channels (20 per chip, with area $W \times L = 25 \times 81\ \mu m^2$) are functionalized using a 1-pyrene-butyric acid *N*-hydroxy-succinimide ester (PBSE) linker to which a 25-mer oligonucleotide probe sequence substituted with an amine group in the 3' position binds covalently via an amide bond. The probe will selectively hybridize with the complementary DNA strand (tDNA) containing a mutation occurring in brain tumor cells, acting as a cancer biomarker. The resulting sensor's signal, limit of detection, and sensitivity are measured and compared. Figure 2 (top) shows a set of calibration curves for tDNA detection on a GFET chip in phosphate buffer (PB) referring to binary mixtures of mutated and healthy DNA in different proportions (0%, 90%, 99%, 99.9 healthy DNA). It can be seen that the multi-transistor array chip can resolve all the mixtures for tDNA concentrations above ~ 100 aM. A critical issue that may hinder the sensor's response, particularly at minor target concentrations, is signal drift [8]. Here, it is quantified, its physical origin elucidated, and the proceedings to circumvent it are discussed. Several electrolytes (DI water, phosphate buffers of different ionic strengths, and ionic liquids) are used in measurements under different polarizations and acquisition rates to clarify the signal drift mechanisms.

The same CVD graphene devices are operated in a two-electrode configuration by shorting the source and drain contacts and using the graphene channel and the gate electrode as the working and counter-electrodes in an electrochemical impedance setup. The graphene electrode is functionalized with the same 25-mer DNA probe sequence as before, using a pyrene-derivative as the linker. It was recently demonstrated [9] that graphene electronic density of states (DOS) can be resolved using an electrochemical setup by experimentally measuring its quantum capacitance (C_q) response through electrochemical impedance spectroscopy (EIS) measurements. The latter is supported by the proportionality between C_q and DOS when plotted as an energy function: $C_q\,(eV) = e^2\,\mathrm{DOS}\,(eV)$, where e and eV correspond to the electron charge and energy, respectively. The biosensing signal is obtained by tracking the minimum graphene quantum capacitance in a series of measurements at a specific low-frequency limit and different offset bias potentials. At this frequency, there is an adiabatic coupling between Dirac electrons in graphene quantum states around the Fermi level and those of the molecular system anchored onto its surface. The displacement current resulting from the AC voltage small perturbation (10 mV RMS) reflects the dynamics of the relativistic electrons occupying states that set this electrode-molecule communication. The biosensing signal obtained in this way measures the degree of hybridization of the

DNA probes with tDNA as those highly charged molecules locally gate the graphene, i.e., change the electrochemical potential at the electrode.
Figure 2 presents the calibration curves obtained for two biosensing assays, in which the transduction signal corresponds to the relative response of the inverse of the C_q (RR), calculated by taking as reference the response of the blank experiment - incubation of PB without tDNA. We use the inverse of the C_q as the transduction signal because it is associated with the electron energy in graphene. Thus, any biorecognition event over the graphene surface, such as a resonant coupling, changes this energy [10]. In the first assay (orange circles), the RR is recorded as a function of the logarithm of tDNA concentration, revealing a behavior roughly linear with an excellent fit parameter (R^2=0.997). Limits of detection and quantification, with values of approximately 0.08 and 0.27 aM, were calculated. The sensitivity of the biosensing assay (the line slope) unveiled a value of 117% per decade of target concentration, demonstrating the technique's performance level. In contrast, the linear fit obtained for the second assay (green circles), made in consecutive PB incubations without tDNA, presented a negligible slope of 2.4%, compared with the previous assay, demonstrating the technique's specificity.

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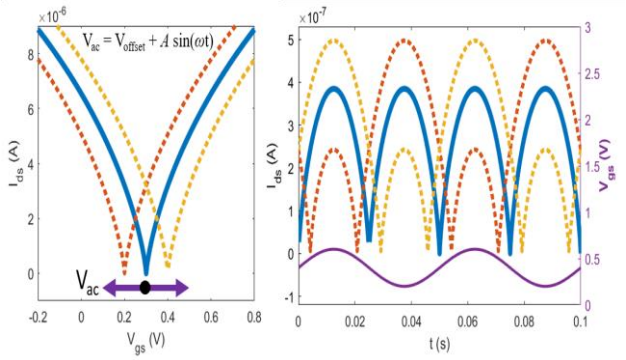

Figure 1. (left panel) A biorecognition event is detected by the shift in the GFET transfer curve minimum. Alternatively (right panel), one can use an AC sinusoidal stimulation (right axis) centered at the initial minimum (solid current line, left axis) and detect the distortion in the frequency-doubled output current upon biorecognition events in the channel (dashed lines).

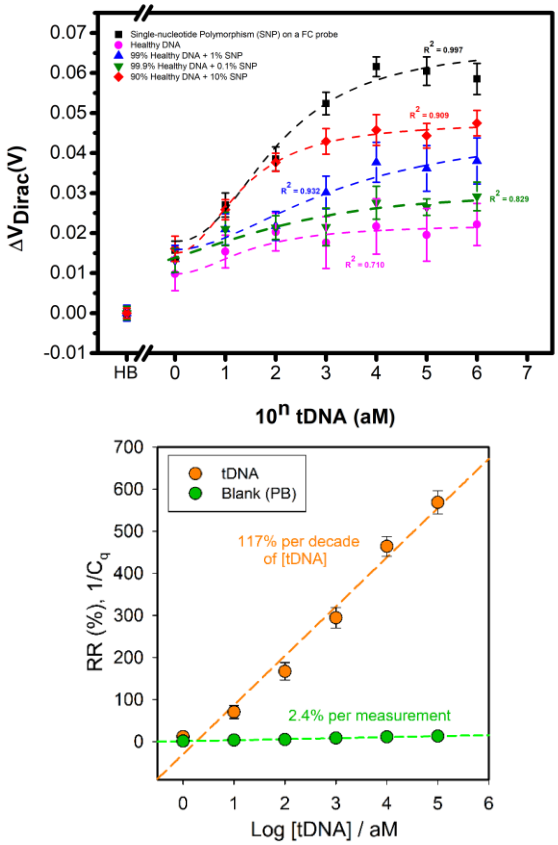

Figure 2. (top) Calibration curves for tDNA detection in PB using GFET devices. tDNA contains one SNP mutation relative to healthy DNA. The family of calibration curves refers to binary mixtures of tDNA and healthy DNA in different proportions (0%, 90%, 99%, 99.9 healthy DNA). (bottom) Calibration curves for two DNA-sensing bioassays with (orange circles) and without (green circles) tDNA. The graphene device was operated in an AC electrochemical setting. Each point of the curves corresponds to the averaged value calculated for three different graphene electrodes.

Precision Targeting Of Solid Tumors Using Smart Magnetic Theranostic Nanocomposites: A Targetless Strategy

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The specificity of cancer treatment is one of the most important parameters that dictate patient outcome. In breast cancer for example, while targeted therapies have been developed for all other subtypes, no specific treatment is available for triple-negative-breast-cancer (TNBC), and survival rates are much worse.[1,2]. Here we present a novel concept to target solid tumors by the combination of magnetic nanocomposites (NCs) [3], cell penetrating peptides (CPPs) and magnetic actuation. Upon local external magnetic stimulation, smart magnetic NCs expose CPPs that promote their internalization in tumor tissues. A simple melt-emulsification protocol was used to prepare in one step smart drug-loaded hybrid magnetic NCs (< 200 nm) displaying two types of ligands on their surface: long PEGylated units and CPPs. The PEG molecules were anchored to the NCs through thermosensitive Diels-Alder (D-A) bonds. Once in the tumor, magnetic hyperthermia (MH) can be locally applied to generate heat, break the D-A bonds and expose the CPPs, which will promote the specific accumulation of the NCs in the tumor. The incorporation of chemotherapeutic drugs in the NCs and their subsequent enhanced release via MH will treat the tumor reducing off-target effects. In addition, the magnetic nature of the NCs enables their tracking non-invasively *in vivo* through MRI. This system was validated *in vitro* (2 and 3D), *ex vivo* (CAM model) and *in vivo* (mice). *In vitro*, the IC50 of the NCs was superior to that of the free drug (55%) and was further improved upon MH application (86%). In 3D spheroids, the NCs could effectively revert their growth (-2%/day). The same was observed *ex vivo* in the CAM model (-45% growth) where angiogenesis was also inhibited compared to saline or free DOX treatment. *In vivo* the vehicle was well tolerated without significant organ damage and in terms of treatment, animals treated with the NCs+MH presented tumors in average ~30% smaller than the saline group and ~15% smaller than the free DOX group. In terms of imaging, T_2 times of the tumor area of animals injected with NCs+MH were in average 24 and 22% shorter than saline or NCs only animals, respectively. The NCs presented here combine imaging and therapeutic capabilities with external actuation (through magnetic fields) to display high specificity. This new concept represents a promising strategy for the treatment and monitoring of tumors for which a targeted therapy has not been developed yet, increasing the efficacy and decreasing off-target side effects.

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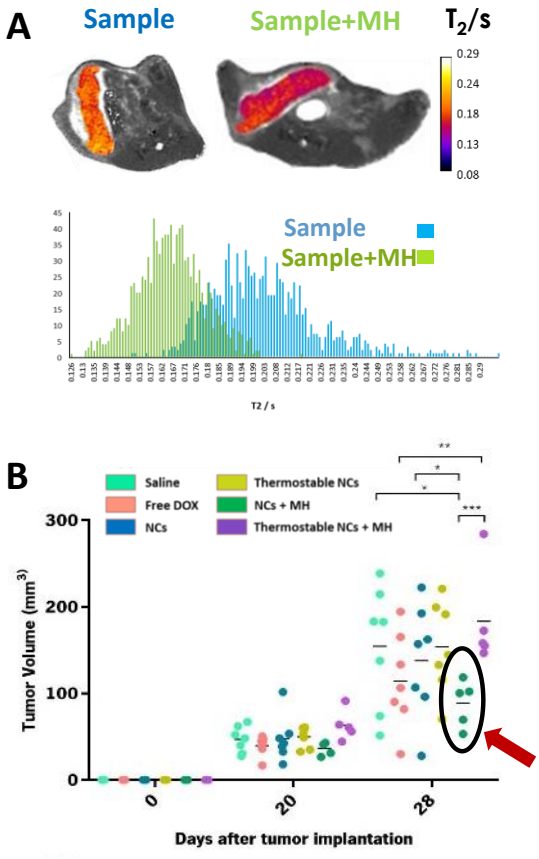

Figure 1. (**A**) T_2 maps of tumor slices of animals injected with NCs and subjected (left) or not (right) to MH. (**B**) Tumor volume evolution for animals under the different treatment schemes and controls.

pBAE polymers: a new delivery platform of biologicals for nucleic acid-based therapies

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Nucleic acid-based targeted therapies are becoming the next-generation standard of care therapeutics for many therapeutics devoted to treat current unmet medical needs, such as cancer and rare diseases. Although traditional small molecules could be used for these purposes, in the last days, there is no doubt on the enhanced performance that biologicals could bring to the field. Beyond the application of viral vectors for monogenic diseases treatment and mRNAs for infectious diseases prophylaxis, it is now the time nucleic acids can be used for therapeutics [1,2].

However, nucleic acids in vivo delivery has still some drawbacks to be overcome. Nucleic acids stability in biological environment is compromised by the presence of nucleases. In parallel, being bigger macromolecules as compared to small drugs, they are easier recognized by the immune system and prematurely cleared up from circulation. Thus, the use of a nanometric carrier system that can overcome both issues, while ensuring a safe and efficient selectively targeted therapy is an urgent need.

Among possible nanocarriers, polymeric nanoparticles, and specifically, our proprietary **oligopeptide end-modified poly (beta-aminoester) polymers (pBAE)** stood as promising carriers, not only for nucleic acids, but also for different type of viral vectors that can be used for tumor and muscular distrophies therapeutics, among others [3-5]. Thus, in here, we **aim** to present pBAE polymeric nanoparticles as a novel platform with demonstrated safety and efficacy for the direct in vivo delivery of nucleic acids for therapeutics of different diseases, such as lung cancer vaccination, Duchenne Muscular Dystrophy gene therapy and Aortic Aneurism reparation.
Through these different example applications, we have already demonstrated the possibility to *selectively target the polymers* and resulting nanosystems to different cells of interest [5,6]. For example, for tumor therapeutics, we can be selective in the tumor microenvironment cell type (i.e. cancer stem cells, antigen presenting cells). Also, we have shown how changing the administration route allows the control and tuning of the particles biodistribution, which could be advantageous for different applications. Additionally, we have demonstrated the potential efficacy of our technology for oncolytic virotherapy as well as for mRNA vaccination, among others [5,7].

In this communication we present and review different studies to demonstrate that our pBAE polymers family successfully deliver nucleic acids and coated viruses in a tunable way, for specific unmet medical needs such as tumor therapeutics. With these results in specific applications as proof-of-concept, we aim to demonstrate the application our pBAE polymers could have for any other disease requiring for the controlled delivery of nucleic acids.

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Fe3O4 Nanoparticles as Multifunctional Theranostic Agents

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Nanoparticles have attracted an enormous interest during the last decades due to their appealing properties which have led to countless applications in very widespread fields. Interestingly, the physicochemical properties of nanoparticles can be efficiently tuned by designing not only their size but also their shape. For biomedical applications, iron oxides, magnetite (Fe_3O_4) and maghemite (γ-Fe_2O_3), are becoming the preferred material due to their excellent biocompatibility. However, most of the research performed in maghemite/magnetite nanoparticles has been carried out on isotropic spherical particles. [1] Here we present a rationally designed synthesis pathway based on the thermal decomposition of to obtain high quality nanocubes [2] and on solvothermal strategies to reach magnetic iron oxide nanorods, both over a wide range of sizes. The nanocubes with an edge length below 17 nm show a great colloidal stability (Figure 1), even after transferring them to water. Moreover, the 17 nm nanocubes exhibit an excellent magnetic hyperthermia and NMR relaxivity performance (better than their spherical counterparts), making them excellent candidates for potential applications in nanotheranostics. In addition, the Fe_3O_4 nanocubes are outstanding heat mediators for photothermia in the near infrared biological windows (680-1350 nm), with heating efficiencies similar to, or better than, the best photothermal agents [3]. In addition, the magnetic and optic anisotropies of the nanocubes have been exploited for a relatively new approach for in situ local temperature sensing.

On the other hand, structural and magnetic properties of elongated IONPs between 25 and 400 nm (length) and aspect ratios between 4 and 8 are presented (figure 2). The magnetic nanorods were synthesized by the solvothermal method using iron organic precursors. Different strategies for their transfer to water have been addressed. We will correlate their magnetic properties with the performance in hyperthermia and MRI applications as a function of the structural and colloidal properties, compared to their spherical equivalents.

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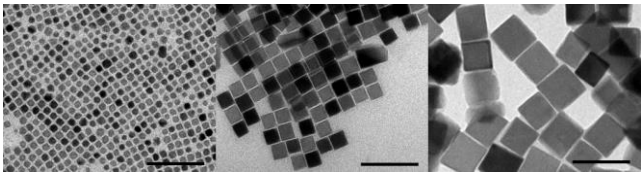

Figure 1. TEM images of magnetite nanocubes with different average sizes (left 10 nm, centre 17 nm and right 30 nm).

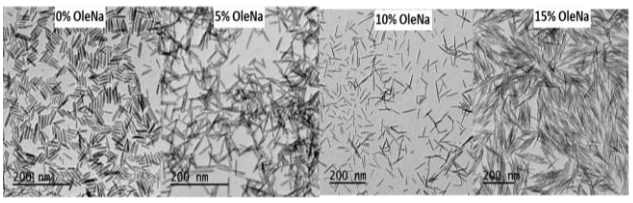

Figure 2. TEM images of magnetite nanorods with different aspect ratios as a function of the amount of sodium oleate.

NFC-enabled on-site precision diagnostics powered by a smartphone

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On-site rapid diagnosis at low cost is key for the monitoring and prevention of diseases, particularly in remote areas. There is, however, a lack of affordable, versatile digital diagnostic platforms available in the market that can be easily adapted to unforeseen situations such as the rapid spread of pathogens and global pandemics.

Lateral Flow Assay (LFA) is one of the most widely used Point-of-Need (PoN) tools for diagnostic testing since it is portable and simple to use [1,2]. Most LFA devices, however, rely on colorimetric detection, which only provides qualitative or at best semi-quantitative results. Many efforts have focused on addressing the lack of quantification on this type of devices by combining LFAs and electrical detection, but the integration of metallic electrodes on nitrocellulose membranes remains a bottleneck.

One of the main challenges for on-site diagnosis is the need of a portable reader amenable for the analysis in the fields. The development of wireless technologies, such as Bluetooth, Wi-Fi and Near-Field Communication (NFC), has enabled the real-time communication of data to accessible platforms, where they can be analyzed and stored [3]. The biggest differentiator of NFC, however, is that it is powered passively without batteries through wireless inductive coupling. NFC allows exchange of electrical power large enough (10 mW) to operate low-power and low-cost electronics, including microcontrollers and sensors. Mobile-operated wireless technology, which combines rapid communication and wireless power supply on the same unit, is an emerging area with promising applications in PoN diagnostics.

In this work, we present two new key technologies to realize the next-generation of rapid digital diagnostics for on-site testing: (i) a disposable nitrocellulose-based platform with integrated hydrophilic 3D metal electrodes (eNC) to facilitate quantitative electrochemical detection on LFAs; (ii) a programmable wireless and batteryless potentiostat powered by NFC technology to perform electrochemical analysis using a smartphone [4]. The versatile NFC-potentiostat enables the use of typical electroanalytical techniques employed in (bio)sensing (cyclic voltammetry, chronoamperometry, square wave voltammetry) by customizing the setup conditions according to the detection process. We have also designed an Android application for use in the field and a 3D-printed phone case to house the NFC-potentiostat and connect the disposable eNCs. The operation of the integrated system (NFC-eNC) was demonstrated by measuring the products of a modified commercial ELISA kit to detect Maize Mosaic Virus, a devastating crop pathogen. The results of the electrochemical measurements were compared to traditional spectroscopic ELISA measurements to validate our on-site testing device.

The NFC-eNC platform reported in this work is highly versatile and can be modified to detect multiple analytes by tailoring the bioreceptors (i.e., antibodies, aptamers) or enzyme conjugates, for applications in other fields, such as healthcare and food safety. We aim to create the next generation of digitized tests for rapid detection of diseases at the PoN.

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Convenient and Rapid Quantification of Therapeutic Biological Drugs in Undiluted Bodily Fluids

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Precision medicine - the ability to tailor treatment precisely to each individual patient - would be greatly advanced by the availability of technologies that enable rapid and convenient measurement of drugs and biomarkers at the point of care. Due to their low cost, ease of use and good analytical performance in complex clinical samples, electrochemical aptamer-based (EAB)[1] sensors appear to be a promising means to this end. Therefore, we present here the development of EAB sensors for the measurement of therapeutic monoclonal antibodies,[2] which are biological drugs used for the treatment of various diseases such as cancer or infectious diseases.[2] The sensors use a previously reported DNA aptamer capable of recognizing the selected monoclonal antibody. We incorporated these into the EAB platform by truncating them (causing them to undergo a binding-induced conformational change), modifying them with a redox-reporting methylene blue, and covalently attaching them to an interrogation electrode. We then adapted the system to a scaffold approach, using the aptamer as the recognition element attached to a redox-reporting methylene blue double-stranded DNA scaffold. The resulting sensors can measure the monoclonal antibody directly in biological fluids such as blood within minutes. The speed and convenience with which this is achieved suggests that the developed sensors could significantly improve the ease and frequency with which biological drugs can be monitored during the therapeutic treatment.

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Carbon nanomaterials and 2D nanomaterials in biomedicine: applications, main challenges, and opportunities

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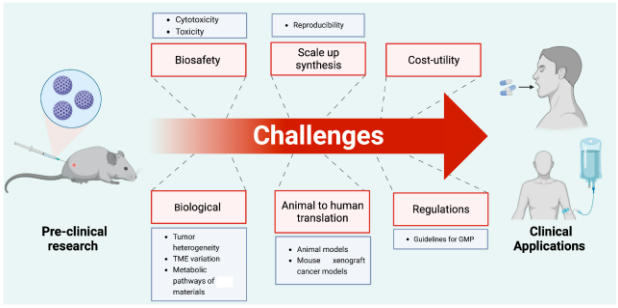
Carbon nanomaterials (CNM) have gained great interest in the scientific community and motivated multiple studies in the biomedical field thanks to their unique properties. CNM colloids can be used as multifunctional agents in phototherapy (photodynamic and photothermal therapy (PTT)), targeting cancer and antimicrobial applications.

The first bi-dimensional nanomaterial (2DnMat) to be isolated was graphene in 2004 by Geim, Novoselov *et al*. Nevertheless, the initial investigations employing graphene-based materials (GBM) for cancer PTT were documented only in 2010, while GBM use for PTT against infections was reported in 2013. The emergence of graphene as a novel material inspired the development of other 2DnMat. Remarkable advances have been achieved in the field of nanomedicine and immunotherapy, since it was found that several nanomaterials can modulate the immune response. CNM, including GBM, and also new 2DnMat have a vast potential on the field, however, little is known about the effects of several of those materials in the immune system.

A general perspective on the work of our team will be presented, focusing on applications of 2DnMat, with emphasis on graphene-based materials for phototherapy, immunotherapy, and 3D-printing for tissue regeneration [1-3].



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Designing nanoporous anodic alumina for nanomedicine applications

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Nanostructured porous materials have been used as versatile platforms in nanomedicine. In this context, nanoporous anodic alumina (NAA) has demonstrated to be an excellent functional platform for diagnostic, drug delivery and tissue engineering. One important characteristic of NAA is its well-defined cylindrical pores, with diameters tunable from a few to hundred nanometers and profiles that can be modulated in depth to design photonic structures [1-3]. Here, we present advances in the structural design of NAA and their application in biosensing, drug delivery and cell culture.

The fabrication of self-ordered NAA structures based on electrochemical etching process is presented and discussed as well as examples of new 2D and 3D micro and advanced nanostructures based on NAA. We analyse the dependence of some technological parameters on the geometry and the photonic properties of NAA. Also, some examples of new nanostructures are presented using NAA as a template and by filling the nanopores with metal, polymers, nanoparticles, etc.

We present and discuss the application of advanced photonic NAA structures for the detection of biomarkers such as Amyloid beta (Aβ) oligomer [4] and tumour necrosis factor alpha (TNF-alpha) [5]. We demonstrate how the pore surface can be chemically and biologically modified for successful biosensing. Furthermore, these structures are evaluated for stimuli drug delivery. Using polyelectrolytes and different shape configuration of nanopores, we can control the released of DOX by pH stimulus [6].

Finally, NAA is also applied for reproducing 3D cellular microenvironments and understanding the complex cellular interactions and behaviors. The effect of the geometry and the functionalization of NAA on cell adhesion and morphology of human aortic endothelial cells is investigated and presented [7-8]. The biocompatibility is demonstrated by analyzing the cell viability and cytotoxicity [9-10]

Results demonstrate that this kind of nanostructures open new possibilities in the diagnostic and treatment of diseases.

Acknowledgments

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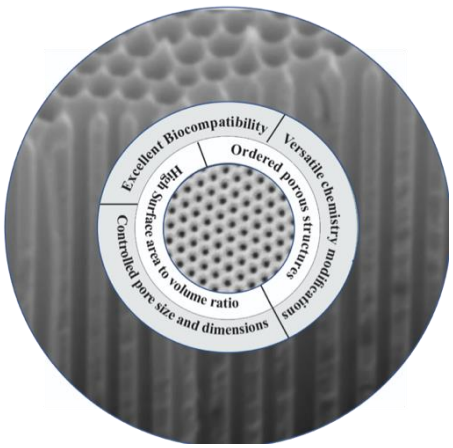

Figure 1. SEM image of an NAA cross-section and major features.

Breaking Barriers in Brain's Health: Liposomes against Neurodegeneration

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Parkinson's disease (PD) is one of the most common neurodegenerative condition, with limited treatment options. The disease is characterized by the loss of dopaminergic neurons and abnormal accumulation and propagation of the neuronal protein alpha-synuclein (AS). An anti-AS antibody (SynO4) has previously shown a high affinity to AS aggregates,[1] suggesting that it can be used as a therapeutic agent to slow PD progression. However, SynO4's penetration into the brain, similarly to other antibodies, is very limited by the highly selective blood-brain barrier (BBB) (only around 0.01% of the injected dose penetrates the BBB), thereby curbing its therapeutic efficiency.[2] In addition, antibodies are limited with their ability to enter cell membranes and specifically neurons. This is a major obstacle for an effective reduction of intracellular AS aggregates and oligomers. During PD, the transferrin receptor is overexpressed on the BBB and in CNS neurons.[3] To overcome the brain-penetration challenge, we encapsulated the therapeutic Syn-O4 antibody within 100-nm lipid nanoparticles decorated with transferrin on their surface (Figure 1)[4]. Transferrin nanoparticles loaded with SynO4 demonstrated enhanced penetration across a supported BBB model and higher neuron cellular uptake and target engagement to intracellular neuronal alpha-synuclein aggregates compared to free SynO4. The efficacy of the TF-SynO4-lipo was tested in primary cortical neurons infected with a viral vector overexpressing A53T alpha-synuclein. The cells were treated overnight with either TF-SynO4-lipo or the free form of the mAb. TF-SynO4-lipo treatment reduced AS aggregation level significantly compared to PD-induced cells or free mAb treatment. Furthermore, *in vivo* studies show that systematic administration of transferrin-targeted liposomes efficiently crossed the BBB and were delivered to the neuronal cells in an AAV-based PD-like mouse model. The nanoparticles improved the therapeutic efficacy, including reducing AS aggregation and neuroinflammation. Taken together, the use of transferrin SynO4 liposomes and their ability to encapsulate therapeutic antibodies represents a promising therapeutic approach and a novel platform for effective drug delivery into the brain. Thereby, they can be considered an improvement of the treatment of PD and other neurodegenerative and CNS disorders[4].

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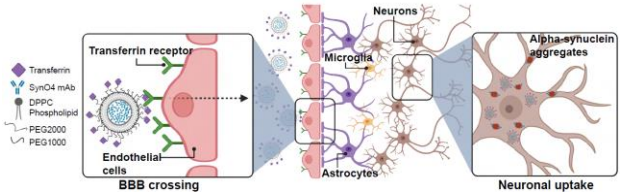

Figure 1. Brain targeted liposomes deliver anti-alpha-synuclein monoclonal antibody to reduce aggregation of alpha synuclein in early stage Parkinson disease mouse model. Through receptor-mediated transcytosis, the liposomes cross the BBB and are taken up by disease neuron cells; the antibody payload then targets the AS aggregation to inhibit neuron cell death.

Unveiling the Promise of Personalized Therapy: Harnessing Cell Membrane-Coated Nanovectors for Targeted Treatment of Glioblastoma Multiforme

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Precise tumor targeting presents a significant hurdle for emerging antitumor therapies that aim at focusing therapeutic effects on tumor cells while minimizing undesirable side effects. Common targeting strategies rely on simple ligand/receptor interactions. Yet, when dealing with heterogenous tumors, selecting a suitable target receptor becomes complicated due to variations in membrane protein expression among patients.

Glioblastoma multiforme (GBM), a highly aggressive brain tumor, displays high genetic diversity among different patients [1]; thus, it is crucial to devise an innovative targeting strategy to improve patient survival and reduce recurrences. Recently, a novel approach known as "homotypic targeting" has emerged, harnessing cancer cells inherent capability to recognize and interact with each other through specific membrane protein [2]. The nanotechnological implementation of homotypic targeting consists in coating nanoparticles with cell membranes directly extracted from the target cells, thus tailoring the targeting approach to each patient's membrane signature and bypassing challenges posed by tumor heterogeneity. Nevertheless, to ensure an optimal targeting efficacy, it is essential to preserve the presence and the natural conformation of membrane proteins in the coating, and the formation of a protein corona upon interaction with serum proteins should be avoided so that the membrane protein in the coating can be freely exposed to the target cells.

In our work, we introduced lipid-based magnetic nanovectors loaded with iron oxide nanoparticles and coated with cell membrane extracts from patient-derived GBM cells (CDMNVs), as an innovative personalized approach to efficiently target tumor cells and treat GBM with magnetic hyperthermia [3]. We thoroughly demonstrated the effective coating of the nanovectors and quantified protein content using different techniques, including X-ray photoelectron spectroscopy (XPS), Fourier-transform infrared (FTIR) spectroscopy, and the bicinchoninic acid (BCA) assay, while the presence of crucial membrane proteins fundamental in the homotypic targeting mechanism, such as CD44, N-cadherin, neuroplastin-1, and beta-catenin, was verified using Western blotting.

We also demonstrated that coating the nanovectors with cell membrane extracts contributed to improved stability and reduced formation of a hard protein corona when interacting with serum-enriched cell culture medium. Since the correct conformation of proteins is also important to guarantee an effective targeting process, we focused on thoroughly characterizing this important, yet poorly studied, aspect. The structural conformation of membrane proteins in the coating was investigated with a straightforward platform based on several spectroscopic techniques such as fluorescence, FTIR, and Raman spectroscopy. This study confirmed that ultrasonication-based coating procures did not induce protein unfolding; thus, membrane proteins are expected to retain their functionality even when confined on the nanoparticles surface. A more detailed characterization with Raman spectroscopy, however, highlighted that after the coating procedure a slight increase in α-helix structures (≈12%) could be observed. Nevertheless, we demonstrated that this change did not affect the efficacy of homotypic targeting of the coated nanovectors, as they showed preferential uptake by GBM cells compared to healthy brain cells such as neurons, astrocytes, endothelial cells, and pericytes in a home-made fluidic bioreactor.

Finally, to better understand the interaction between the nanovectors and patient-derived GBM cells, we employed label-free techniques to directly detect iron oxide internalized within cells. In 2D cultures (Figure 1) and 3D tumor spheroids, synchrotron X-ray fluorescence (XRF) imaging displayed co-localization of iron signal from CDMNVs with native elements in cells (Na and C), confirming effective internalization. In 3D spheroids, we quantified the depth of CDMNV penetration over time with respect to an analogous nanovector named CD*MNVs, coated with cell membrane extracts previously deprived of membrane proteins. Results showed that CDMNVs were able to interact more efficiently with patient-derived GBM spheroids compared to CD*MNVs, validating the effectiveness of cell membrane coating and unveiling the crucial role of membrane proteins in the targeting process.

This work offers a platform for rapid assessment of cell membrane-coated nanovector functionality, a critical aspect when implementing new formulations and coating procedures. Moreover, it further validates the promising features of this personalized approach for the treatment of GBM.

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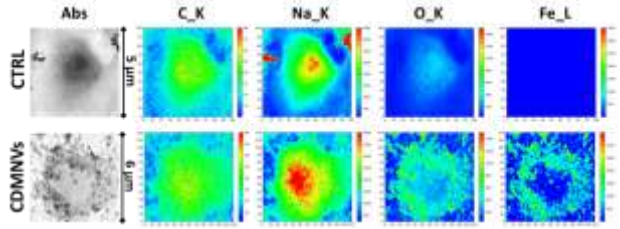

Figure 1. Absorption images and XRF maps of C, Na, O, Fe, (1.3 keV) of patient-derived GBM cells (control cells and cells treated with CDMNVs).

Nanoparticle-based lateral flow assays for diagnostic applications

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Abstract

In the wake of the COVID-19 pandemic, the critical need for swiftly deployable, cost-effective and easily scalable diagnostic tools has never been more evident. Rapid diagnostic tests, also known as lateral flow assays, emerged as indispensable tools in the identification of SARS-CoV-2. These biosensors, characterized by their portability, user-friendliness, lack of reliance on batteries or equipment, and quick response that can be interpreted with the naked eye in less than 10 minutes, proved to be invaluable tools.

This talk is designed to unveil the intricacies of the lateral flow paper strip, shedding light on its components, elucidating the assay mechanism, and showcasing its diagnostic potential. Utilizing nanomaterials as transducers for generating a colorimetric signal, these paper-based biosensors stand as a testament to simplicity in operation, while harboring vast untapped potential for refinement and advancement.

Join us on this enlightening journey as we explore the hidden depths and promising horizons of lateral flow assays, a technology poised to revolutionize diagnostic applications!

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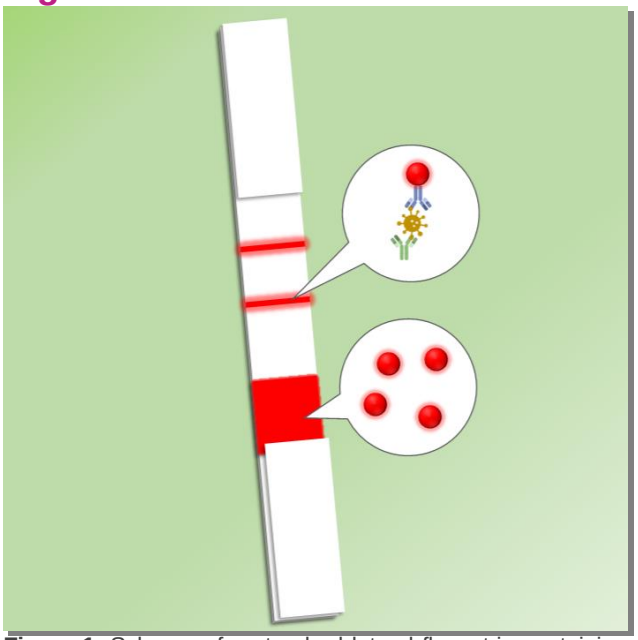

Figure 1. Scheme of a standard lateral flow strip containing nanoparticles as colorimetric labels.

Photoporation for enhanced mRNA delivery to the ocular surface

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Introduction
Diseases of the ocular surface such as dry eye disease can severely impact vision. Current therapies typically rely on traditional approaches such as the use of artificial tears, antibiotics, or anti-inflammatory drugs. Recently, nucleic acid (NA) research have grown interest in ophthalmology by investigating the potential of siRNA and mRNA as for the treatment of ocular surface diseases. However, a major pitfall for using NAs lies in limited delivery efficiency to the ocular surface, as extra- and intracellular barriers impede their cell uptake and function[1]. To overcome this challenge, we introduce the use of photoporation to enhance mRNA delivery into cells at the surface of the eye. Photoporation makes use of pulsed-laser light to create transient pores in cell membranes through mechanical forces which arise by implosion of vapor nanobubbles (VNBs; **Figure 1**)[2]. Making use of polydopamine nanoparticles (PD NPs) as photosensitizers, we show improved delivery of mRNA in the corneal epithelium by using the mechanical forces induced by the implosion of VNBs to disrupt the mucus layer covering the ocular surface and form pores in the membrane of ocular surface cells. Our research demonstrates that photoporation using low fluences allows for safe and efficient mRNA delivery in rabbit corneas, offering a promising new approach for laser-based engineering of the ocular surface and potential treatment of various ophthalmological diseases.

Methods
Bovine eyes were used for the purpose of isolating and culturing primary bovine epithelial corneal cells (pBCECs). Additionally, intact bovine eyes were used as an *ex vivo* model to assess the impact of different NP sizes (90 nm versus 250 nm) and concentrations on photoporation efficiency. PD NPs were exposed to a pulsed laser (<7 ns; 561 nm) to produce VNBs, and the effectiveness of photoporation in delivering FITC-dextrans (FD; 10 kDa, 150 kDa, and 500 kDa) and mRNA to pBCECs and bovine corneas was investigated. To evaluate the transfection efficiency *in vivo*, rabbits were administered with PD NPs and eGFP-mRNA and their corneas were exposed to the pulsed laser (532 nm; <7 ns; 1.8 J/cm^2). The extent of transfection was measured by assessing fluorescence at the corneal level. Safety evaluations were performed through optical coherence tomography (OCT), H&E staining, TUNEL assay and electroretinography (ERG) to assess any potential corneal and retinal damage.

Results
The delivery of FD of different molecular weights was achieved with high efficiency in primary epithelial corneal cells. There was no significant difference in delivery yield between 90 nm and 250 nm NPs, but the latter demonstrated increased toxicity (**Figure 2a-d**). When applied to the surface of intact bovine eyes with FD10, optimal concentrations determined *in vitro* did not result in increased uptake when compared to FD alone. However, increasing the concentration of PD NPs clearly improved the uptake. Single particle tracking (SPT) measurements in corneal mucus reveals that photoporation induced mucus liquefaction suggesting facilitated diffusion of the cargo through the mucus, a major barrier to delivery to the ocular surface. In rabbits, photoporation using 250 nm NPs was found to be an efficient method for mRNA transfection (**Figure 3**) and to be safe, as there were no morphological changes in the cornea (determined via OCT, H&E staining and TUNEL assay) and no impact on retinal functions (determined via ERG; **Figure 4**).

Conclusions/impact
Our findings indicate that PD NPs were able to generate VNB, which effectively disrupted the corneal mucus and facilitated photoporation of corneal epithelial cells, resulting in a significant increase in delivery efficiency observed *ex vivo*. Furthermore, in an *ex vivo* setting, we were able to efficiently deliver FDs and mRNA to the corneal epithelial layer. In rabbits, we were able to safely and efficiently deliver mRNA to the cornea at low laser fluences, which is a promising development for the treatment of ocular surface diseases.

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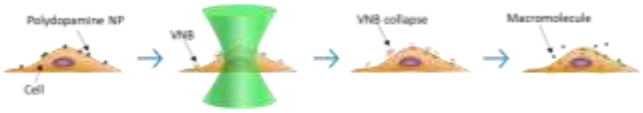

Figure 1. Principle of photoporation.

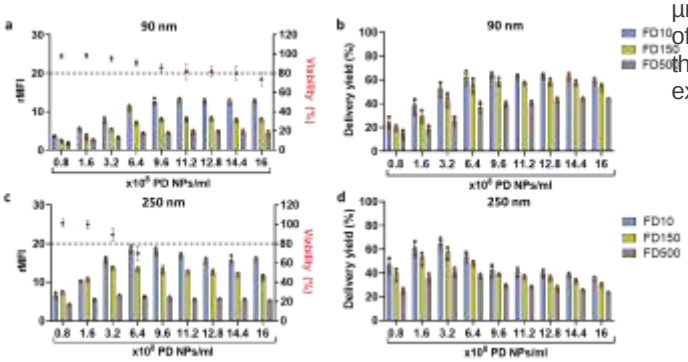

Figure 2. Photopoation of primary epithelial corneal cells. Cells were incubated with 90 nm (a and b) or 250 nm (c and d) PD NPs before being irradiated with a pulsed laser (1.8 J/cm2; 532 nm; pulse duration < 7 ns).

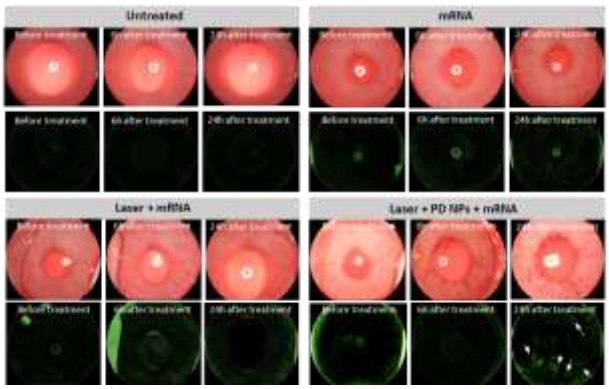

Figure 3. Fundus imaging (top row) and fluorescence imaging (bottom row) of corneas respectively untreated, treated with mRNA (control), treated with mRNA and laser irradiated (control) and treated with PD NPs (4.8x1011 NPs/ml) and mRNA (0.1 µg/µl) and laser irradiated (1.8 J/cm²; 532 nm; <7 ns). The images were taken before, 6 hours after, and 24 hours after the treatment, and are representative of the images obtained from three rabbits for each condition. The white arrows indicate eGFP-expression. Fundus imaging did not reveal any damage of the corneas.

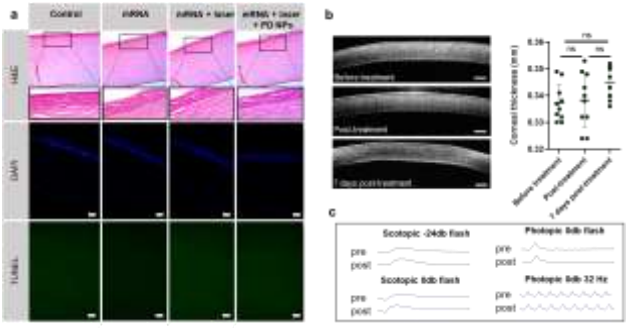

Figure 4. mRNA delivery in the corneal epithelium of rabbits in vivo by photoporation. (a) Fundus imaging (top row) and fluorescence imaging (bottom row) of corneas respectively untreated, treated with mRNA (control), treated with mRNA and laser irradiated (control) and treated with PD NPs (4.8x10^{11} NPs/ml) and mRNA (0.1 µg/µl) and laser irradiated (1.8 J/cm²; 532 nm; <7 ns). The images were taken before, 6 hours after, and 24 hours after the treatment, and are representative of the images obtained from three rabbits for each condition. The white arrows indicate eGFP-expression. Fundus imaging did not reveal any damage of the corneas. (b) Cryo-sections of the corneal epithelium 24 hours after treatment. Nuclei were stained with Hoechst; scale bar = 20 µm. eGFP-fluorescence could be detected in the epithelium of rabbits treated with PD NPs and mRNA and irradiated with the laser (1.8 J/cm²; 532 nm; <7 ns). In all control experiments, eGFP-expression did not occur.

Targeting macrophage polarization states for precision immunotherapy

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Macrophages are crucial immune system components, safeguarding our tissues from external threats such as injuries, toxins, and infections [1]. When faced with an insult, resident macrophages initiate the inflammatory process, transitioning from a resting state (M0) to an activated state and changing their effector function into a pro-inflammatory (or M1) and anti-inflammatory (or M2) phenotype [2]. This dynamic activation of macrophages plays a pivotal role in disease progression and can lead to unresolved inflammation if impaired. To address this, macrophage-targeting nanomedicines have emerged as a revolutionary approach for treating a wide range of human diseases, including infections, chronic inflammatory disorders, neurodegenerative diseases, and cancer. Traditionally, targeted strategies have relied on high-affinity ligands like antibodies. However, these interactions can lead to indiscriminate targeting of any cell expressing the corresponding receptor, resulting in a loss of selectivity. One strategy to overcome such a challenge involves employing low-affinity ligands within a multivalent scaffold, thereby achieving super-selectivity [3]. This approach relies on the collective effect of individual affinities, ensuring that associations only occur when receptors are expressed at specific densities, effectively targeting cells expressing the desired receptor while minimizing non-specific interactions. We propose using engineered polymer-based self-assembled nanoparticles (polymersomes) where multiple ligands are expressed alongside polymers that prevent non-specific interactions and act as steric modulators [4]. In vitro experiments show that nanoparticle binding to the cell surface is non-linear, dependent on the number of ligands present. This behavior allows for identifying an optimal ligand density, creating on-off association profiles that enable precise targeting of specific macrophage phenotypes. Through this approach, we can achieve phenotypic targeting of macrophages while enhancing selectivity and therapeutic efficacy.

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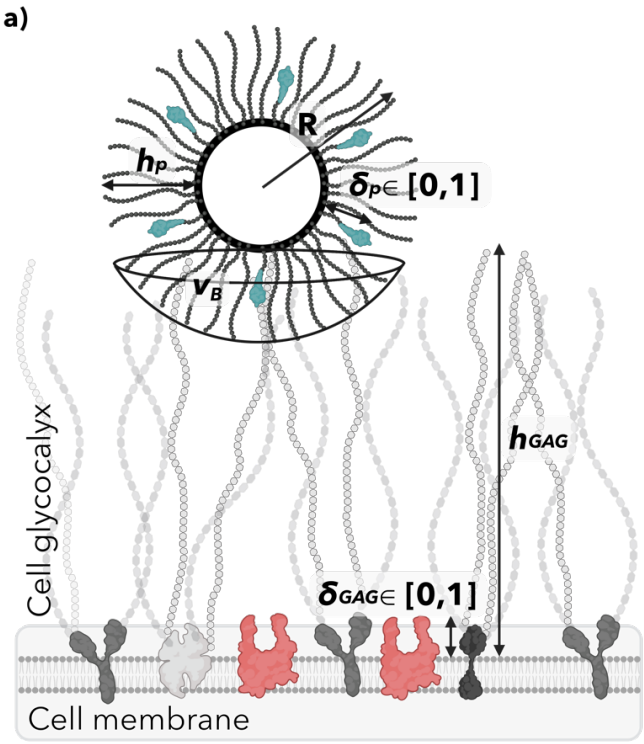

Figure. Nanoparticle (NP) - cell interaction. a) The multivalent system is described by the NP topology, size (radius, R), number of ligands, and length of the ligand tether with respect to the polymer brush length, h_P, given by the polymer inference parameter, δ_P. The target, the cell, is relatively larger that the NP, hence considered as a flat surface with a density of receptors (targeted receptors and glycans) that characterizes the cell phenotype. NP effective binding to the cell requires high avidity associations that can overcome the repulsive forces arising from the NP polymer brush and from the sugar-rich cell membrane brush, the so called glycocalyx that can reach an extension of a hundred nanometers long, h_{GAG}. Abbreviations. V_B: Binding volume. δ_{GAG}:

Engineered Nanoparticles as An Emerging Platform to Fight Antimicrobial Resistance

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Abstract

Antimicrobial resistance (AMR) is one of the biggest public health issues, causing more than 700,000 deaths per year worldwide [1]. This number is expected to rise to 10 million by 2050 unless preventive measures are taken. Specifically, the World Health Organization (WHO) has designated the development of therapeutic strategies against *Staphylococcus aureus* (*S. aureus*) as a top priority, as it is the most common and one of the most dangerous pathogens involved in numerous infections [2]. A promising approach to fight *S. aureus* is using engineered nanoparticles (NPs) capable of targeting *S. aureus* and delivering antibacterial agents directly to the site of infection. In this work, polymeric NPs made of poly(lactic-co-glycolic acid) (PLGA) were produced by the emulsion solvent evaporation method. The surface of the PLGA NPs was modified with different ligands, including folic acid and cell-penetrating peptides (CPP), to increase the nanocarriers' specificity and penetration into *S. aureus*. NPs were characterized by dynamic light scattering (DLS), electrophoretic light scattering (ELS), and Fourier-transform infrared spectroscopy (FTIR). The different NPs showed monodispersed distribution with mean hydrodynamic diameters lower than 220 nm and negative zeta potential values. Moreover, all the produced engineered NPs remained stable for at least two months. The ability of the functionalized NPs to target and penetrate S. aureus was demonstrated by microscopy using rhodamine B-loaded NPs. Overall, the developed NPs show promising characteristics for the targeted treatment of *S. aureus*.

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Inhibition of the photosynthesis of *Coccomyxa subellipsoidea TL4* algae by diuron– comparison with the cyanobacteria *Synechococcus elongatus PCC 7942 and analytical exploitation*

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Introduction

Water pollution is a major concern due to its threat to animal's and human's health [1]. Among early warning systems that can alert about water pollution, the sensors based on the inhibition of photosynthesis of cyanobacteria, algae and plants show great potential for the detection in water of heavy metals and several classes of herbicides. widely used in agriculture [2]. Diuron, a phenylurea herbicide that persists longtime in water and can cause skin irritation, cancer, and mutations [3] was often employed as a model inhibitor of photosynthesis when developing biosensors. While cyanobacteria *Synechococcus elongatus* PCC 7942 was often chosen as biological recognition element in such biosensors, there is a continuous quest for novel phototrophs that are not only more sensitive to pollutants but can also provide stable sensors [4].

The present study compares whole cells and subcellular preparations (thylakoids and photosystem II) from the cyanobacteria *Synechococcus elongatus* PCC 7942 and the microalgae *Coccomyxa subellipsoidea* TL4, in terms of morphology and degree of photosynthesis inhibition by diuron. The stability of the various cellular and subcellular preparations was also investigated as a critical step in the development of a biosensor.

Experimental

Synechococcus elongatus PCC 7942 were from the collection of Babes Bolyai University, Cluj Napoca, Romania while *Coccomyxa subellipsoide*a TL4 microalgae were isolated from Scarisoara ice cave, Romania. Thylakoids and photosystem II preparations were obtained according to known protocols [5]. Optical microscopy and Atomic Force Microscopy (AFM) were used for imaging the whole cells and respectively the thylakoids from *Synechococcus elongatus* PCC 7942 and *Coccomyxa subellipsoide*a TL4. AFM measurements were performed with a Nanowizard II AFM instrument (from JPK, Germany) operating in intermittent contact in air. Thylakoids were diluted 20 times in 10mM Tris buffer pH 7.4, 150 mM KCl, and adsorbed onto poly(diallyldimethylammonium) (PDDA)-modifiedmica for 30 min. The modified mica was washed with buffer and water to remove loosely bond thylakoids.

Chronoamperometry was used to measure the current generated by the photosynthesis of the various biological preparations in presence and in absence of diuron. Carbon nanotubes electrodes (Metrohm Dropsens, Spain, DRP110D CNT), were polarized at +650 mV versus Ag and 2,6-dichlorobenzoquinone 0.5 mM was added in the electrolyte as an electrochemical mediator. For *Coccomyxa subellipsoide*a TL4, the assays were conducted at an applied potential of +400 mV in the presence of 0.7 mM hexacyanoferrate (II) as mediator. Cycles of 1 min light followed by 15 minutes dark were applied and the increase in the current intensity after 1 min illumination was taken as the analytical signal. The inhibition was calculated as: Inhibition (%) = $(I_0-I_1) / I_0*100$ (1), where I_0 and I_1- are the signals in the absence and in the presence of herbicide, respectively.

Results

The obtained AFM images of thylakoids from *Coccomyxa subellipsoidea* TL4 show disk shaped structures with heights of either 4-5 nm (corresponding to a horizontally sectioned thylakoid disk) or 10-12 nm (corresponding to a complete thylakoid disk) (see Fig. 1A). The AFM images of thylakoids from *Synechococcus elongatus* PCC 7942 showed similar disk-shaped structures (often stacked on top of each other). Higher magnification AFM images also revealed nicely organized, 20 nm diameter protein complexes, protruding 1-3 nm above the lipid layer of the thylakoid disk (see Fig. 1B).The images are in good agreement with the literature.

Inhibition investigation was conducted for whole cells, thylakoids and photosystem II (PSII) of both species. The presence of 60 ppb diuron caused a 14.9±8.6 %, 25.5±0.17 %, 25.8±9.3 inhibition of the photosynthetic activity of whole cells, thylakoids and PSII, respectively of *Synechococcus elongatus* PCC 7942. For *Coccomyxa subellipsoide*a TL4 the same concentration of diuron induced a 10.2±5.3 % inhibition of the whole cell's photosynthetic activity and 40.2±7.3 % for thylakoids.

From the various stabilizers (glycerol, PEG 8000, mannitol, sucrose and DMSO) used with PSII from *Synechococcus elongatus* PCC 7942, glycerol was the most efficient. After being stored for 12 days at -20˚C and with glycerol, PSII still showed 57.0 ±4.0 % inhibition by 2.57 µM diuron, while PSII stored without glycerol was completely insensitive to diuron. Thylakoids from *Coccomyxa subellipsoide*a

TL4 were stabilized with sucrose 2M. After 6 days at -20˚, their photosynthetic activity was 55.6 % of its initial value, and inhibition by 60 µM diuron decreased from 40.2±7.3 % to 14.9±2.5 %.
Calibrations curves for diuron were also obtained using optimum chlorophyll concentrations for *Synechococcus elongatus* PCC 7942 (7.5 µg/mL) and *Coccomyxa subellipsoide*a TL4 thylakoids (85 µg/mL). The calibration experiment emphasized detection limits of 13 ppb and 6 ppb diuron for *Synechococcus elongatus* PCC 7942 and *Coccomyxa subellipsoide*a TL4 thylakoids, respectively.

Conclusion

The studies on different cellular preparations of *Synechococcus elongatus* PCC 7942 and *Coccomyxa subellipsoide*a TL4 showed their sensitivity to diuron, thylakoids representing the best compromise between stability and sensitivity for both microrganisms. Stability of the preparations needs further improvement. An immobilization method for a future disposable platform assay is under study.

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Figures

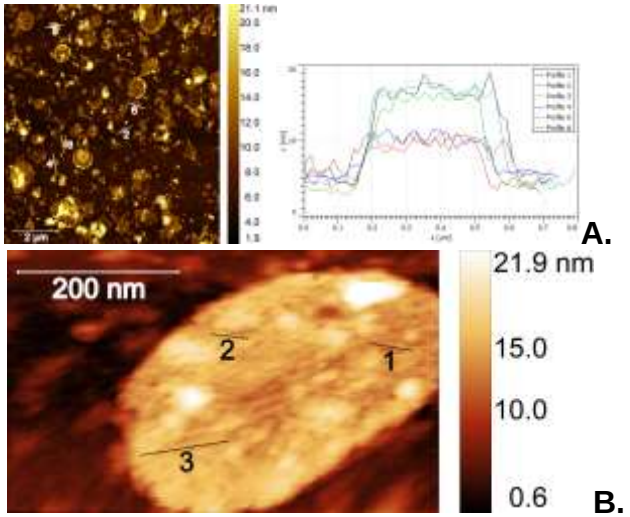

Figure 1. A. AFM image of several thylakoid disks from *Coccomyxa subellipsoidea* TL4 and cross sections highlighting the heights of such disks; B. AFM image of a single thylakoid disk from *Synechococcus elongatus* PCC 7942 showing semicrystalline arrays of complexes adjacent to disordered regions.

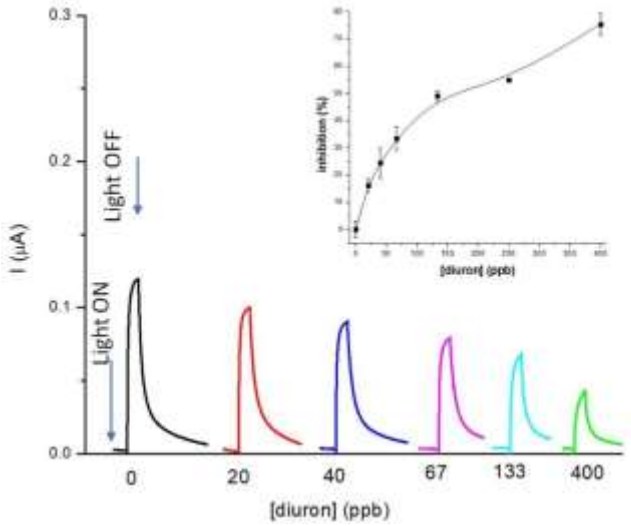

Figure 2. Photocurrents observed with *Synechococcus elongatus* PCC 7942 exposed to different concentrations of diuron. Inset: Calibration curve linking the extent of inhibition to the concentration of diuron.

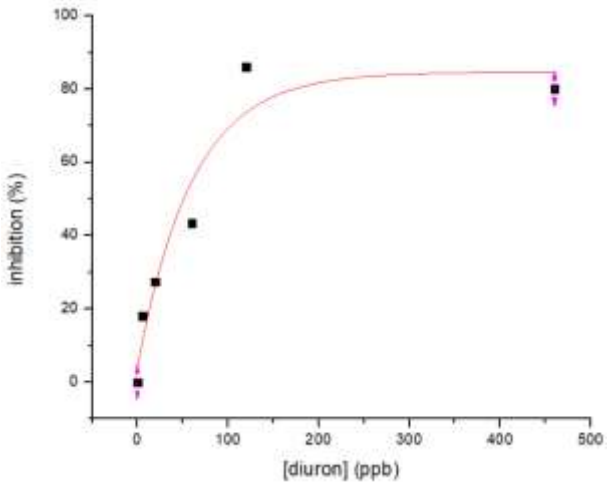

Figure 3. Calibration curve linking the extent of the inhibition of the photosynthetic current observed for *Coccomyxa subellipsoide*a TL4 thylakoids to the concentration of diuron

Acknowledgements

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Chemotaxis of porated liposomes with encapsulated enzymes

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Active systems have gained significant interest across various scientific disciplines. Active motion is an important phenomenon in nature, particularly when related to concentration gradients, a behavior known as chemotaxis. Examples include the directed movement exhibited by bacteria and neutrophils, orchestrated through intricate signaling pathways. In synthetic systems, active and chemotactic behavior has been demonstrated in Janus particles and colloidal structures such as polymersomes and liposomes.

This study investigates a novel active system composed of liposomes with encapsulated enzymes. Asymmetry is introduced by incorporating the pore protein alpha-hemolysin, accelerating the diffusion of substrate and products across the vesicle membrane. The resulting asymmetric distribution of species creates a slip velocity on the liposome's surface, resulting in self-propulsion. Experimental exploration of this motion was conducted within a microfluidic channel.

The nature of the substrate proves to be a critical factor influencing the direction and velocity of the drift. Phenomena such as diffusioosmosis and diffusioosmophoresis emerge as inherent components of the motion. Specifically, amine-modified polystyrene beads exhibit distinctive drift behaviors in a gradient of urea and glucose, attributed to interactions between the substrate with the channel walls and the surface of the beads. These phenomena are also present in the movement of porated liposomes. In addition to them, a chemotaxis component is also observed for the liposomes that have pores. The direction and velocity of the drift is a result of all these events and depend on the enzyme/ substrate pair.

This research sheds light on some fundamental principles governing liposome chemotaxis with encapsulated enzymes. This system can offer insights into the active behavior of some natural vesicles, like exosomes or synaptic vesicles. Also, it could have potential applications in diverse fields, including drug delivery. The interplay between substrate properties, surface interactions, enzyme reactions, and asymmetry opens new horizons for further exploration of chemotaxis in biochemical systems.

Figure

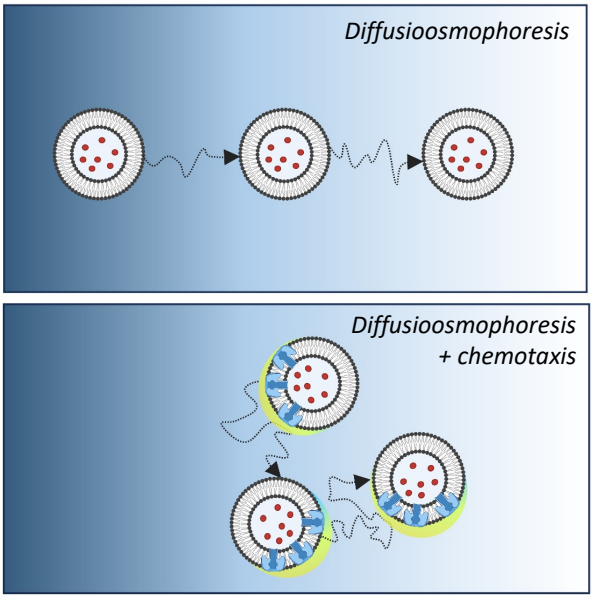

Figure 1. Liposomes with encapsulated enzymes and no pores present a drift due to diffusioosmophoresis. In liposomes with pores, a chemotactic component results in a total drift aligned with the substrate concentration gradient. In both cases, the direction and velocity of the drift depend on the enzyme and substrate.

Glyco-Gold Nanoparticles: Exploiting Multivalent Sugars for Addressing Rare Lysosomal Storage Disorders

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Lysosomal Storage Disorders (LSDs) are a family of genetic and pediatric pathologies caused by a lysosomal enzyme misfolding and dysfunction that lead to accumulation of unmetabolized substrates in the lysosomes [1].

Enzyme replacement therapy (ERT) is a therapeutic strategy for LSDs consisting of the infusion of the enzyme involved in the disorder in its recombinant form. This therapy shows some crucial disadvantages like the low stability *in vivo* of the recombinant enzyme and the high cost.

An emerging therapy for LSDs involves the use of pharmacological chaperones (PC), molecules capable of stabilizing mutated enzymes and restoring their physiological activity [2]. The identification of new PCs is also useful for the PC/ERT combined therapy, a strategy already investigated for some LSDs with the aim of reducing the dose of the infused recombinant enzyme and the administration frequency with great benefits for the patients' life quality [3].

Most PCs are identified among molecules able of mimicking the natural substrates of the enzyme involved in the pathology. Particular attention is on sugar molecules and their nitrogen-containing analogues since most of the enzymes involved in LSDs are carbohydrate-processing enzymes.

In addition, some of these enzymes, such as β-glucocerebrosidase (GCase) and *N*-acetylgalactosamine-6-sulfatase (GALNS) recently demonstrated enhanced affinities for multivalent ligands [4]. In particular, gold nanoparticles (AuNPs) have already been used as scaffolds for the multimerization of sugars, leading to biocompatible and water dispersible systems and guaranteeing the possibility of the simultaneous grafting of different thiol-ending ligands in a controlled manner [5]. In this context, AuNPs were selected as valuable scaffolds for the design and synthesis of multivalent nanosystems decorated with sugars and/or sugars analogues, properly chosen to mimic specific lysosomal enzyme substrates. After the chemical-physical characterization of the AuNPs, the biological evaluation towards enzymatic targets will be presented, to prove their affinity for lysosomal enzymes as well as their ability to stabilize the enzyme tertiary structure, acting as PCs (Figure 1).

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Figure 1

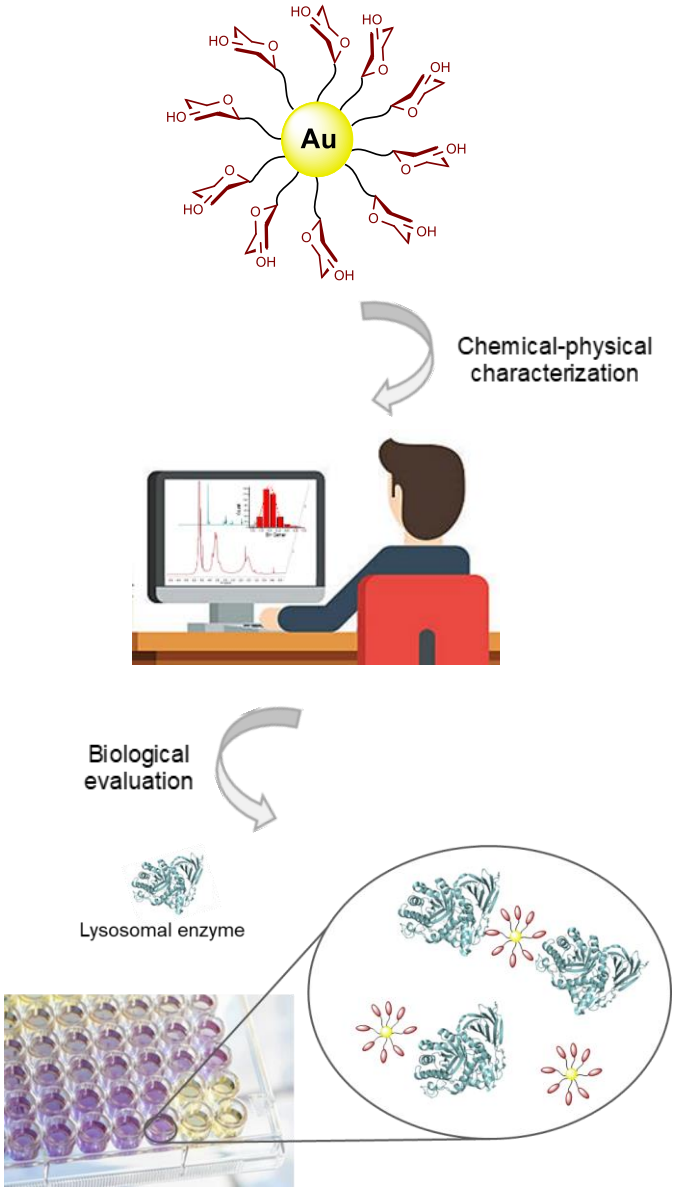

Figure 1. Graphical abstract

Engineering gold nanoclusters-decorated on nanoporous anodic alumina for biosensing applications

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Self-ordering nanoporous anodic alumina (NAA) is a material obtained through a two-step anodization process of aluminium. This process is carried out by an electrochemical reaction based on an acid solution. Different interpore distances and pore sizes can be obtained depending on the anodization conditions [1]. The possibility to control the different parameters of the anodization (temperature, potential and charge), allow us to fabricate NAA with different morphologies such as bilayers, rugates, nanotubes, etc., that can be used to detect different analytes [2], [4-6]. Figure 1 shows the top-view and cross-section field emission scanning electron microscopy (FESEM) images of an NAA structure fabricated using oxalic acid. The pore diameter is 30 nm and the interpore distance is 80 nm.

In this study, we developed a photonic biosensor that uses a monolayer of NAA with gold nanoclusters to detect Endoglin 105. Nanoporous anodic alumina (NAA) and gold nanoclusters (AuNCs) were used to combine their optical and geometrical properties.

The effect on the photoluminescence of NAA attached gold nanoclusters on its structure following their functionalization with a specific antibody, for the detection of Endoglin 105 was investigated [9]. Figure 2 shows the changes in the PL for different concentrations of Endoglin 105. The results obtained indicate that this can be a promising platform for the detection of different biomolecules.

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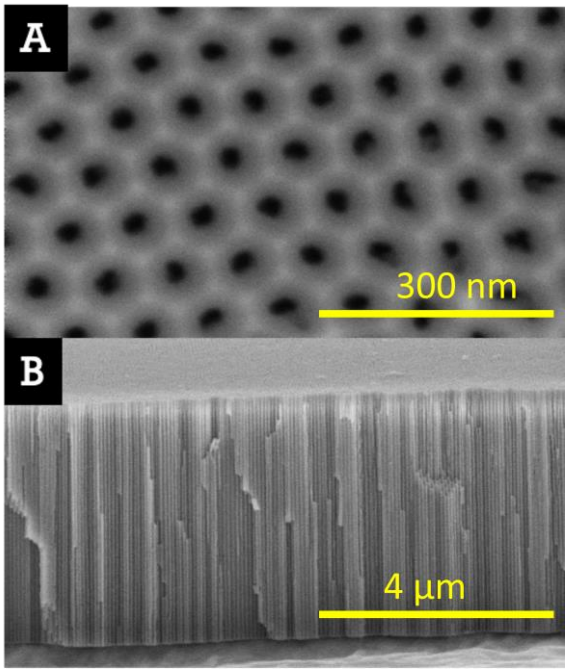

Figure 1. FESEM images of a NAA sample after a two-step anodization process. A) top-view and B) cross-section.

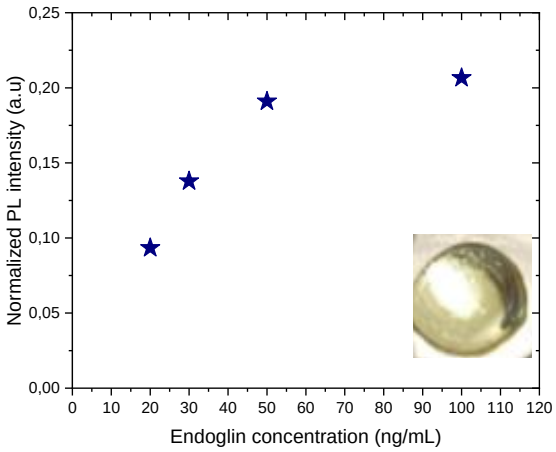

Figure 2. Photoluminescence response of the sensor for different protein concentrations. Inset shows the image of AuNC - nanoporous anodic alumina used in the experiments.

Acknowledgements

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A patient-personalized lipid-based magnetic nanovector for a selective glioblastoma multiforme treatment through oxidative stress induction and metabolic impairment

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Abstract

Glioblastoma multiforme (GBM) is the most aggressive type of brain tumor with limited options for long-term cure. Cases of recurrences are very common, caused by the impossibility of removing all the neoplastic cells even after an invasive surgical resection, followed by radio- and chemotherapy [1]. Finding a more targeted and effective solution for this type of tumor is a challenge that current medicine has not yet addresses. In this context, new nanotechnological solutions seem to offer good hopes. Nanoparticles can deliver anticancer drugs to their destination more effectively; in addition, they can be functionalized to bare the same biological features of the tumor to target. This strategy, named “homotypic targeting”, can be achieved by coating nanoparticles with cell membrane extracts of the target cells, and exploits the well-known abilities of cancer cells to interact with each other [2].
In our work, we developed lipid-based magnetic nanovectors, loaded with the anticancer drug regorafenib (Reg) and with iron oxide nanoparticles in order to combine the action of the drug with the magnetic hyperthermia induced by the proper stimulation with an alternated magnetic field (AMF) [3]. Furthermore, the nanovectors were coated with membranes deriving directly from primary GBM cells in order to provide a selective and patient-personalized targeting of GBM.
In this study, we investigated in details the induction of intracellular damages [3], and in particular, oxidative stress related to intracellular Fenton reaction, related with the production of excessive reactive oxygen species (ROS) [4]. Nanoparticle selective tumor targeting was studied with dynamic flow experiments, focusing also on the particular preference of nanovectors for the specific patient’s source cells. For this experiment, five different primary GBM cell cultures were obtained from GBM surgical samples (ethical permission CER Liguria 341/2019), here called “Pat1”, “Pat2”, “Pat3”, “Pat4”, and “Pat5”. Nanoparticles were coated with “Pat1” cell membrane extracts. The inter-patient targeting experiments were performed in dynamic conditions and evaluated with confocal acquisitions and iron colorimetric assay. Results showed differential uptake among patient cell lines. In particular, the “Pat 1” cells showed a greater uptake with respect to the other primary patient cells (“Pat1”: 33.07 ± 9.50%, “Pat3”: 8.60 ± 6.6%, “Pat4”: 1.00 ± 0.6 %, “Pat5”: 6.70 ± 2.76 %), with the exception of "Pat2"(42.14 ± 14.91%); all % indicate nanoparticle / cell area signal co-localization (Figure 1).
The production of intracellular ROS was investigated by flow cytometry, showing that in presence of nanovectors and nanovectors + AMF there is a significant increase in oxidative stress.
To better understand the effect of the increased intracellular oxidative stress on GBM cells, their metabolic state was investigated by fluorescence lifetime imaging (FLIM), a technique that monitors the decay times of the enzyme-bound and free forms of reduced nicotinamide adenine dinucleotide (phosphate) (NAD(P)H). Fluorescence imaging of NADH is extremely useful for monitoring the metabolism of live cells, since the fluorescence lifetime of NAD(P)H is sensitive to its bound-to-enzyme or free state. In general, more abundance of longer lifetime (e.g., 3.4 ns) are associated to an OX/PHOS oriented metabolism (bound-to-enzyme NAD(P)H), while an abundance of short lifetime values (e.g., 0.4 ns) is associated to an increase of the glycolytic flux [5]. Generally, tumour cells have a higher rate of glucose uptake with respect to healthy cells, and they produce ATP preferentially through aerobic glycolysis (Warburg effect) [6]. Nevertheless, an impairment at the level of the mitochondria or anaerobic environments can further drive cell metabolism towards glycolysis. Our results showed a shift towards free NADH in the nanovectors-treated cells, with and without AMF stimulation compared to the control GBM cells: in these conditions, a further tendency towards glycolysis is observed (Figure 2), suggesting an impairment of mitochondria, induced by the intracellular oxidative stress due to the nanovectors, in agreement with our previous results. This intracellular damage was ultimately demonstrated to induce apoptosis in patient-derived GBM cells, especially after a chronic AMF stimulation.
The obtained results demonstrate that the combined action of magnetic nanovectors + AMF exerts a clear antitumor action based on localized hyperthermia, as previously demonstrated, and that the oxidative stress promoted by iron oxide-induced Fenton reaction and hyperthermia support the tumor cell death, increasing the effect of the drug. In addition, the effective targeting could render this strategy a valid option for personalized nanomedicine against GBM, enhancing the selectivity towards tumor cells and reducing side effects.

ACKNOWLEDGMENTS
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Figures

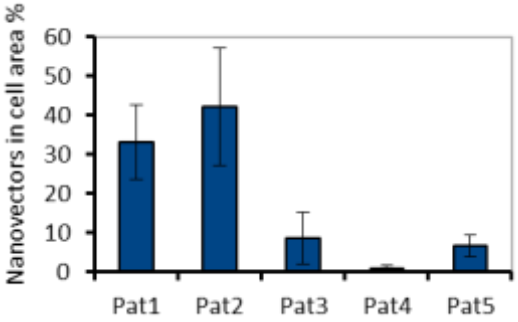

Figure 1. Nanovectors uptake analysis in five primary GBM cultures

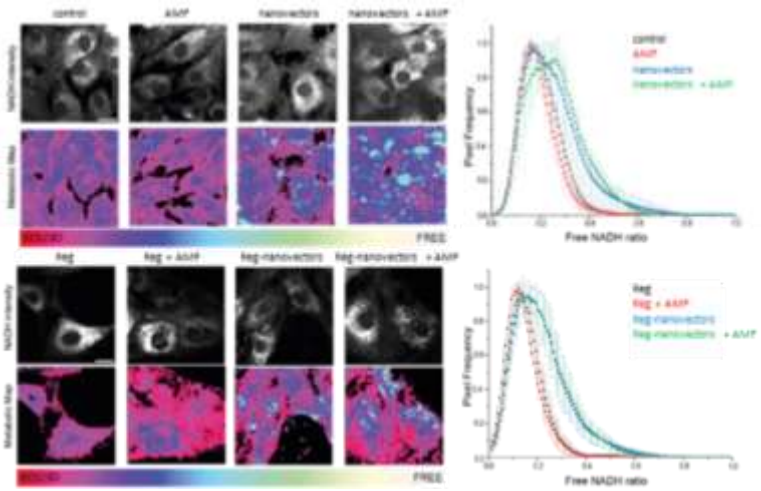

Figure 2. FLIM acquisitions and analysis of primary GBM cells undergoing different treatments.

Effect of surface functionalization and loading on the mechanical properties of soft polymeric nanoparticles used as delivery systems.

Aurora Dols-Perez[1,2,3], Cristina Fornaguera[3,4], Natalia Feiner-Gracia[3], Santiago Grijalvo[3], Conxita Solans[3,‡] and Gabriel Gomila[1,2,‡]

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Recent studies have evidenced the importance of nanoparticle mechanics in their uptake and efficacy [1-3]. Different strategies have been developed to tune NP mechanics but is still unknown the effect of loading or functionalization on the NP mechanical properties. The main drawback to study these factors is the difficulty to find a fabrication technique that allows to modify the inner part of the NP, to functionalize the surface, to change the composition, but obtaining comparable particle structures and sizes.

In here, we performed a study of the effect of these parameters using Phase Inversion Composition method (PIC) [3] to create NPs with similar composition, structure and sizes, and determine the effect of functionalization on the NP mechanics. Samples studied were PLGA NPs, PLGA NPs containing rhodamine 6G (PLGA-Rho), PLGA functionalized with antibodies (PLGA-Ab), PLGA functionalized with dendrons (PLGA-dendron), Ethyl cellulose NPs (EC) and cationic Ethyl cellulose NPs (EC cationic).

NPs were measured individually by Atomic Force Microscopy (AFM) force spectroscopy and a multiparametric nanomechanical study was performed including the determination of the Young's modulus, breakthrough force, total indentation and adhesion, covering from small to large deformations and the NPs' rupture, thus containing all relevant mechanical information.

Results [4] showed an effect of composition, functionalization and loading on the NP mechanics evaluated and a graphical representation method has been proposed to identify formulations with similar properties.

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Figure

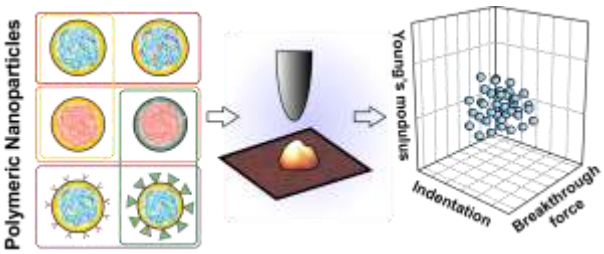

Figure 1. Graphic representation of the steps followed in this study.

Hemoglobin based Oxygen Nanocarriers: An exogenous supply of O_2 for applications in photodynamic therapy

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Photodynamic therapy (PDT), a minimal invasive technique for cancer treatment, is based on generation of reactive oxygen species (ROS) in tumor tissue by the activation of O_2 through light pulses[1]. The energy of light is transferred to O_2 by photosensitizing molecules (PS). The increase of ROS results in the activation of apoptotic pathways in tumor tissue, activates the immune system and may induce the collapse of tumor vasculature[2].

One of the main drawbacks of photodynamic therapy in cancer treatment is the limited O_2 content in tumour tissue, which limits the ROS production. The use of hemoglobin (Hb), the natural O_2 carrier in blood stream, could be an interesting approach for oxygen supply to the tumor during PDT[3].

However, the use of native protein as exogenous oxygen carrier at the therapeutic level is highly conditioned since, free Hb can lead to vasoconstriction and renal injuries[3] . Hemoglobin based Oxygen Nanocarriers (HOBC) prepared by entrapping hemoglobin in polymeric or protein nanoparticles could provide a means to transport oxygen to tumor tissue without the drawbacks from free Hb delivers[4]. Also, HOBCs size should be below of 500 nm for optimal tumor penetration and reduced immunogenic response[5]. Established methodologies for HOBCs synthesis result in particles with sizes over 800 nm with limited tumor penetration.

In this framework our aim is the synthesis of small-size HOBCs that could be applied for tumor targeting. For this purpose, different procedures have been designed to obtain Hb nanoparticles (Hb-NPs). Synthesis protocols were first stablished with Bovine Serum Albumin NPs (BSA-NPs) as a model protein NPs.

Two different approaches were tested for protein NPs fabrication. **Co-precipitation** with carbonate templates and **polymer complexation**.

Co-Precipitation

Crosslinked particles: Based on the CCD[6] procedure, protein is co-precipitated with a carbonate template (MnCO3) and the amine groups are crosslinked. Subsequently the template is dissolved, resulting in a protein particle(Figure 1). Once optimized this synthesis procedure and purification steps, we were able to produce BSA particles crosslinked with size of 200-400 nm.

Layer By Layer (LBL): In this approach, after we performed the coprecipitation stage a multilayer coating of polycations and polyanion was assembled on top of the precipitates. By doing so, protein structure was not altered after the carbonate template dissolution without using crosslinking. With this procedure BSA-NPs were produced with size of 300-350 nm (Figure 1).

Polymer complexation

Protein is complexed with polyelectrolytes through electrostatic interactions. Different polyelectrolytes were tested and both proteins (Hb and BSA) were complexed in nanosystems with size of 30-50 nm (Figure 2).

Dynamic Light Scattering (DLS), Transmission Electron Microscopy (TEM), UV-Visible Spectra, Circular Dichroism (CD) were applied for an in-deep characterization of physico-chemical properties of our systems,

In conclusion, the nanosystems designed could be the basis, for the implementation of PDT by exogenous oxygen supply.

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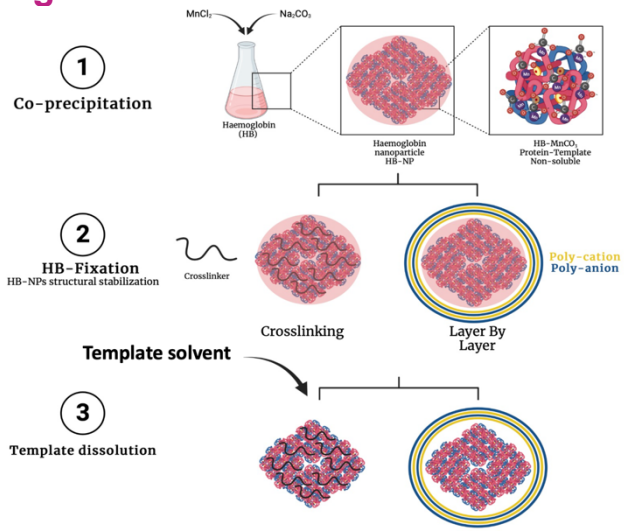

Figure 1. Scheme of synthesis of HOBCs by co-precipitation processes. Crosslinked Hb NPs (left) and NPs with LBL coating.

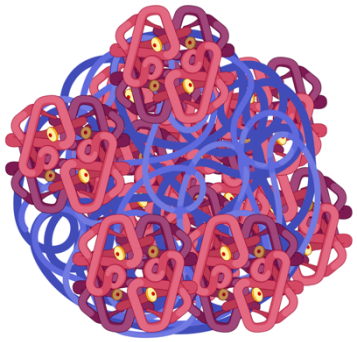

Figure 2. 1) Scheme of HOBCs produced by Polymer complexation of Hb.

Surface functionalization of graphene oxide with alkyl chains allows high rates of capture and release of viral particles in aqueous solutions

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Waterborne viral diseases can affect any population in the globe, but they are especially recurrent in poor rural regions of developing countries. Many of those affected by these diseases do not seek medical attention because of the existing limitations on the clinical detection of viral infections [1]. Moreover, further complications can arise from the fact that detecting the sources of infection when viruses are highly diluted, impedes their monitoring and successful identification. For this, in this study we intended to develop a new carbon-based material to increase virus capture and release performances beyond conventional methods, hoping it may be used as an alternative method to capture and detect pathogenic viruses in water.

Graphene oxide (GO) was prepared using a modified version of Hummer's method [2, 3] and exfoliated in water until obtaining a 1% w/v solution of GO. After that, we assessed the capacity of the produced pristine GO to capture viruses in aqueous solutions. For this, we prepared a solution of ~10^5 PFU/mL phage Q_β (NBRC, Tokyo, Japan) in 100 mM Phosphate Buffer (PB) solution and proceeded as follows. Briefly, 9 mL Q_β solutions were supplemented with 1 mL 1%, 0.5%, 0.1%, 0.05% or 0.01% w/v GO and were incubated at room temperature for 30 minutes. After that, the mixtures were centrifuged at 10,000 rpm for 30 min and 1 mL of the supernatant was assessed in duplicate for the presence of phages by plaque assay [4]. Next, to evaluate the capacity of GO to release the captured viruses, we resuspended the GO pellet in 10% beef extract solution and incubated the mixture at room temperature for 30 min. After that, the materials were centrifuged at 10,000 rpm for 30 min and 1 mL of the supernatant was cultured in duplicate by plaque assay to detect any phages present.

Results indicated that a minimal concentration of 0.01% w/v of pristine GO was able to capture phage Q_β to the order of 3 log10 (99.9%), and even lower concentrations, to the order of 0.005% and 0.001% w/v were able to capture more than 2 log10 (99.2%) of the suspended viruses (**Table 1**). When evaluating the release capacity of the material, our method could not release the virus attached to the surface of GO effectively (4.3%).

Concentration of GO (w/v)	Phage concentration [(PFU/mL) ± SD]	Adsorption (% ± SEM)
Initial phage	3.99×10^5 ± 2.12	-
0.1%	2.09×10^2 ± 1.13	99.96 ± 0.004[a]
0.05%	3.99×10^2 ± 4.63	99.92 ± 0.02[a]
0.01%	6.20×10^2 ± 4.12	**99.88 ± 0.01[a]**
0.005%	2.04×10^3 ± 1.06	99.27 ± 0.20[b]
0.001%	2.24×10^3 ± 1.30	99.28 ± 0.14[b]

Table 1. Capacity of different concentrations of pristine GO to reduce the amount of Q_β phage in a water-based solution. Data was obtained from at least 5 independent replicated experiments. [a,b]Different superscript letters indicate significant differences amongst values in the same column ($P < 0.01$).

Since it became clear that pristine GO was unable to release viruses once captured on its surface, we decided to change its properties by functionalization with other molecules. Since it is already known that viruses tend to attach to slightly hydrophobic surfaces [5], and that alkyl amines can be functionalized to the surface of GO [6], we added the following alkyl chains to GO: 1-butylamine (C_4NH_2) and 1-octylamine (C_8NH_2), but since C_8NH_2-GO displayed a subtle hydrophobicity, we further researched the influence of hydrophilic terminal groups such as NH_2 and OH by using 1,8-diaminooctane ($C_8(NH_2)_2$) and 8-amino-1-octanol (C_8NH_2OH) to improve the solubility of the materials. To prepare the functionalized GOs, a mixture of 0.5:1 w/w alkyl chains:GO was mixed in ethyl alcohol at room temperature for 1 h. After several washes to remove any non-attached compounds, the materials were either resuspended in water at a final concentration of 0.1% w/v or freeze-dried for further structural analysis.

Structural characterization was performed to assess the successful grafting of alkyl chains onto the GO sheets. FT-IR analysis of functionalized GO samples revealed the presence of alkyl chains around 2900 cm^{-1}, accompanied by a decreased C=O peak at 1732 cm^{-1}, indicative of a shift to approximately 1600 cm^{-1} due to amine neutralization (**Figure 1-A**). The degree of functionalization was further confirmed by TGA (**Figure 1-B**). GO exhibited a weight loss of 33.29% at 300 °C, whereas the functionalized materials exhibited a weight loss of 29.71%, 27.31%, 24.89% and 28.27% for C_4NH_2-GO, C_8NH_2-GO, $C_8(NH_2)_2$-GO and C_8NH_2OH-GO, respectively. The weight loss of pristine GO at a lower temperature is due to the degradation of the oxygen functional groups on its surface as opposed to the more resistant amine molecules present in all the other materials. XRD analysis (**Figure 1-C**) showed that the GO sheet distance increased with the addition of the longer chains from 0.86 nm in GO and C_4NH_2-GO to ~0.95 nm in the materials containing longer alkyl chains.

To further understand if the functionalized GOs could be used for the virus capture and release experiments, the materials were subjected to a dispersibility analysis of the supernatants after centrifugation by UV-Vis measurements. According to the results, we decided to exclude $C_8(NH_2)_2$-GO since complete precipitation could not be achieved even after 60 minutes.

Finally, we assessed the capacity of our materials to capture Q_β phage following the same protocol as with pristine GO. Since 0.01% GO was the minimal amount to obtain a 99.9% viral capture, we used this amount to compare the efficiency of our functionalized alkyl-GO materials. While no differences were observed amongst the adsorption rates for all the samples (> 98% reduction), significant differences were observed after phage release by 10% beef extract treatment, depending on the functionalized molecules (**Figure 2**). The lowest release rates (3.8%) were those of pristine GO, whose surface strongly interacted with the phages. Functionalization of GO with short alkyl chains (C_4NH_2-GO) slightly increased the release rates to 15.4%, and longer chains (C_8NH_2-GO), enhanced viral release performance to 36.2%. Remarkably, amongst all the materials analyzed, the presence of a terminal hydrophilic group at the end of a long alkyl chain (C_8NH_2OH-GO) showed the best release performance (55.8%).

This study allowed us to evaluate the capacity of GO to capture a model coliphage (Q_β phage) from aqueous solutions in controlled laboratory conditions. Furthermore, we successfully developed several GOs functionalized with alkyl chains with a simple protocol and evaluated their effectivity when capturing and releasing Q_β phages. All the alkyl-functionalized GOs were able to capture the virus to more than 2 log10, but the material with the best performance was C_8NH_2OH-GO, because of its ability to release the adsorbed phages to > 50%. Further evaluation on the practical applications of this material is required to determine its successful application for viral capture and release under field conditions.

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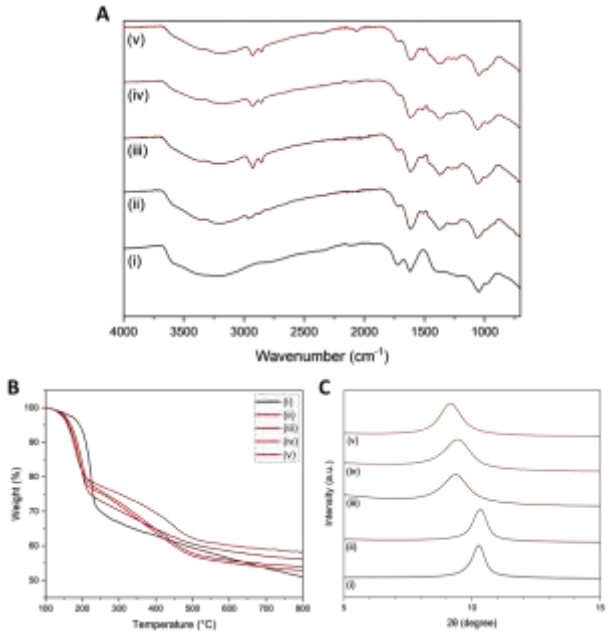

Figure 1. A. FT-IR spectra of (i) pristine GO, (ii) C_4NH_2-GO, (iii) C_8NH_2-GO, (iv) $C_8(NH_2)_2$-GO and (v) C_8NH_2OH-GO. The bands at ~ 2900 cm^{-1} indicate the presence of alkyl chains. **B.** TGA analysis of (i) pristine GO, (ii) C_4NH_2-GO, (iii) C_8NH_2-GO, (iv) $C_8(NH_2)_2$-GO and (v) C_8NH_2OH-GO. The samples containing alkyl chains show more stability than pristine GO at ~300 °C, confirmed by the lower weight loss. **C.** XRD analysis of (i) pristine GO, (ii) C_4NH_2-GO, (iii) C_8NH_2-GO, (iv) $C_8(NH_2)_2$-GO and (v) C_8NH_2OH-GO, the sheet interlayer space increases with the length of the alkyl chains attached to the surface of GO.

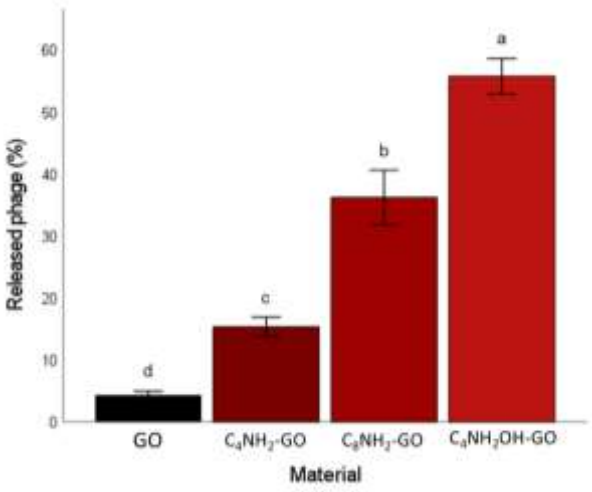

Figure 2. Average rates of Q_β phage release by functionalized GO after resuspension in 10% beef extract solution. Data was obtained from at least 4 independent replicates. $^{a\text{-}d}$ Different letters indicate significant differences amongst the values ($P < 0.01$).

Anticancer and immunological properties of selenium nanoparticles in 3D Breast Cancer Spheroids

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Introduction: Selenium Nanoparticles (SeNPs) have shown antitumor properties while having high biocompatibility, although a stabilizing agent is required to prevent their aggregation.[1,2]. BSA-SeNPs have also been demonstrated not only to induce apoptosis in cancer cells but also to function as an immunostimulant [3]. Since solid tumours develop in a 3-D shape, the use of 3-D cultures preserves better the biological characteristics of the original tumour than conventional 2-D monolayers, especially when including primary cancer cells removed from a mice tumour [4]. Therefore, the aim of this work was to develop 3D multicellular spheroids of breast cancer to characterize the anti-tumour immune-modulatory effect of SeNPs.

Methods: SeNPs were produced using sodium selenite, ascorbic acid as reducing agent and bovine serum albumin (BSA) as stabilizing agent, adjusting the pH reaction environment to 1. The stability of BSA-SeNPs was performed in human plasma, cell medium, pH 7.4 and 5.5.
Spleen was removed from C57BL/6J mice and the splenocytes were extracted and cultured with BSA-SeNPs for 48 h. The BSA-SeNPs effect on the viability of splenocytes was evaluated by flow cytometry.
A breast cancer orthotopic model was established by inoculating 1 × 10^6 E0771 cells in the fourth inguinal mammary fat pad of C57BL/6J mice, being divided in non-vaccinated and vaccinated with KRAS-loaded PLGA-Mannose NPs on days 7 and 14 after tumor inoculation. After 3 weeks, the mice were sacrificed and the tumour of the non-vaccinated mouse was extracted, while the splenocytes from the vaccinated mouse were isolated as well. 3D spheroids created using E0771 murine cells, were embedded in Matrigel. The splenocytes were divided into non-stimulated and stimulated with CD3 and KRAS peptide. Finally, the 3D-spheroids were treated with BSA-SeNPs and co-cultured with splenocytes, non-stimulated and stimulated according to the Scheme in **Figure 1**. The spheroids were observed under a microscope for 120 h.

Results: BSA-SeNPs demonstrated a size below 50 nm, low polydispersity, and a positive surface charge. The nanosystem produced was stable at cell medium, human plasma, and physiologic pH, although degraded at acidic pH, characteristic of the tumour microenvironment. The BSA-SeNPs at 20 ng/mL did not affect the viability of splenocytes after 48 h of incubation. Furthermore, BSA-SeNPs at 20 ng/mL presented synergic activity with both non-stimulated and stimulated T cells in inhibiting 3D-spheroids sprouting (**Figure 2**).

Conclusion: Stable SeNPs at physiologic pH and plasma were produced. These NPs aggregated at acidic pH. *Ex vivo* studies indicated that the BSA-SeNPs potentially present an immune-modulatory effect, as their anti-tumor effect was potentiated by activated T-cells , being thus an interesting approach for cancer therapy.

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Figures

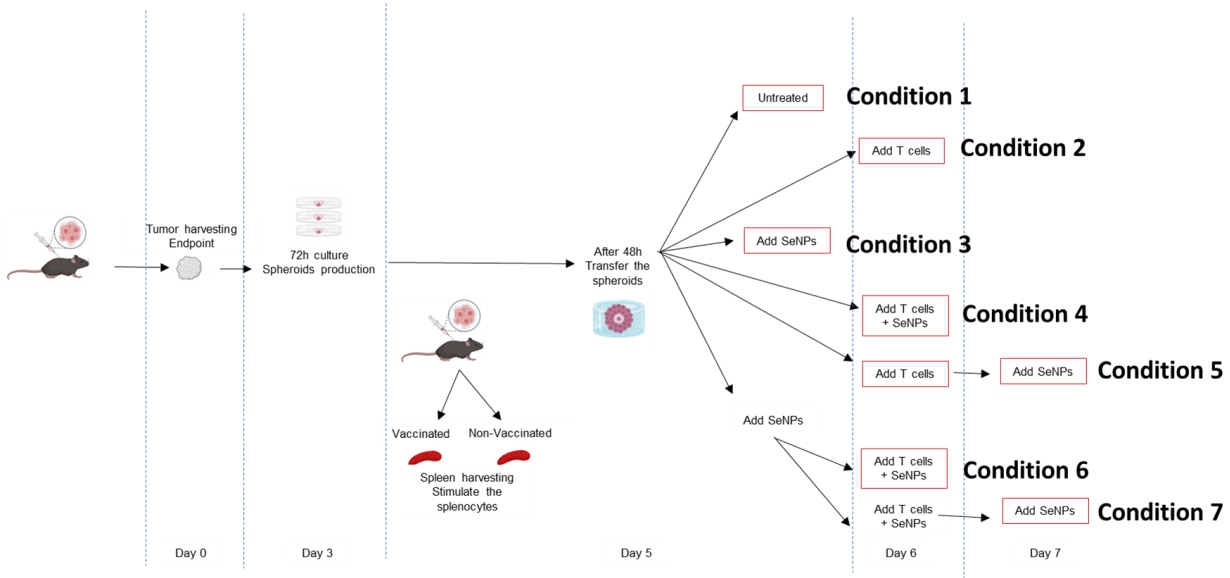

Figure 1. – Experimental design of the spheroids production, and the time schedule of the several treatment conditions (BSA1-SeNPs and splenocytes)

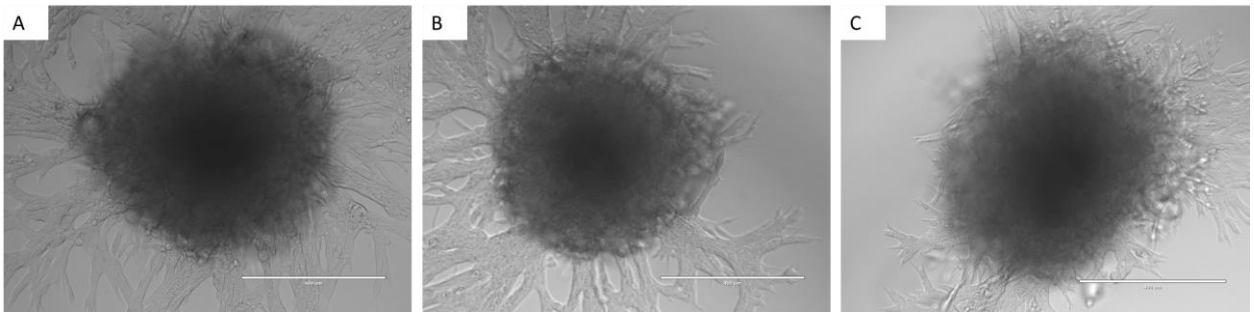

Figure 2. – Spheroids and sprouting formation after 120h in reduced growth factor Matrigel: (A) untreated, (B) incubated with BSA-SeNPs and (C) treated with BSA-SeNPs and co-cultured with splenocytes.

Sensitivity of the ice cave microalgae *Coccomyxa subellipsoidea TL4* to marine water pollutants: potential for biosensors

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Introduction

Pollution of marine bodies is a serious problem, aggravated in case of closed seas such as the Black Sea. One pathway to design cost-effective systems that can alert about the pollution due to agricultural runoff or intensive industrial activities close to the sea side relies on the inhibition of photosynthesis of microorganisms [1]. New phototrophs isolated from cold environments can provide the sensitivity and stability to low temperature required for on-site testing of marine water. The present study reports the characterization for the first time of the photosynthetic activity of microalgae *Coccomyxa subellipsoidea* TL4 isolated from the Scarisoara ice cave in Romania [2] as measured by an electrochemical sensor. The effect of water salinity and of various pollutants on the photosynthetic activity was examined with the aim of designing a biosensor for the screening of marine water. Artificial sea water (ASW) spiked with pollutants as well as real samples collected from the Black Sea were tested. Towards the development of a biosensor, the stability of thylakoids was investigated by freeze-frying in the presence of various stabilisers. Moreover, microalgae-impregnated paper was used in conjunction with screen-printed electrodes to devise a practical test for the on-site screening of marine water.

Experimental

*Coccomyxa subellipsoide*a TL4 microalgae was isolated from Scarisoara ice cave, Romania and grown in liquid BG-11 at 20 ±3 °C for 3 weeks, until DO 660 nm exceded 0.8AU Cells were lysed by bead-beating and ultrasonication. The cell lysate was exposed to osmotic shock to disrupt residual intact chloroplasts, and the resulting mixture was loaded on a sucrose density gradient and subjected to ultracentrifugation [3]. Two fractions of thylakoid membranes were obtained (Figure 1), united and analysed by amperometry.

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Chronoamperometry experiments were conducted using a potentiostat from Princeton Applied Research equipped with EC-Lab software and a an ekectrochemical cell housing screen-printed electrodes (from Metrohm Dropsens, Spain). A white light lamp was used to illuminate the solution in the cell. The electrolyte was 20 mM phosphate buffer pH 7.1 containing 5g/L NaCl and contained thylakoids of *Coccomyxa subellipsoidea* solutions with chlorophyll concentration 85 µg/mL and the sample or calibration standard. was used to measure the current generated by the photosynthesis of the various biological preparations in presence and in absence of diuron. The assays were conducted at an applied potential of 400 mV in the presence of 0.7 mM hexacyanoferrate (II) as mediator. Cycles of 1 min light followed by 15 minutes dark were applied the increase in the current intensity after 1 min illumination was taken as the analytical signal. The inhibition was calculated as: Inhibition (%) = $(I_0-I_1) / I_0*100$ (1), where I_0 and I_1- are the signals in the absence and in the presence of herbicide, respectively. Artificial sea water was prepared according to a literature protocol [4]

Results

The *Coccomyxa subellipsoidea* C-169 cells had ellipsoidal shape with a diameter ranging from 4 to 10 µm (Figure 1). Thylakoids lyophilized in the absence and in the presence of stabilisers were tested after 1 week of storage at -20° C and compared to the fresh preparation prior to lyophilization. When no stabilisers were added the degree of inhibition by 60 ppb diuron dropped to an average of 10%, indicating significant degradation (Figure 2). Thylakoids stabilized with either 0.5M trehalose or 0.5M sucrose presented on average 58% and 63% inhibition in the same conditions, similar to values recorded with fresh preparations. Based on these results, papers impregnated with thylakoids and 0.5 M sucrose were stored in the fridge at 4° C and tested regularly.

The salinity of the medium (the electrolyte) greatly influenced the photosynthetic current, both by increased conductivity of the electrolyte and due to the effect on the thylakoids. It was found that the response was relatively stable in the range from 5 to 20 g/L NaCl. Further on, artificial sea water (ASW) was spiked with diuron and analysed. It was observed that the thylakoids' photosynthetic activity was not negatively affected by the ASW and that the sensitivity to diuron was very similar to that in the 20 mM phosphate buffer pH 7.1 with very low salinity. A 10 ppb diuron solution in ASW caused an inhibition of 26% of the photosynthetic activity (Figure 3). Sensors ontained from paper impregnated with thylakoids had lower sensitivity than thylakoids in solution, due to both the diffusional barrier brought by the paper and lower chlorophyll concentration in the cell (Figure 4).

Conclusion

While optimization is underway, both use of free-dried thylakoids for tests in solution and thylakoid impregnated paper appear as valid solutions for fast testing of marine waters. Surprisingly, while CS was isolated from an ice cave, is not sensitive to salinity (at least up to 20 g/L, the maximum level in the Black Sea). Its sensitivity to diuron and heavy metals shows promise for the development of biosensors as early warning systems for screening the quality of marine water close to coastal area characterized by intense agricultural or industrial activities.

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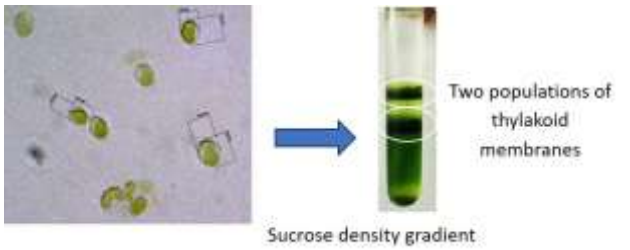


Figure 1. Phototrophic cells of *Coccomyxa subellipsoidea* C-169 observed by light microscopy and the fractions with thylakoid membranes separated by sucrose gradient .

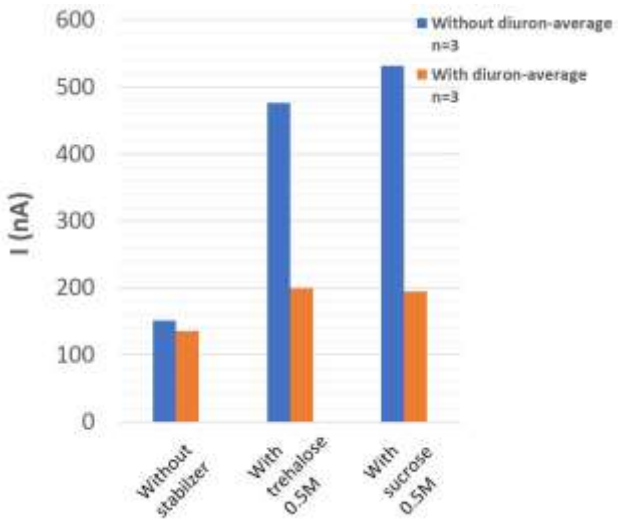
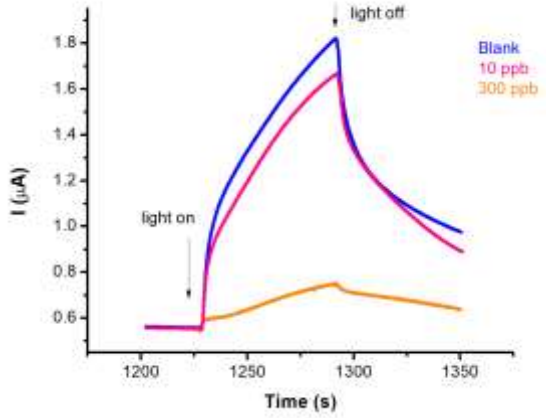


Figure 2. Sensitivity to 60 ppb diuron of lyophilized preparations of *Coccomyxa subellipsoidea* C-169 obtained in the absence and presence of stabilisers.

A



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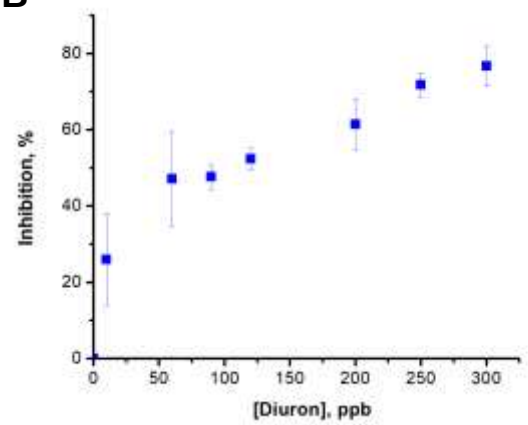

Figure 3. A. Typical amperometry signals obtained with thylakoids of CS tested in solution with a CNT electrode polarized at +0.4 V vs Ag in 0.7 mM hexacyanoferrate (II), in the presence of various concentrations of diuron in artificial sea water. B. The calibration curve for diuron in ASW.

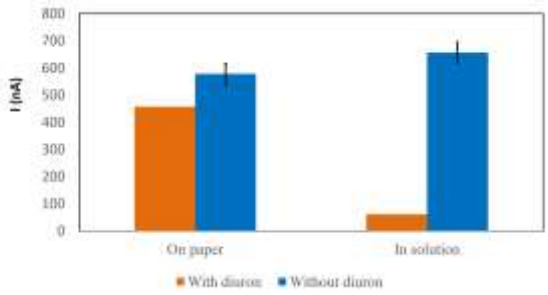

Figure 4. Photosynthetic activity of *Coccomyxa subellipsoidea* TL4 thylakoids in solution an on paper and their sensitivity to 60 ppb diuron

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Nature-inspired biomaterials: from interactive coatings to granular hydrogels exhibiting specific interactions with living matter

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Biointerfaces play a key role in regulating and controlling cell-biomaterial interactions. Uncontrolled protein adsorption and cell adhesion to biomaterial surfaces can lead to adverse outcomes such as inflammatory responses, harmful immune reactions, infections, and even implant failure. A fundamental strategy to mitigate non-specific and potentially harmful interactions is to introduce selectivity at the interface. A cornerstone for this is to be able to confer the interface with a cloak of invisibility to which specific molecules can be tagged. [1] In this talk, I will first introduce our concept of the Kill&Repel coating strategy.[2] This coating system combines the synergistic action of antifouling polymer brushes assembled in situ with a bacteriophage-inspired bacteria killing mechanism. As a result, this coating specifically kills bacteria with a low risk of resistance development, while remaining completely harmless to human cells. When the coating was applied to wound dressings, it was able to specifically eradicate antibiotic resistant bacteria such as *S. agalactiae*, *S. epidermisdis*, *S. aureus* and MRSA in a simulated infection. These dressings also showed unique resistance to cellular debris adhesion, indicating a self-cleaning mechanism with inexhaustible antimicrobial activity. In the second part of my talk, I will present our latest advances on the design of stealth granular hydrogels for wound regeneration. These granular hydrogels are composed of jammed microgels based on zwitterionic poly(carboxybetaine) polymers, which renders them superhydrophilic, non-immunogenic and resistant to protein fouling. We prepared a diverse set of microgels in the scale of 60-300 µm using different fabrication techniques - batch emulsion, microfluidics, and mechanical extrusion fragmentation. Furthermore, we achieved microgels with storage moduli ranging from ~10-10,000 Pa, which span the stiffness regime of mammalian soft tissue and cartilage. The microgels were cross-linked by covalent and reversible non-covalent linkages to form interconnected porous (~8-30%) granular hydrogels that allow cell migration and proliferation, both of which are essential for wound regeneration.
We envision that our work may contribute to the next generation of specific cell-instructive biomaterials capable of directing cell behavior in a superior-manner.

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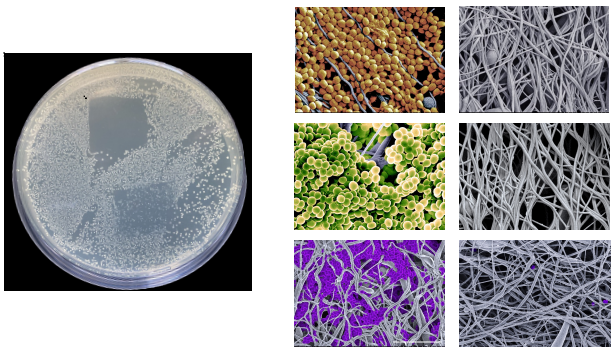

Figure 1. Kill&Repel coating was able to completely eradicate bacteria and prohibiting adhesion of residues.

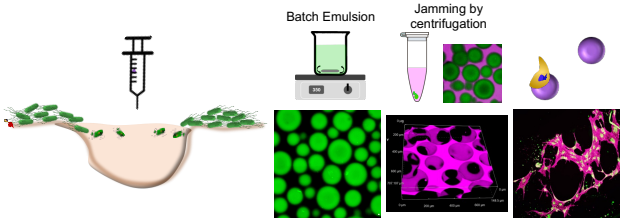

Figure 2. Stealth granular hydrogels feature an interconnected porous network that allows for cell migration, proliferation and mass transport. They also have shear-thinning and self-healing properties that make them ideal for injection, extrusion and 3D printing applications, enabling constructs that better mimic the complexity and heterogeneity of native tissues.

Polyallylamine-plasmid complex: A physico chemical study

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Plant genetic engineering for phytoremediation approaches is still poorly explored due to difficulties in genetic manipulation of non-model-plant species, often used for this approach. A promising strategy for enhancing phytoremediation is to generate plants with enlarged root system and an increased absorption capacity, obtained by modifying genetic traits that regulate root development.

Conventional DNA delivery methods for plants transformation are time consuming and have important limitations such as host- species barriers and low transformation efficiency.

In plant genetic engineering, nanomaterials have been used as vehicles for the delivery of plasmid DNA, short-interfering RNA (siRNA) and proteins to plants through the infiltration of leaves. However, so far there are no works reported about the use of polymeric nanoparticles for generating non-food crop with enhanced abilities for phytoremediation purposes.

Furthermore, exogenous DNA and RNA delivery into plants is a great challenge because the cell wall provides an important barrier, limiting molecules diffusion inside the plant cells thereby reducing the efficiency of plant genetic engineering.

Recently, in animal cell studies, modified polyallylalamine hydrochloride (PAH) polymeric nanoparticles have found applications as vectors of genetic material. These cationic polymers interact electrostatically with negatively charged nucleic acids[1,2,3]. Among PA, polyallylamine phosphate (PAN) polymeric nanoparticles have been used successfully as vectors for siRNA. Nano polymer-siRNA complexes are stable at pH values between 7 and 9, while dissociation occurs at pH less than 6 or greater than 9. These complexes enter the cells by endocytosis and are directed to lysosomes where the acid pH induces the dissociation of the complexes, leading to the release of siRNAs in the cytoplasm[4].

In this work we have studied the complexation of nucleic acids for plant genetic with PAH molecules with different modifications: oleic acid and dextran, aiming at having a more physical insight on the properties of the polyplexes formed. We have studied the process of complex formation and characterized the polyplex nanoparticles by a combination of techniques: Fluorescence Correlation Spectroscopy (FCS), Dynamic Light Scattering (DLS), Small Angle X-ray Scattering (SAXS) and Transmission Electron Microscopy (TEM).

FCS is used to study the changes in diffusion time of fluorescently labelled PAH after complexation. Stability of the polyplexes in relevant pH's is studied by FCS. Using PAH and nucleic acids both fluorescently labelled but with two different dyes with non-overlapping emission we have determined the actual stoichiometry of the complexes calculating the fraction of free polymer for different N/P ratios (amount of protonable amine groups per each phosphate group) considered for polyplex formation FCS data are complemented with TEM/DLS/SAXS

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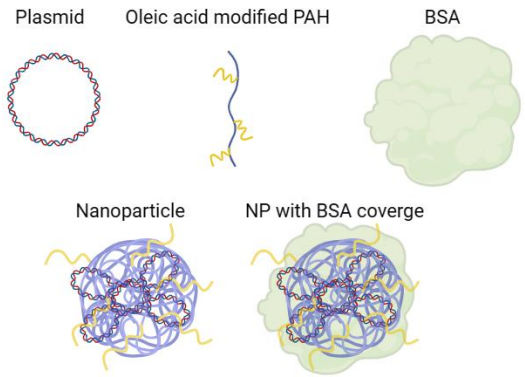

Figure 1. Scheme of the self-assembled nanoparticles with and without BSA coverage.

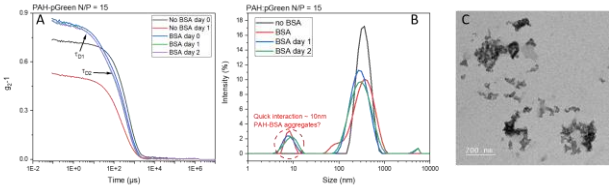

Figure 3. NP's with BSA at higher N/P ratio after 0, 1 and 2 days of incubation. A) Autocorrelation functions, B) size distributions by intensity obtained by DLS and C) TEM image of them.

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Intranasal Administration of Pt(IV) nanoparticles for Glioblastoma treatment

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The progressive population aging in developed countries has favored a steady prevalence increase over the years of many pathologies of the central nervous system (CNS), such as neurodegenerative diseases (e.g., amyotrophic lateral sclerosis, Alzheimer's, Parkinson's, Huntington's, and prion diseases), genetic deficiencies (e.g., lysosomal storage diseases, leukodystrophy) and brain cancer (i.e. glioblastoma multiforme).

Most of neurological diseases (ND) clearly diverge in their origin, overall population incidence and treatment though all of them share the same problem, namely the lack of efficacy of their state-of-the-art therapies originated largely by the existence of the blood-brain barrier (BBB). This barrier limits the access of most therapeutic agents to the brain area, and for those crossing requires ever-increasing drug doses increasing side effects. More specifically, current therapies for treating GB, and brain tumors in general, are inefficient and represent numerous challenges and treated patients face dismal prognosis with a median survival below 15-18 months.

Intranasal administration represents an alternative route to transport drugs from the nasal mucous membrane through the trigeminal nerve, and from there to the brain while largely avoiding the systemic dispersal of the drug and the limitations of BBB. However, as far as we know no IN products for GB are nowadays commercialized mostly due to the poor mucosa penetration of most drugs, the rapid mucociliary clearance and the enzymatic degradation. In this sense, the use of nanotechnology-based approaches is of special interest as allows for control of the formulation, surface charge, hydrophilicity, and mucoadhesion and favors the transcellular transport to the brain as well as induce both a systemic and local immune response. It also allows for the use of effective chemotherapies against GB, such is the case of Platinum (Pt) complexes. So far, and despite the multiple benefits reported in the literature, the administration of Pt complexes is often associated with severe systemic toxicity resulting from long-term treatment. BBB produces scarce drug arrival to the brain when administered orally or intravenously.

To circumvent these limitations, our group has recently reported neuromelanin bioinspired coordination polymer nanoparticles containing Pt (IV) prodrugs as building blocks. This nanoformulation has showed dual pH and redox sensitivity in vitro, showing controlled release and comparable cytotoxicity to cisplatin against HeLa and GL261 GB cells. In vivo intranasal administration in orthotopic preclinical GL261 GB-bearing mice demonstrated increased accumulation of platinum in tumors, leading in certain cases to cure and prolong survival of the tested cohort. For comparison purposes we will also show the activity of the monomeric Pt(IV) complexes as well as other nanoparticles obtained by melanization reactions.

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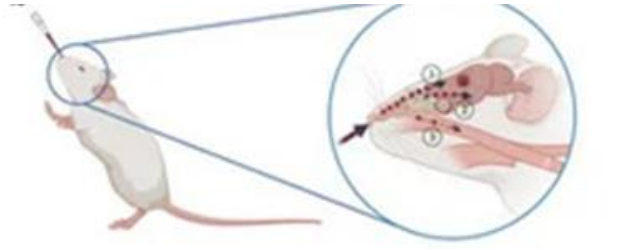

Figure 1. Schematic representation of the intranasal delivery of nanocarriers for Glioblastoma treatment

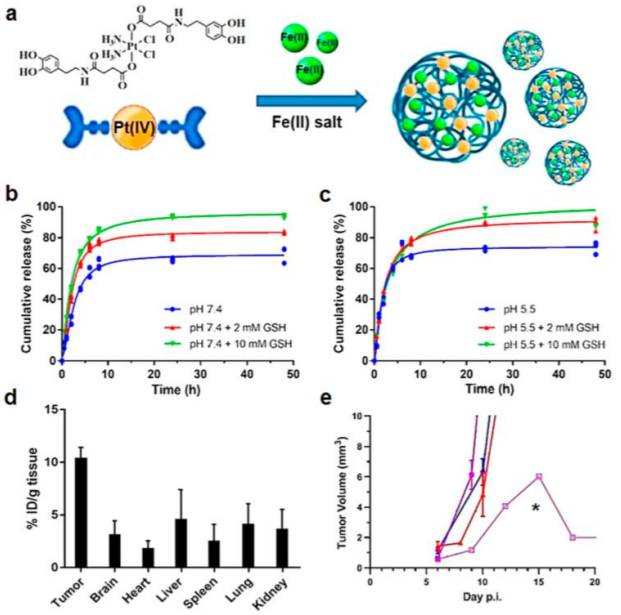

Figure 2. (a) Scheme of the Pt-Fe NCPs synthesis upon polymerization of a Pt(IV) prodrug with iron ions as metal nodes; Cumulative release profiles of Pt from Pt-Fe NCPs at 37 ◦C at (b) pH 7.4 and (c) pH 5.5 in PBS using the dialysis method in the absence or in the presence of glutathione (GSH); (d) Biodistribution of Pt-Fe NCPs in mice organs 1 h after administration (dosage of 1.5 mg/kg, n = 3); (e) Tumour volume evolution in the period 0–20 days post-implantation (p.i.) [blue: control; red: therapy starting point at day 10 p.i.; pink: therapy starting point at day 6 p.i.;

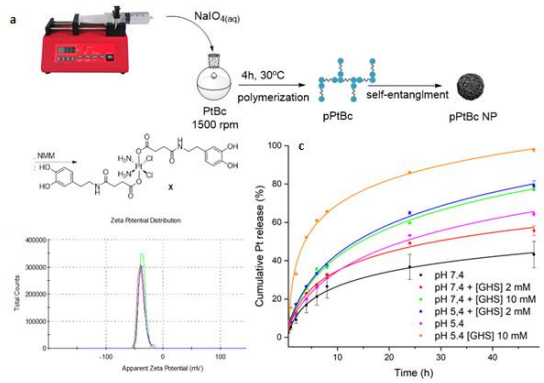

Figure 3. (a) Scheme of the Pt-Fe NCPs synthesis upon polymerization of a Pt(IV) prodrug with sodium periodate. (b) zeta potential of the pPtBc NCPs nanoparticles. (c) Cumulative release profiles of Pt from pPtBc NCPs at 37 ◦C a using the dialisys method in the absence or presence of glutathione (GHS) and at pH 7.4 and pH 5.4

PEGylated Nanoemulsions as immune-PET imaging agents for early Glioblastoma diagnosis

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Glioblastoma is the most common and lethal type of brain cancer, with 12-15 months median survival. The current treatment relies on surgical removal and chemotherapy.[1, 2] However, chemotherapy is limited by the challenge of crossing the blood-brain barrier (BBB), which prevents more than 98% of chemotherapeutic drugs from reaching the brain. [3] Nanoparticles (NPs) can be tailored to facilitate penetration through the BBB, thereby enabling encapsulated drugs to get to the tumor, making nanotechnology-based drug delivery a promising strategy to reach glioblastoma. [4]

Different types of nanoparticles are investigated as imaging agents (Figure 1) since molecular imaging techniques allow to monitor their pharmacokinetics and biodistribution. [5,6,7] Due to their biodegradable and biocompatible composition, the organic NPs are still the most demanded ones. Nevertheless, the use of nanoemulsions (NEs) in nuclear imaging is still recent and there are only few reports describing radiolabeled NEs. [5, 6]

In this work, we present the rational design of nanoemulsions as imaging agents for the detection of glioblastoma using positron emission tomography (PET). The design takes into consideration the different barriers to overcome from the intravenous injection to the tumor, including crossing the BBB (Figure 2). Apart from the composition needed for reaching the tumor, the nanoemulsions would be marked with ^{89}Zr for PET imaging. PET is a very sensitive and versatile technique that provides a full and quantitative characterization of the NPs biodistribution. [6, 8] To best of our knowledge, this is the first example of combining the NEs and PET technologies for the diagnosis of glioblastoma.

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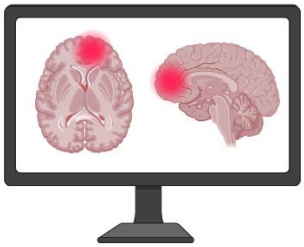

Figure 1. Simplified example of an image obtained using radionuclides by PET.

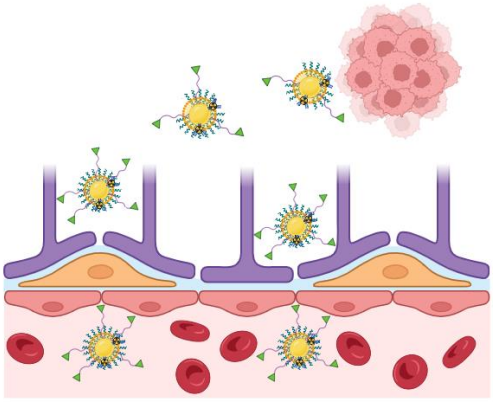

Figure 2. Representative scheme of the NEs crossing the BBB and reaching the tumor.

Magnetically Navigated Polysaccharides-based Capsules as Smart Delivery Systems

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Nanomedicine as a dynamically developing part of nanotechnology utilizing novel and effective approaches to eliminate many diagnostic and therapeutic limitations. Complexity of the proposed solutions, as well as potential biomedical applications require interdisciplinary cooperation combining knowledge, techniques and experience in the field of chemistry, materials engineering, biology, physics and medicine. For this purpose solutions based on targeted and controlled delivery and release of biologically active substances play a significant role as a tool in novel medicine.
Obtaining biocompatible systems capable of controllable transport and release of a bioactive substance can significantly contribute to the development of modern therapy and diagnostics. Solutions based on externally stimulated carriers represent a very forward-looking alternative to conventional methods of biologically active substances administration, which are limited by the problems of maintaining the appropriate therapeutic dose, absorption or the occurrence of strong side effects.
Considerable efforts have been dedicated to fabrication and characterization of magnetically controllable polymeric carriers in the form of capsules with oil cores stabilized by modified polymers of natural origin. Researches have been focused on core-shell nanosystems, their magnetically controllable navigation, as well as externally induced release of the transported substance. Two types of carriers were developed: with a negative and with a positive surface charge, containing encapsulated magnetic nanoparticles inside. The therapies which provide carriers that will enable precise and targeted therapeutics delivery contain an innovative and very promising approach, especially in the treatment of neoplastic diseases. The main advantages of this form of treatment include the ability to maintain an appropriate concentration of the active substance at the target site, leading to the death of diseased cells without damaging healthy cells, i.e. without causing additional side effects of the therapy.

The first stage of the research was to obtain and characterize the substances needed to create biopolymer capsules. Due to the dedicated biomedical use of the carrier, syntheses were performed by the modification of a natural origin polymer and reactions leading to the production of magnetic iron oxide nanoparticles. The second stage of the research was the optimization of the capsules preparation procedure and the physicochemical analysis of two types of magnetic carriers obtained. Capsules with a positive surface charge were produced by self-assembly of an amphiphilic chitosan derivative, modified with cationic groups and with alkyl chains grafted on the surface of oil droplets containing dispersed magnetic nanoparticles. The additional application of an anionic chitosan layer on the capsule surface by the LbL technique led to the second type of carrier (with a negative surface charge). Physicochemical properties and stability of the structures obtained at the first and the second stage of the research were investigated using various techniques (light scattering and zeta potential measurements, infrared spectroscopy, X-ray diffraction, Mössbauer spectroscopy, magnetometry and Scanning Transmission Electron Microscopy).
The systems developed were subjected to cellular tests, which constituted the third stage of the experimental part of this work. The research conducted was aimed at verification of the application potential of the capsules in the context of magnetically controlled carriers capable of targeted and controlled transport and release of the encapsulated hydrophobic substance. All assays were performed against a mouse mammary gland tissue derived breast cancer cell line (4T1). The cytotoxicity of the capsules was checked using the XTT test, and the obtained results revealed the appropriate concentration of anionic and cationic capsules, which do not show toxicity to healthy cells. Then, with the use of a constant magnetic field, an experiments relying on the controllable introduction of the capsules, containing the model hydrophobic fluorescent dye, inside the tumour cells were performed. [1,2]

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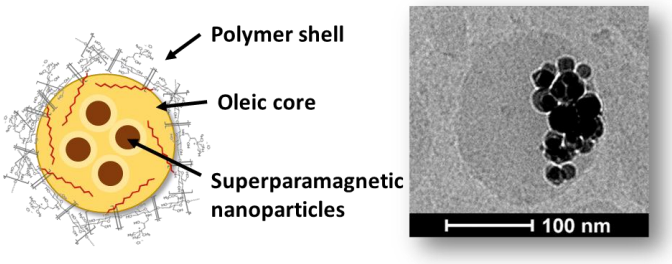

Figure 1. Scheme of the polymer capsule with oleic core and encapsulated magnetic nanoparticles (left picture); cryo-TEM image of magnetic cationic capsules based on oil cores (right picture)[2]

Nano-intervention to stop therapy-induced cellular senescence.

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Cellular senescence is a phenomenon that causes genetic, epigenetic, metabolic, and structural changes to original cells, causing cell cycle arrest and prevents them from growing. It is naturally caused by telomere shortening, when cells are unable to divide anymore, and is an inherent part of our lives. In general, this mechanism is a protector from over proliferation, what makes this a tumor-suppressive mechanism [1]. Senescent induction is an effect of various factors, such as epigenetic stress, proteotoxic stress, oxidative stress, telomere damage and DNA damage [2]. Such stressful environment may lead to accumulation of those cells in tissues which are a source of inflammation or tissue disfunction and causes many age-related diseases. Chemotherapy is one of the sources to induce cellular senescence but after induction it is ineffective for those cells making them apoptosis-resistant. It is caused by their growth arrest, while chemotherapeutics are mostly working on highly proliferative cells such as cancer cells. Moreover, Senescent Associated Secretory Phenotype (SASP), which is a very characteristic feature of senescent cells, containing cytokines chemokines, growth factors and proteases, plays a crucial role in tumorigenesis and inflammation [1]. In summary, it is necessary to use drugs that are working selectively, which are senolytics, causing induction of apoptosis on senescent cells (senolysis). Increase of cancer senescent cells after chemotherapy is a considerable problem, as those cells can reenter cell cycle to become cancer stem cells and leads to cancer relapse. The idea is to introduce additional therapy – senotherapy – to eliminate remaining senescent cells [3-5]. This study focused on preparing the protocol of therapy-induced senescence (TIS) with doxorubicin on two cell lines – WI38 (fibroblasts) and A549 (cancer cells). To confirm the effectiveness of the method experiments with senescence markers were conducted, such as evaluation of β-galactosidase level and proliferation of the cells. Followed by investigation of its morphology on confocal microscopy to prove the size changes which were additionally corroborated by the change in complexity as measured by flow cytometry. Next, liposomes were prepared encapsulating the senolytic drug, Fisetin, and administrated to senescent cells. Liposomes were characterized CryoSEM and DLS. Cell uptake of liposomes with encapsulated Fisetin was studied by flow cytometry. Cell viability studies after administration of liposomes were conducted by IN Cell Analyzer, using Live/Dead Assay. This investigation showed that therapy-induced senescence by doxorubicin is greatly effective and senotherapy through fisetin encapsluated in liposomes is highly potential.

Acknowledgements
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Figures

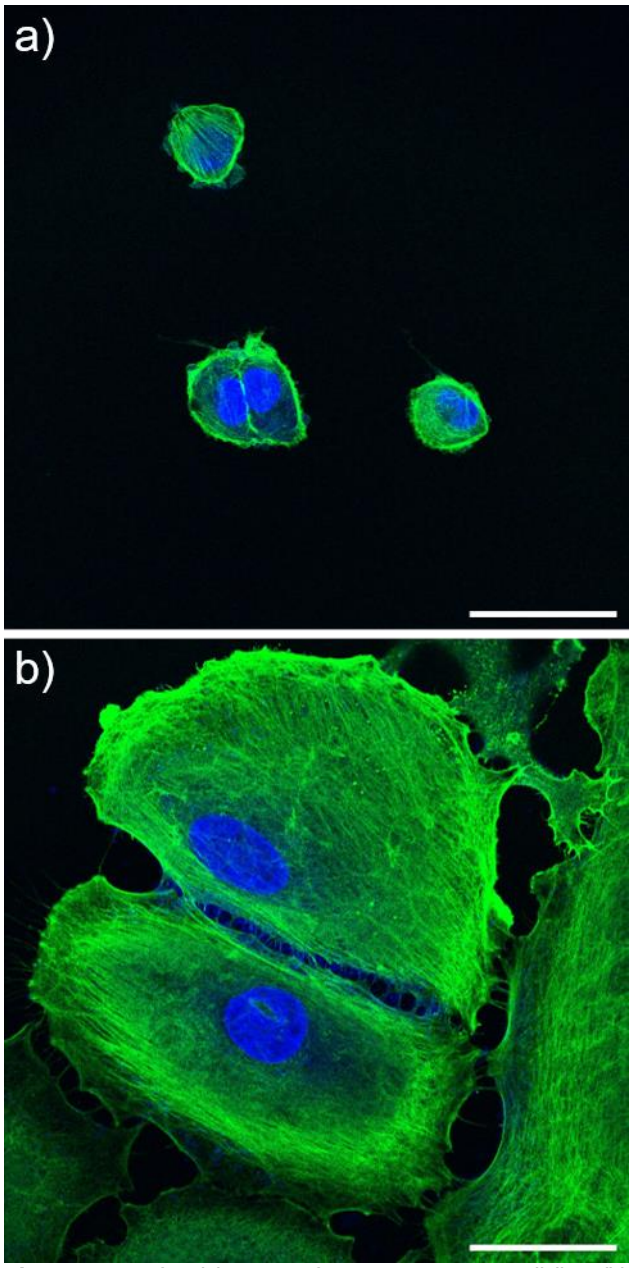

Figure 1. Confocal images of senescent A549 cell line (b) compared to control, non-senescent cells (a). Scale = 50 µm

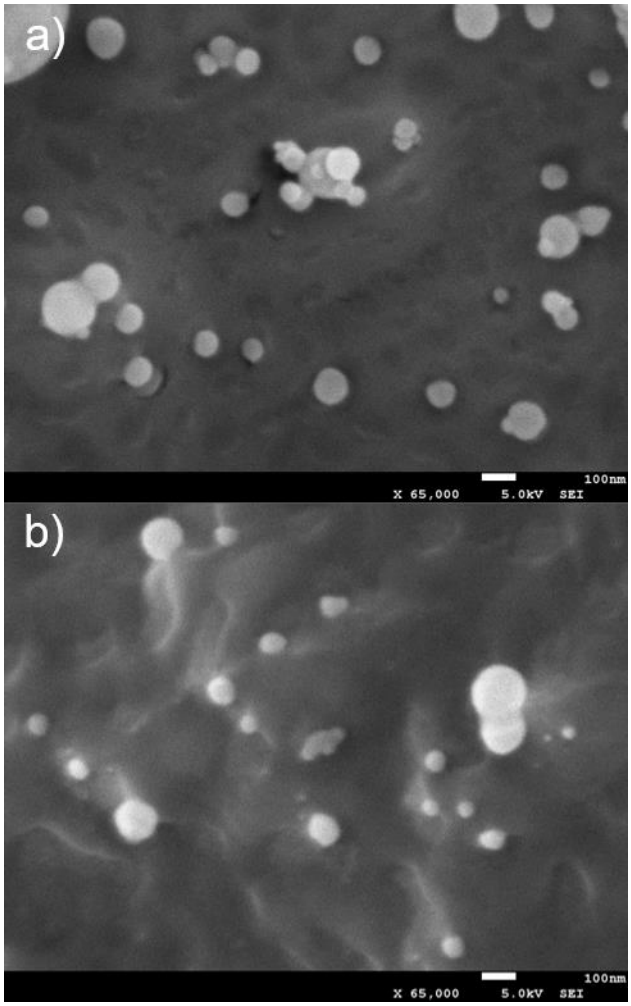

Figure 2. CryoSEM images of liposomes without Fisetin (a) and with Fisetin (b). Scale = 100 nm

Introducing NIAGARA project. Safeguarding Human Health through Real-time Assessment of water pollutants.

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The quality of the environment, as well as the quality of the surroundings in which we live, has a relevant and significant influence on our health. In fact, this impact is known to be greater in children under 5 years of age and in people between 50 and 75 years of age. It is estimated that around 12 million people in the world die annually from living or working in highly polluted spaces and environments [1]. Environmental pollutants, which can range from chemical to microbiological agents, are responsible for the development of respiratory and cardiac diseases, as well as favouring the development of certain types of cancer. For example, in urban areas where industrial activity is high, access to clean water is very limited and soil degradation is notable due to the presence of chemical agents, the likelihood of developing these types of diseases is much higher [2]. In addition, the recent pandemic has highlighted the value of tracking different areas to trigger early warnings, significantly shortening response times [3]. On the other hand, people affected by chemical pollution of drinking water is projected to increase from 1.1 billion in 2000 to 2.5 billion in 2050. It is a harsh consequence of global change: rapid industrialization (industrial chemicals like polyaromatic hydrocarbons -PAHs- and heavy metals are among the most frequent chemicals found in natural water bodies), urbanization (megacities hold 50% of global population and are responsible of large wastewater discharges), the increase of intensive livestock for an increasing of global population (agricultural pesticides are also one of the most frequent pollutants in natural water bodies) [4].Therefore, there is a clear need to develop devices capable of monitoring the presence of environmental pollutants, which can directly affect the quality of the living conditions and have a direct impact on our health. At present, diagnostic techniques for environmental analysis (classical chromatographic, spectroscopic and cell culture methods) are often expensive and laborious, require several sample preparations steps, use toxic chemicals and are time-consuming. In contrast, biosensors, that are analytical tools that combine materials and nanomaterials, such as carbon nanotubes, metal nanoparticles or graphene, with biological elements, such as antibodies, aptamers or enzymes, can provide real-time and robust responses, as well as being portable and cost-effective devices [5]. The construction of a biosensor must consider the complexity of the environmental sample. In this sense, the integration of biosensors with devices capable of sampling automatically, as well as the different pre-treatment steps necessary to guarantee the reliability of the analysis by means of the biosensor, are of special relevance.

In this work, different approaches developed by ITENE in the field of environmental monitoring will be presented, most of them based on the use of biosensors integrated with devices capable of automating both the sampling and its pre-treatment, as well as the detection step. In addition, the NIAGARA project will be presented, which aim is to develop solutions capable of mitigating the impact of certain pollutants on drinking water and, consequently, on human health.

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Figures

Figure 1. Overview of the different threats that the NIAGARA project intends to address throughout the drinking water treatment process.

BIOMATERIAL INCORPORATED HUMAN MESENCHYMAL STEM CELL SECRETOME FOR CARDIAC REGENERATION

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The therapeutic effect of stem cell transplants for heart disease is now attributed to the pro-regenerative microenvironment created through secreted bioactive molecules such as growth factors (GF), cytokines, and extracellular vesicles known collectively as the secretome. Mesenchymal stem cell (MSC)-derived factors have been shown to protect the heart against hypertrophy or promote synchronous contraction.[1,2] To address the short duration of secretome activity, we aimed to incorporate it into a biomaterial for sustained release and enhanced cardiac regeneration.

To produce bone marrow-derived human MSC secretome, cells were cultured for 48 hours in low serum media under three different conditions: 2D and 3D normoxia (2D and 3D), and 2D hypoxia (Hyp.). A human cytokine array was carried out to determine the cytokines and GF levels, selected factors were quantified by ELISA. The secretome activity was tested on human cardiac fibroblasts (hCFb) before encapsulation: we synthesised poly(lactic-co-glycolic acid) (PLGA) nanoparticles, using the water-oil-water emulsion method.

Several factors and cytokines such as angiogenin, VEGF, IL-6 and IL-8 were detected. ELISA analysis indicates that there was no difference in the levels of IL-6 between the secretomes, however higher levels of VEGF were detected in the secretome obtained by hypoxia. In cellular studies all three secretomes showed no cytotoxicity, the 2D and Hyp. secretomes showed the greatest proliferation of hCFbs over 7 days. Based on these results, we are currently attempting to encapsulate the secretome in PLGA nanoparticles.

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Figures

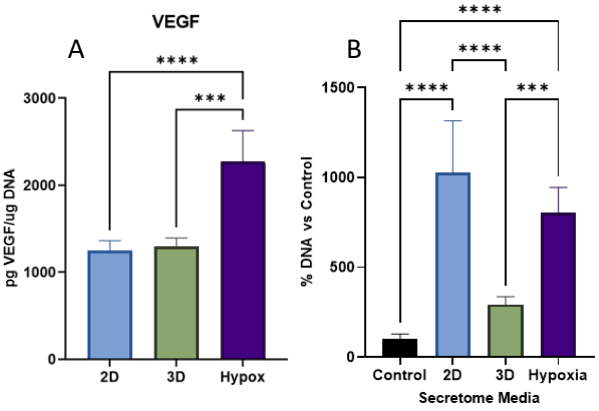

Figure 1. A) Results of the ELISA analysis of human VEGF production under different growth conditions, normalized to hMSC DNA, and **B)** Picogreen DNA analysis of the proliferation of hCFbs treated with 0.5x secretome media for 7 days.

Green Synthesis of Near-Infrared Plasmonic Gold Nanostructures by Pomegranate Extract and Their Supramolecular Assembling with Chemo- and Photo-Therapeutics

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In the last few years, gold (Au) nanostructures have collected a tremendous attention thanks to their manifold applications in different fields spanning photonics, catalysis, sensing and medicine [1]. Among the unique properties of Au nanostructures, photothermia is one of the most intriguing in view of its biomedical applications [2]. It is based on a straightforward working mechanism, based on the ability of the noble metal to absorb light in the visible (Vis)/near infrared (NIR) region thanks to its localized surface plasmon resonance (LSPR), and to convert the excitation light into heat with superb efficiency while preserving excellent photostability. This phenomenon is at the basis of the photo-thermal therapy (PTT), which represents one of the emerging unconventional treatments for cancer and bacteria diseases, with great prospects in the burgeoning field of nanomedicine [3]. The ease of manipulation of light in terms of intensity, wavelength, duration and location, combined with the nanodimensions of Au structures offer the great advantage to confine a rapid in increase of temperature in a very small volume with high spatiotemporal precision, inducing cellular death with great efficiency and selectivity [4].

Au nanostructures exhibiting a localized surface obtained in a single, green step by pomegranate extract in the presence of a biocompatible β-cyclodextrin branched polymer, without the need of preformed seeds, external reducing and sacrificial agents, and conventional surfactants [5]. The polymeric component makes the Au nanostructures water dispersible water, stable for weeks and permits their supramolecular assembling with the chemotherapeutic sorafenib (SRB) and a Rhodamine- bond nitric oxide (NO) photodonor (RD-NO), chosen as representative for chemo- and photo-therapeutics [6-7] SRB is a multi-kinase inhibitor, already approved by the U.S. Food and Drug Administration and currently widely employed in hepatocellular and advanced renal cell carcino-mas treatment [8]. Irradiation of the plasmonic Au nanostructures in the “therapeutic window” (650-1300 nm), with 808 nm laser light, results in a good photothermal response, which (i) is not affected by the presence of either the chemo- or the phototherapeutic guests and (ii) does not lead to their photoinduced decomposition. Besides, irradiation of the hybrid Au nanoassembly with the highly biocompatible green light results in an efficient release of NO, a well-known anticancer species if produced within a specific concentration range. The suitability of these nanoassemblies in the prospect of multimodal anticancer applica-tions has been demonstrated by preliminary experiments against Hep-G2 hepatocarci-noma cell lines which revealed synergistic action between the cytotoxic species involved.

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Figure

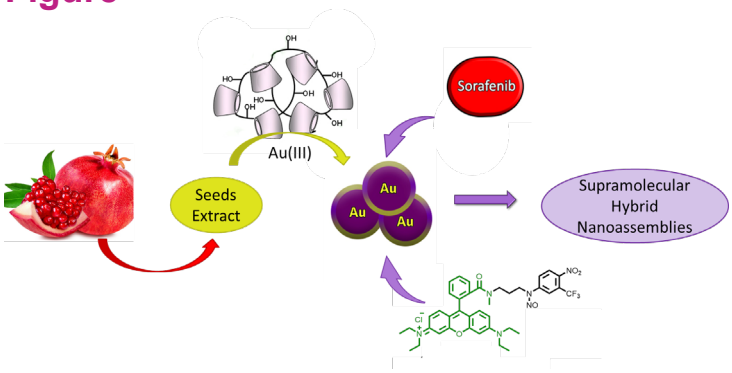

Figure 1. Schematic preparation of the NIR plasmonic Au nanostructures and their supramolecular assembling with SRB and RD-NO.

Nano-Programmed Cross-Kingdom Communication Between Living Microorganisms

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The engineering of chemical communication at the micro/nanoscale is a key emergent topic in micro/nanotechnology, synthetic biology and related areas. However, the field is still in its infancy – previous advances, although scarce, have mainly focused on communication between abiotic micro/nanosystems or between microvesicles and living cells.

Here, we have implemented a nano-programmed cross-kingdom communication involving two different microorganisms and tailor-made nanodevices acting as "nanotranslators".[1] Information flows from the sender cells (bacteria) to the nanodevice and from the nanodevice to receiver cells (yeasts) in a hierarchical way, allowing communication between two microorganisms that otherwise would not interact.

Figures

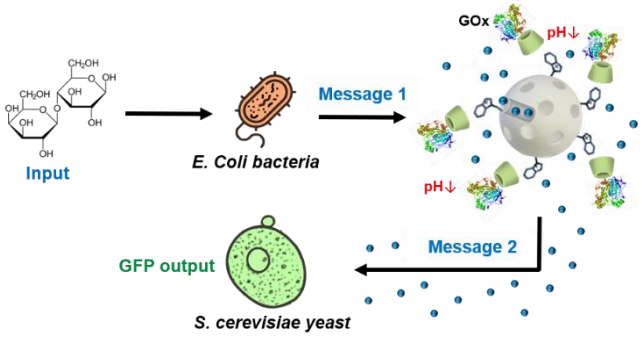

Figure 1. Schematic representation of cross-kingdom communication enabled by nanoparticles acting as nanotranslators.

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Ultrasensitive interferometric detection of beta-lactam specific IgE antibodies

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Driven by the technological advances in lithography and material science, integrated photonic biosensors have been presented in the most recent decades as small, label-free, high-sensitivity alternatives to more traditional label-based biodetection methods such as the different immunoassay techniques. Moreover, photonic solutions offer unmet detection limits with the added advantage of real-time monitorization.
This work presents an interferometric sensor based on the well-stablished silicon-on-insulator platform with state-of-the-art figures of merit, developed at Bioherent (Malaga, Spain). The chosen platform ensures high production reliability while maintaining fabrication costs low. The sensor was tested detecting amoxicillin specific IgE antibodies (AXO-sIgE), illustrating its potential use for drug allergy diagnostics, an area where in-vivo tests, such as the drug provocation, are still the gold standard, not without risk for allergic patients.

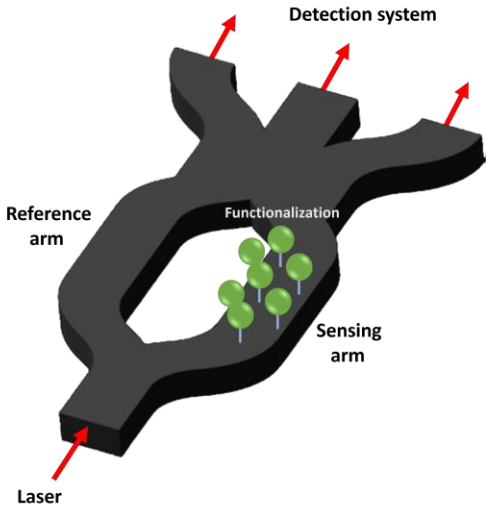

Figure 1. Basic structure of a Mach-Zehnder biosensor with 2x3 coherent readout system.

The sensor is based on a silicon nitride Mach-Zehnder architecture with a 2x3 coherent readout, operating at a wavelength of 1550 nm. In this structure, the incoming light gets split into two waveguides. One acts as reference arm, completely covered by SiO_2, encapsulated from the environment, while the other waveguide, the sensing arm, is exposed to the sample solution that flows over the surface of the chip. Refractive index changes close to the sensing arm surface affect the light modes propagation, as a small part of the modes power penetrates the surrounding medium (evanescent field sensing). Mode velocity changes, due to the refractive index variation in the medium, being this change linearly translated into a phase shift at the end of the sensing arm, when compared with the modes of the reference arm.
The 2x3 multimode interference coupler splits the incoming light of reference and sensing arm into three interferometric light signals with a relative phase offset of 120° between one another. The linear phase response is derived from the periodic interferometric signals of these three channels.
Prior to its use in biosensing, the performance of the device was characterized by homogeneous refractive index changes. Sensitivity was studied first. For this, the sensor was exposed to sodium chloride aqueous solutions of increasing concentrations ranging from 1.5% to 6% (w/w). A washout injection with water was introduced between NaCl injections. The phase obtained from the experiment is presented in figure 2.

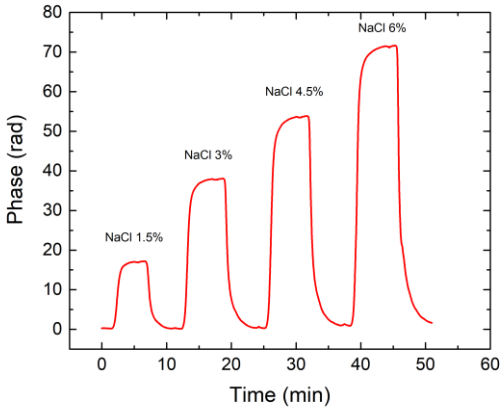

Figure 2. Phase response of the sensor to increasing concentrations of NaCl in water.

The homogeneous sensitivity of the sensor is the slope of the phase shift obtained as a result of the refractive index change introduced. To achieve this, we mapped the NaCl concentrations to their respective refractive indices based on the relationship studied by Saunders [1]. A sensitivity value of S = 7061 rad/RIU was reported, being RIU the refractive index units.
A most appropriate figure of merit to compare our sensor with competing technologies is the limit of detection (*LOD*), defined as the smallest detectable physical parameter change, generally derived by the ratio between three times the sensors uncertainty (noise) and its sensitivity:

$$LOD = 3\sigma/S$$

The measured noise level during the experiment was $6.5 \cdot 10^{-4}$ rad, which results in a *LOD* of $2.7 \cdot 10^{-7}$ RIU. The noise level can be further reduced by

applying digital filters to the signal. This way, a *LOD* of ~1·10^{-8} RIU can be obtained. This is a state-of-the-art value that outperforms commercial competitors such as surface plasmon resonance (~1·10^{-7} RIU, Biacore) and ring resonators (~1·10^{-5} RIU, Genalyte), and the most recent, and usually more complex, advances in the academic space, that are not available in the diagnostics market [2].

Our sensing structure has also been tested in a practical biosensing scenario. The goal was to detect specific IgE antibodies against beta-lactam drugs, which are clinically related to immune-mediated, and potentially life-threatening, allergic reactions.

The concentration of drug specific IgE antibodies is extremely low in individuals (0.2% of total IgE) compared to other allergens [3]. This explains why there are commercially available in vitro diagnostic tests for a wide variety of inhalant and food allergens with high specificity and sensitivity, while for drugs, there are few tests available. Notably, average beta-lactam specific IgE values in patients' serum samples often fall below the detection limit of the few available in vitro techniques like ImmunoCAP® (ThermoFisher, USA). This underscores the need for more sensitive technologies, such as the ultra-sensitive photonic biosensor presented in this work.

For IgE biorecognition, a specific functionalization of the sensing waveguide was performed using amoxicillin, but amoxicillin alone does not elicit an immune response. The allergic response in sensitive individuals is triggered when the amoxicillin bioconjugates to a serum transport protein like albumin, forming the hapten-carrier. Our surface chemistry introduces a linker molecule that mimics the function of these serum proteins, allowing the binding of the AXO-sIgE present in patients' serum samples.

The experiment performed registered the sensor response to the interaction between the hapten-carrier immobilized on the surface and mouse AXO-sIgE in a PBST buffer solution at increasing antibody concentrations. These concentrations spanned from clinically relevant values (0.24 to 2400 ng/ml, equivalent to 0.1 to 100 kUI/L). In between antibody samples injections, the transiently bonded molecules were washed out of the system by injecting buffer solution. The phase shift that corresponds to the binding process was calculated by subtracting the phase baseline prior to the IgE injection to that well after the IgE pass, during the washout phase. The phase response of the sensor can be completely characterized by its IgE concentration vs phase curve (figure 3).

At low IgE concentrations, the steep slope of the curve corresponds with a sensitivity to IgE concentrations of 88 mrad/(ng/ml). Even non-clinical levels of IgE result then in lectures well above the noise level of the sensor. Beyond antibody concentrations of 100kUI/L, the rate of phase change per unit concentration decreases, suggesting the onset of saturation on the sensor's active surface.

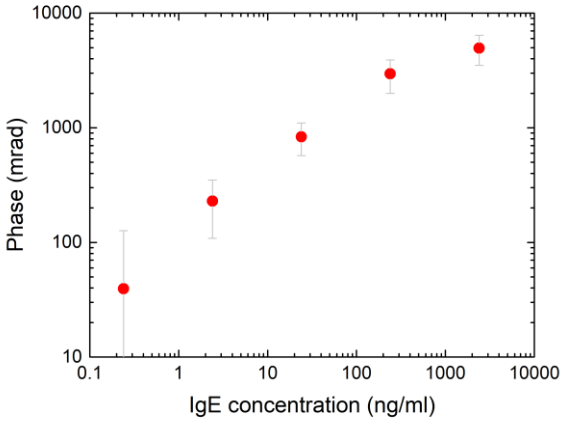

Figure 3. Phase shift vs mouse AXO-sIgE at increasing concentrations.

The specificity of the reported response was assessed through the repetition of the experiment where the mouse AXO-sIgE was replaced with a non-specific mouse IgE variant.

To summarize, the biosensing architecture presented here shows state-of-the art *LOD*s compared not only with commercial competitors but also with equivalent academic research. Preliminary experiments for specific IgE quantification demonstrate that the photonic biosensing technology developed at Bioherent, with minimal optimization, reached detection limits comparable with other in vitro commercial techniques based on label-based immunoassays.

While this work illustrates the potential use of the sensor for drug allergy diagnostics, the photonic technology is label free. The use of the sensor can be extended to any potential biomarker, given a reliable surface functionalization that guarantees the efficient recognition of the target.

In addition to that, this technology inherits all the well-known benefits derived from the silicon-on-insulator platform on which it is based. Those are the maturity of the processes that ensure high reliability and low cost, being an optimal option for commercial scalation.

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Red-Light-Photosensitized NO Release and Its Monitoring in Cancer Cells with Biodegradable Polymeric Nanoparticles

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Nitric Oxide (NO) is a small, free radical involved in the regulation of several physiological and pathophysiological processes [1] including cardiovascular diseases, bacterial infections [2] and cancer [3]. This multifaceted role has stimulated, over the last few years, a massive interest for the development of unconventional therapeutic approaches based on the NO use to tackle important diseases. However, the strict dependence of the biological effects of NO on its concentration [3] requires the generation of the radical with precise spatiotemporal control. Light represents a powerful tool to fulfil this need in a minimally invasive way and with high accuracy by using appropriate photoprecursors namely NO Photodonors (NOPDs) [4]. The development of organic NOPDs activatable in the so-called “therapeutic window” with highly biocompatible and tissue-penetrating red light is desirable and challenging [5].

In this contribution, we demonstrate that one-photon red-light excitation of Verteporfin, a clinically approved photosensitizer (PS) for photodynamic therapy, triggers NO release from a blue-light activatable NOPD (NBFNO) with an improvement of about 300 nm toward longer and more biocompatible wavelengths [6]. NO photorelease is photosensitized by the lowest triplet state of the PS, more likely by a catalytic photoinduced electron transfer. In view of biological applications, the water-insoluble PS and NOPD have been co-entrapped within water-dispersible and biocompatible polymeric nanoparticles (NPs) of mPEG-PCL without affecting the NO release process. Moreover, the spectroscopic prerequisites and the restricted environment of the NPs permit the green-fluorescent co-product (NBF) formed concomitantly to NO photorelease to communicate with the PS via Förster resonance energy transfer (FRET). This results in an enhancement of the typical red emission of the PS offering the possibility of a dual colour optical reporter useful for the real-time monitoring of the NO release through fluorescence techniques (figure 1).

Biological tests were performed using different types of cancer cell lines to ascertain the validity of this strategy applied to the polymeric NPs as potential nanotherapeutics. The results of cell viability experiments and fluorescence investigation in cells demonstrate the occurrence of the NO release under red-light illumination also in the biological environment. This confirms that the adopted strategy provides a valuable tool for generating NO from an already available NOPD, otherwise activatable with the poorly biocompatible blue light, without requiring any chemical modification and the use of sophisticated irradiation sources and opens intriguing prospects in biomedical research for studies where precise and spatiotemporally controlled concentrations of NO are required.

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Figures

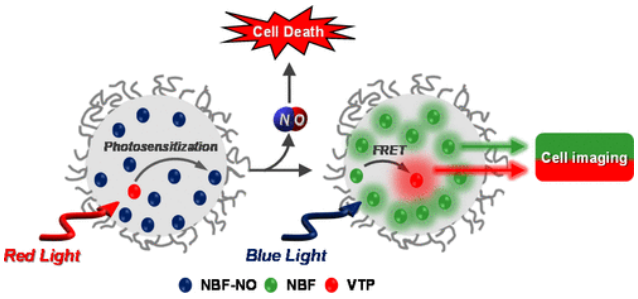

Figure 1. Schematic for the photosensitized NO release and its monitoring.

Transport of Single-Chain Polymer Nanoparticles across the Blood-Brain Barrier

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Neurological disorders, such as brain injuries, infections, stroke and dementia, affect many millions of people worldwide and are the cause of death of nearly 7 million people every year. [1] Therapies to treat these disorders often fail, due to the difficulties encountered with therapeutics delivery. The brain is protected by the blood-brain barrier (BBB), which is extremely effective in keeping foreign substances from entering the brain. A variety of nanocarriers has been developed over the past decades, that exploit the transport systems of the BBB, and promote transport of therapeutics to the brain. However, a reliable, efficient and easy to use nanocarrier for brain delivery is still lacking. [2]

Our research efforts have been focused on developing single-chain polymer nanoparticles as nanocarriers for controlled therapeutics delivery. Single chain polymer nanoparticles (SCNPs) are prepared by intramolecular crosslinking of individual polymer chains into a nanoparticle, and therefore particle size and dispersity originate directly from the employed precursor polymer. As such, SCNPs are highly uniform and display sizes in the 5-20 nm range. This size range is of particular interest, as it matches the sizes of proteins and small viruses, and is therefore likely to display promising behavior in vivo. We developed strategies to easily develop functional SCNPs through post-formation conjugation of different surface ligands. This has enabled us to rapidly prepare SCNPs with increasing content of negative surface charge, allowing targeting of malaria parasites. [3] Further, a series of SCNPs with increasing amounts of tertiary amines provided control over their cellular uptake behavior, even on a sub-cellular level. [4]

Many nanocarriers are based on assembled or crosslinked polymers, utilizing their highly modular nature, and display sizes in the range of 50-200 nm. Interestingly, several studies outlined the importance of particle size on brain transport efficiency, with the smallest 10 nm-sized particles displaying substantially higher transport than larger 100-250 nm-sized particles. [5,6] We therefore reasoned that single chain polymer nanoparticles may display enhanced transport across the BBB.

We report here our investigations into the cellular uptake of SCNPs equipped with varying ratios of 1-aminoglycerol and *N,*N-dimethylaminoethylamine, as well as SCNPs equipped with increasing densities of 1-glucose ligands, by hCMEC/D3 brain endothelial cells, as well as their promising transport behavior across an in vitro blood-brain barrier model.

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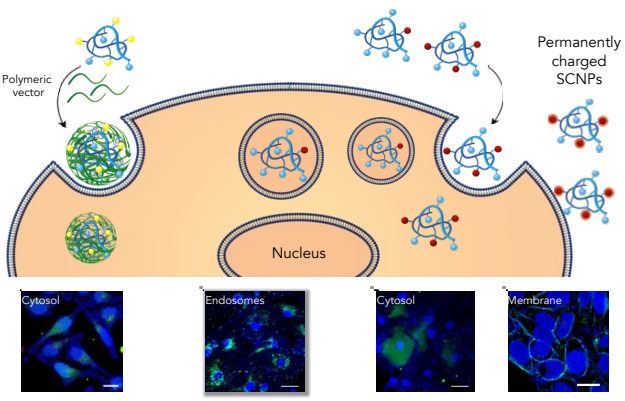

Figure 1. Cellular uptake pathways of single chain polymer nanoparticles depending on their surface functionality.

Graphene microtransistor array derivatized with modified aptamers as a functionalization alternative for biosensing applications

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Graphene solution-gated field-effect transistors (gSGFETs) (Figure 1) offer a high potential for chemical and biochemical sensing applications to perform label-free, rapid, and highly sensitive analysis coupled with a large ample throughput. These properties, combined with the potential for integration into portable instrumentation makes graphene-based transistors suitable for point-of-care diagnostics. [1] However, practical applications of gSGFETs require reliable and suitable functionalization steps for detection of different types of analytes. In this aspect, the surface modification of graphene has a significant impact on the electronic properties of these devices. Hence, it is crucial to perform robust and reproducible strategies for surface biofunctionalization. [2]

In this work, we report a single-step strategy of functionalization based on derivatized aptamers with fluorenylmethyl and acridine moieties (Figure 2), [3] using multiplexed arrays with 48-gSGFETs (Figure 3), for simultaneous measurements with a custom characterization electronic system [4] which allows the acquisition of significant data on each assay.

Here we demonstrate the potential of this system for biosensing, paying special attention to the robustness and sensitivity, performing the modification of graphene channel of the transistor by means of different novel strategies for immobilizing aptamers to achieve label-free detection of thrombin as a model. This study is focused on the simplification of the functionalization strategy and a better understanding of the parameters that affects the development of gSGFETs for biosensing.

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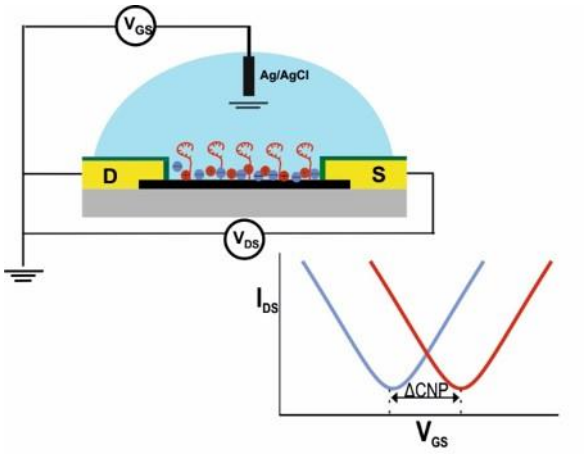

Figure 1. graphene SGFET scheme. Current to voltage curve shift when functionalization is performed

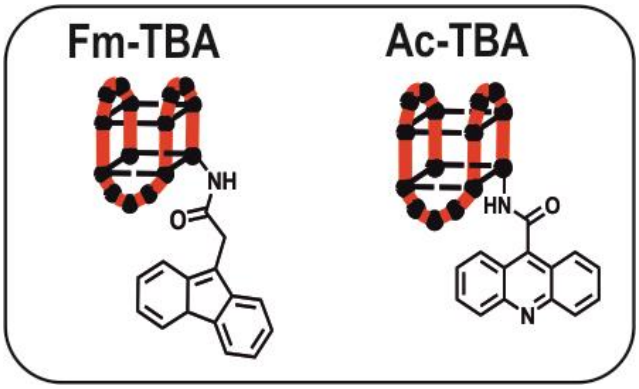

Figure 2. Thrombin Aptamer (TBA) derivatized with Fluorenylmethyl and Acridine molecules

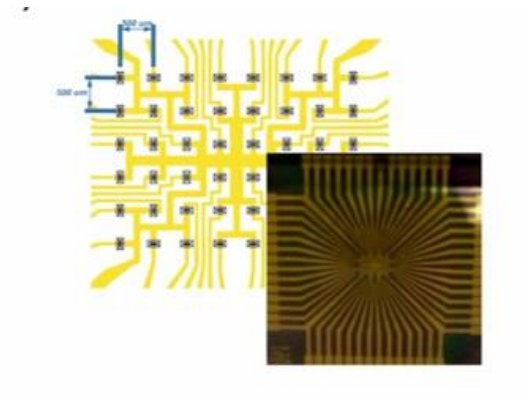

Figure 3. Array of 48 transistors of graphene

Implementing Horizon Scanning as a Tool for the Strategic Development of Regulatory Guidelines for Nanotechnology-Enabled Health Products

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INTRODUCTION

The global nanotechnology market, particularly nanotechnology-enabled health products (NHPs), is seeing significant growth (1). Yet, a gap exists between research and marketable products due to slow regulatory advancement (2). Horizon Scanning, a tool used by policy makers worldwide, identifies significant developments and threats and could be key in supporting to bridge this gap (3-6). Implemented through exploratory or issue-centered scanning, Horizon Scanning focuses on short to medium term topics rather than broad predictions. Regulatory agencies use it to identify future needs and establish guidelines, streamlining the path to market for innovative products. In this work we implemented Horizon Scanning methodology for NHP advancements, predicting trends and enhancing regulatory guidelines' strategic development.

MATERIALS AND METHODS

Regulatory database

A regulatory database was gathered, including guidelines and documents related to nanotechnology-enabled health products (NHPs) up to August 31st, 2023. Sources included competent authorities like EMA and FDA, European Commission, OECD, and standard-emitting organizations like ISO and ASTM.

Horizon Scanning methodology

This research applies the Horizon Scanning methodology in four stages based on the exploratory scanning approach (including signal detection, filtration, prioritization, and assessment). Signal detection uses various sources such as scientific publication databases and international patent registries, with searches conducted over distinct periods from January 2020 to June 2022. Irrelevant results were discarded. Filtration and prioritization utilized novelty as a criterion to eliminate irrelevant signals and categorize results into potential trends or disruptive elements, based on metrics proposed by Shah et al., 2003. This approach measures novelty by comparing how unusual an idea is relative to others. Finally, an assessment was carried out on the detected trends for their potential impact on the current regulatory state-of-the-art. This analysis aimed to identify how these signals fit within the existing regulatory science and potential areas of insufficient regulation.

Classification system for nanotechnology-enabled health products

Detected signals on NHP development have been indexed following the classification system described in a previous work of our group (7). This classification system considers both scientific and regulatory criteria and allows the grouping of NHPs into different categories, expected to share similar relevant characteristics when evaluated by regulatory authorities (refer to Figure 1). The system assigns a unique four-digit code, or classification signature, to each category of NHP. This code is based on the NHP's principal mode of action, chemical composition, intended purpose, and the approach followed in its nanofabrication (7).

RESULTS AND DISCUSSION

Signal detection for Horizon Scanning implementation involved databases that represent NHPs at various stages. Scopus, the European Patent Office (EPO) Database, and ClinicalTrials.gov were used for signal detection. These databases cover NHPs in early stages and also those assessed by a regulatory authority (either products preliminary assessed before a clinical study is initiated or product that already available in the market).

For filtration and prioritization of detected signals, novelty was used as a key criterion. A novelty quantification methodology was applied, which assigned higher novelty scores to the typology of NHPs less repeated within the whole distribution (Figure 2).

The results show a clear trend towards the development of drug delivery systems (DDS) and a growing trend in the development of nanomaterials for dental applications. Furthermore, the most disruptive elements involve NHPs that are multicomposite and multifunctional, harnessing nano-scale properties to combine therapeutic and diagnostic purposes within a single product. When compared with the regulatory landscape, current regulations are gradually adapting to accommodate emerging trends, with specific guidelines being developed. However, for the most disruptive elements, multicomposite and multifunctional NHPs, their novelty still poses significant regulatory challenges, requiring a strategic development of guidelines by regulatory agencies to ensure their safe and effective integration into healthcare practices. This study underscores the importance of proactive regulatory planning to bridge the gap between NHP innovation and market implementation.

In conclusion, there is a disparity between the innovative NHPs being developed and those that achieve regulatory approval. This is primarily due to

the lack of adequate regulatory guidelines. The application of Horizon Scanning methodology can be key for anticipating future regulatory needs. This study identifies DDSs and dental applications as leading trends in NHP development, and multifunctional and multicomposite products identified as the most novel. The continued focus on developing robust and adaptable regulatory guidelines for these types of NHPs is of utmost importance.

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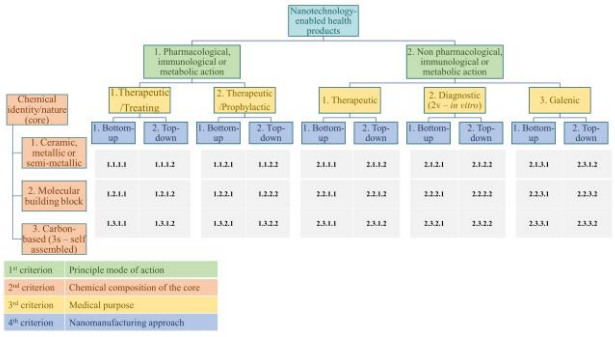

Figure 1. Nanotechnology-enabled health products classification system (taken from Rodríguez-Gómez *et al.*, 2023).

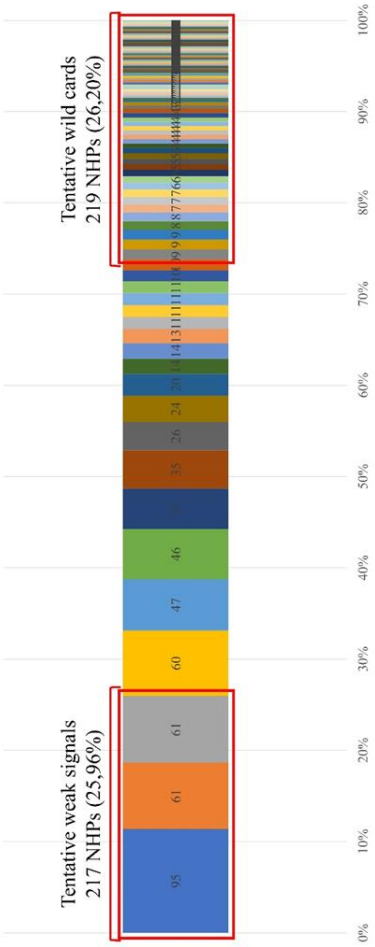

Figure 2. Cumulative percentage of the frequency of design solutions. Novelty analysis of the design solutions of detected signals. Design solutions are in descending order of frequency (i.e., in ascending order of novelty). The red squares show the 0.25 and 0.75 percentiles, Q1 and Q3, respectively. All design solutions with a frequency higher or equal to Q1 are considered tentative trends, all design solutions with a frequency lower or equal to Q3 are considered tentative wild cards.

Biofabrication of Self-assembling Protein Nanomaterials through Histidine-templated Cysteine Coupling

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Nanoscale protein materials have gained significant importance in the fields of biotechnology and biomedicine due to their diverse applications in catalysis, drug delivery, and tissue engineering [1-3]. Protein oligomerization can be achieved through various engineering methods, such as the use of divalent cations to target histidine-rich regions [4], however, the success of cation-histidine binding is contingent upon factors such as protein structure, media composition, and the presence of chelating agents [5].

Therefore, we have developed a robust, cross-linker-free, and versatile platform for oligomerization, centered around histidine-templated cysteine coupling: a H6-derived His-Cys tag (H3C). This innovative hybrid peptide tag enables the spontaneous and efficient self-assembly of proteins into covalently bound nanoparticles. The resulting nanostructures are stabilized by the formation of disulfide bridges and can be readily disassembled using reducing agents, but not affected by chelating agents.

Moreover, the incorporation of cysteine residues into the tag does not compromise the metal-binding capabilities of the histidine residues. This particularity allows to keep the His-associated properties of one-step IMAC-based protein purification as well as the cation induced formation of higher-order microparticulate materials [6], serving as nanoparticle-releasing depots for protein delivery.

The dual interaction modes exhibited by the engineered H3C tag, along with the structural robustness and stability of the resulting nanoparticles make the biofabrication approach presented here broadly applicable for advancing in the development of novel therapeutic protein materials.

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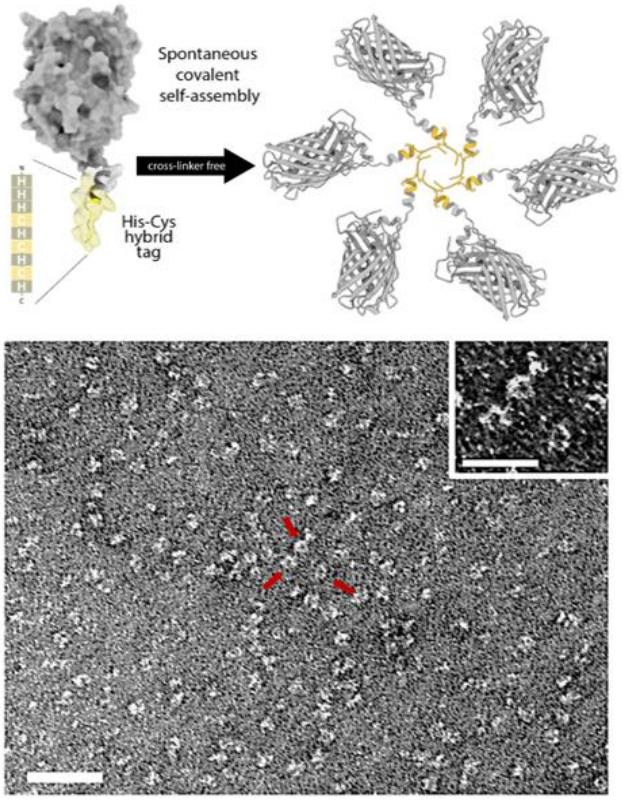

Figure 1. Architecture of GFP-H3C nanoparticles (Cys interaction in yellow) and it's morphometric analysis by transmission electron microscopy (TEM).

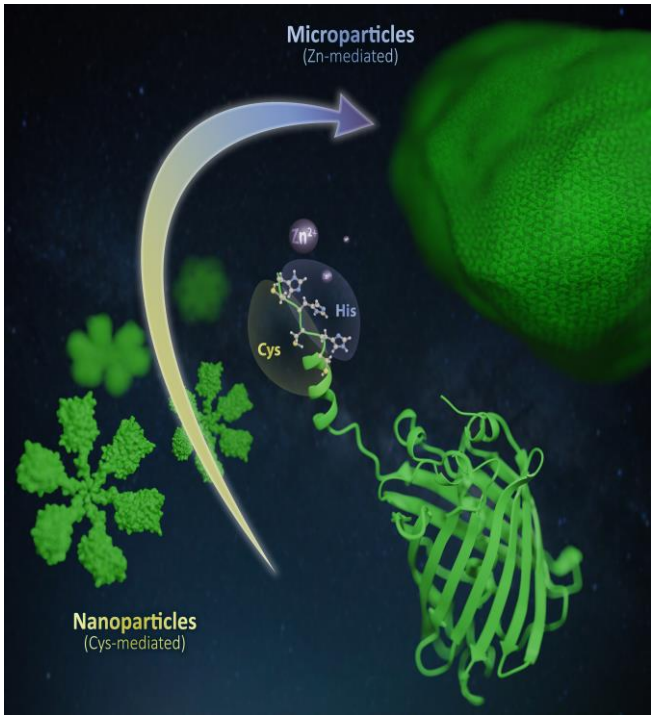

Figure 2. Versatility of the H3C tag. Formation of covalent hexametric nanoparticles through the histidine templated cysteine coupling process. The use of cations can then induce supramolecular organization into microparticle depots.

Artificial internalizing receptors for mammalian cells

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Receptor-mediated endocytosis is one of the core cellular functions and is often exploited for targeted drug delivery. The natural presence of cell surface receptors enables the action of antibody-drug conjugates (ADCs), a highly successful modality of drug delivery. However, targeting natural receptors is often associated with a high risk of off-targets and side effects. We therefore try to develop new dedicated artificial communication routes, which could be essential in the improvement of current cell-based therapies e.g. CAR T cell therapy. Inspired by Nature, we have designed chemical, artificial internalizing cell receptors that mimic the natural process of receptor-mediated endocytosis. The chemical receptor design includes a cholesterol amine anchor that enables association with the phospholipid membrane of mammalian cells, and a spacer moiety, which separates the anchor from the crucial recognition motif, fluorescein (Figure 1A). This xenobiotic, fluorescent moiety supports the visualization of the artificial receptor and enables extracellular targeting with a cognate anti-fluorescein antibody (Figure 1C). Targeting of the artificial receptor in primary human T-cells using a Monomethyl auristatin E (MMAE)-based ADC was proved to be of nanomolar potency (Figure 1B, D). Furthermore, the eradication of a receptor-equipped 3D cell spheroid was achieved. This emphasizes the ability to use our artificial receptor in tumour-infiltrating engineered cells, where on-demand deactivation would not only lead to the killing of the equipped cell but also of the surrounding cancerous tissue, by the inherent bystander effect of the released drug (MMAE) [1]. In new unpublished data, we made significant improvements in the receptor-mediated ADC delivery by demonstrating enhanced potency relative to the free drug (70-fold), increased selectivity by elimination of the bystander effect, significantly faster action, and long sustainability of the receptor. These improvements increase the applicability of using artificial receptors in the design of a selective communication route only to the pre-engineered cells, such as a functional “suicide switch” installed in CAR T cells in case unwanted immunological responses or side effects arise. Additionally, we work on investigating the scope and potential limits in terms of cargo that can be internalized using artificial receptors. To do so, we inverted the design and equipped the anti-fluorescein antibody itself with an anchor moiety. This allowed easy incorporation into mammalian cells, with both fluorescein-labelled antibodies and serum albumin being successfully internalized. This highlights that the internalization of cargo is potentially only limited by the ability to be labelled with the commonly used fluorescein moiety. Our artificial fluorescein-based recognition system illustrates how the use of xenobiotics can overcome the problems faced when targeted drug delivery is based on naturally occurring antigens. Furthermore, our designs demonstrate how artificial receptors can be very useful tools with many future applications within important areas of biomedicine and biotechnology.

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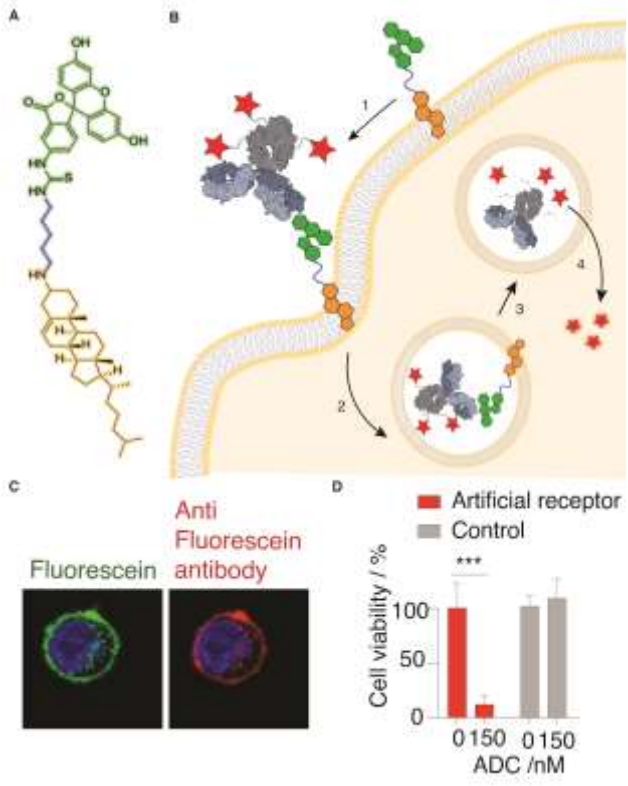

Figure 1. Artificial internalizing receptors [1].
A. Structure of the artificial receptor including the anchor (orange), spacer (purple), and recognition motif (green). **B.** Illustration of the artificial receptor-mediated internalization mechanism. 1) Binding of the antibody-drug conjugate to the artificial receptor 2) Internalization and pH-dependent cargo dissociation 3) lysosomal degradation 4) drug release. **C.** Nanomolar potency of the antibody-MMAE conjugate in artificial receptor-equipped primary human T cells.

Development of Essential Oil based Nanoemulsions loaded with Dimethyl Fumarate for Intranasal Drug Delivery

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Dimethyl fumarate (DMF) is a first line medication for the treatment of multiple sclerosis (MS), a neurodegenerative disease characterized by inflammation with demyelination and gliosis. Despite a good therapeutic effect of DMF, it is associated with ambiguous safety profile and several adverse events. These adverse events are attributed to the high doses of DMF, and consist of GIT problems, flushing, skin irritability and in severe cases can cause progressive multifocal leukoencephalopathy (PML).[1-3] Now-a-days, intranasal drug delivery is in the limelight among pharmaceutical innovations owing to its advantages over conventional drug delivery routes. Intranasal drug administration has the potential to deliver the drug directly to brain by passing the blood brain barrier and generating a therapeutic effect promptly. A relevant hindrance in this route is the mucociliary clearance that may cause flushing of the formulation reducing absorption.

Intranasal drug delivery of DMF can help in utilization of minimum doses and can directly initiate a therapeutic response in the brain. It would avoid first pass effect as well as GIT related adverse events. Furthermore, essential oils in combination with drugs can help in achieving a synergistic effect and can help in reducing the adverse events. Carvacrol (CV), a monoterpene phenolic compound, have neuroprotective effects and in combination with DMF can help in ameliorating the need for higher doses leading to mitigation of adverse events.

Nanoemulsions of CV loaded with DMF intended for intranasal route can help in addressing the problems associated with the drug, patient compliance, and disease management. Essential oils have gained recognition in these past few years as potential therapeutic entities, but it is necessary to evaluate them for solubility studies and molecular interactions with the drugs.

First, solubility of DMF in CV was determined and found to be 150 mg/ml at 25±1 °C. Physical mixtures of DMF-CV in molar ratios of 1:1, 1:2, and 2:1 were prepared and analyzed by means of FT-IR and thermal analysis (TGA). In the next phase, oil in water (o/w) nanoemulsions by self-nanoemulsification method, incorporating CV oil and encapsulating DMF in the oil were prepared. Chitosan was utilized as surfactant with oleic acid to physically stabilize the nanoemulsions and to impart mucoadhesive properties required for efficacious intranasal drug delivery.[4] Oil and chitosan-oleic acid was fixed at 1:1, with chitosan utilized at 0.1% w/v of the overall volume for chitosan and oleic acid mixture in accordance with previously reported method.[5]

Blank and drug loaded nanoemulsions were investigated for particle size, PDI, zeta potential, and entrapment efficiency. The particle size was below 200 nm for blank and for drug loaded nanoemulsions below 250 nm with a PDI of less than 0.5. Encapsulation efficiency was found to be 90%. For the stability studies, nanoemulsions were stored at room temperature (25ºC) and at refrigerated temperature (4°C) for 8 weeks. It was inferred from the obtained results that at room temperature, particle size increased to range of 300 to 400 nm and PDI was found to be more than 1. On the other hand, nanoemulsions at refrigerated temperature were able to sustain their mean particle size of below 250 nm and PDI below 0.5. Thus, it is concluded that nanoemulsions based on CV as oil loaded with DMF are more stable at refrigerated temperature.

Furthermore, design of experiment is planned to investigate different parameters such as surfactant ratio, speed of addition of organic phase to aqueous phase, and probe sonication time to better optimize the formulation before leading to cell permeability studies on cell lines.

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Interactive coatings direct blood components to modulate coagulation in medical devices

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In medical devices, the contact of blood with the artificial surface inevitably causes the activation of coagulation. Immediately after the contact of blood with the surface, protein adsorption occurs. This leads to the reciprocal activation of factor XII and plasma prekallikrein generating large amounts of thrombin resulting in clot formation. Thus, the use of blood-contacting medical devices can lead to life-threatening complications such as thrombosis and stroke. In nature, the lining of healthy endothelium can sense and maintain a tightly regulated equilibrium called hemostasis that prevents hemorrhages and excessive coagulation. Our goal is to develop coatings inspired by the endothelium that turn the surface of medical devices hemocompatible to prolong their use without negative outcomes. Towards this aim, we develop nanoscale coatings that go beyond passivation by interacting with blood components and orchestrate a cascade of reactions to enhance their hemocompatibility and performance. The ultrathin nano-coatings include three hierarchical levels: a passive, a modulatory, and an interactive one. The passive level consists of antifouling polymer brushes or brush-like coatings that create a physical barrier to protein adsorption and cell adhesion, prohibiting surface-induced activation of coagulation.[1-3] The modulatory level is achieved by decorating the brushes with small biomolecules capable of binding to key elements of the coagulation cascade and inactivating them directly at the surface of the device. In contrast to anticoagulants, this approach allows a local inhibitory effect at the surface and does not interfere with hemostasis.[4,5] The interactive level can sense the presence of a thrombus formed somewhere else in the system and orchestrates its disintegration. We developed a fibrinolytic coating that is only active in the presence of a thrombus and directs its destruction using components present in the blood.[6] We envision that our ultrathin nano-coatings are a promising route toward the improvement of the hemocompatibility of medical devices.

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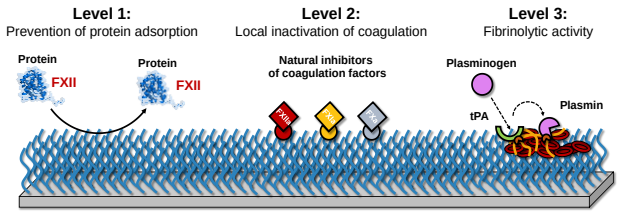

Figure 1. Scheme of the hemocompatible coatings that consist of three different levels. (1) The passive level prevents protein adsorption, (2) the modulatory level locally deactivates surface-induced coagulation, and (3) the interactive level digests an unwanted blood clot if it is necessary.

Radical Dendrimer-Based MRI Contrast Agents: In Vivo Applications and Studies on Influencing Relaxivity Factors

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Abstract

This abstract focuses on the in vivo applications of radical dendrimer-based Magnetic resonance imaging Contrast Agents (MRI CA) and investigates the factors influencing relaxivity.

MRI, a highly versatile and widely employed clinical diagnostic tool, relies heavily on gadolinium-based contrast agents (GBCAs) for enhancing image quality. These agents work by shortening the T1 relaxation time of nearby water protons, thereby intensifying the signal and contrast between normal and abnormal tissues. [1] However, it's important to note that GBCAs have been associated with the potentially lethal nephrogenic systemic fibrosis for over a decade. [2] In light of this concern, there is growing interest in alternative options, particularly stable organic radicals like nitroxides (e.g., TEMPO, PROXYL). These paramagnetic species can serve as MRI CA similar to Gd(III) chelates but with minimal toxicity. To enhance molecular relaxivity and protect against bio-reduction, one effective approach is anchoring multiple nitroxide units to conventional linear or hyperbranched polymers. Dendrimers with controlled structures are excellent candidates for this purpose. [3] Consequently, we have successfully synthesized a series of free radical dendrimer-based CA characterized by exceptional relaxivity, high water solubility, low toxicity, biocompatibility, and safety. [4]

We have developed a completely organic, metal-free MRI CA (G0 to G3) using polyphosphorhydrazone (PPH) dendrimers, functionalized with up to 48 PROXYL radical units. [4] Notably, G3 demonstrated robust contrast enhancement in murine GL261 glioblastoma tumors, on par with commercial GBCAs. G3 exhibited selective accumulation within brain tumor tissues, extending imaging sessions to over 2.5 hours. Importantly, G3 showed no signs of toxicity and maintained stability within biological environments (Figure 1). [5] All of these features allow us to suggest that radical dendrimers could be a viable alternative to metal-based MRI CA.

Nonetheless, the synthesis of G3 is complex, and our next research focus revolves around simplifying the production of molecules with high relaxivity. The first thing is to simplify the synthesis of dendrimers, and the second is to increase the relaxivity. We have also synthesized fluorescent and magnetic bimodal imaging probes, [6] oligoethylene glycol dendrimers [7] and various other types of radical dendrimers, each exhibiting distinct relaxation rates per nitroxide unit. Understanding the key factors affecting relaxivity is important for the synthesis of good CAs.

Research on the relaxation mechanisms of GBCAs already has theoretical support, [8] such as the SBM theory. [9] However, there are few studies that focus on the relaxation mechanisms of nitroxides especially when they are tethered to macromolecules. Therefore, in this study, we aim to synthesize various types of radical dendrimers and subsequently employ molecular dynamics to investigate the microenvironment surrounding these radicals. We will analyze critical factors including the presence of water molecules, rotational correlation times, molecular configurations, and their impacts on the relaxivity. These discoveries will provide essential theoretical groundwork for future molecular design efforts.

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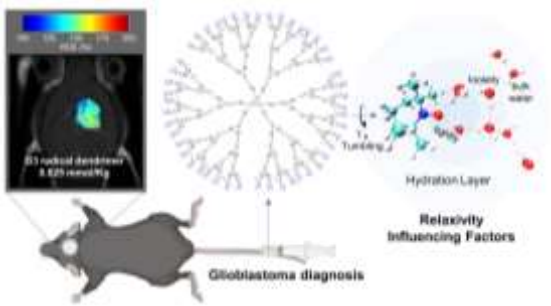

Figure 1. Middle) Structure of G3 based on polyphosphorhydrazone dendrimer and PROXYL (in blue). Left) Color-code scale for relative contrast enhancement (RCE) of GL261 glioblastoma tumour-bearing mice with intravenous administration of G3. Right) Representation of the key factors governing the relaxivity of nitroxides.

Effect of Tyrosol Functionalized Gold Nanocomposite on Biofilm Milieu and Wound Healing

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Abstract

Candidiasis, a fungal infection caused by opportunistic pathogen Candida which range from superficial to deep invasive manifestations, and is of significant concern in immunocompromised individuals [1]. Moreover, the fungal infections provide conditioned space to bacterial pathogens for colonization and results in mixed/ polymicrobial surface adhered community called polymicrobial biofilm. These communities are particularly responsible for emergence of multidrug-resistant strains. Thus, posed formidable challenges for clinicians in effectively managing these infections using conventional antimicrobial therapies. Hence, we synthesized chitosan mediated gold nanocomposite of tyrosol, for their efficacy against *Candida albicans, Staphylococcus aureus, Pseudomonas aeruginosa* and their mixed communities. Compared to tyrosol alone, Chi-TY-AuNP's nanocomposite demonstrated excellent inhibition potential on both planktonic and sessile growth of *C. albicans, S. aureus, P. aeruginosa, C. albicans + S. aureus, and C. albicans + P. aeruginosa* at lower concentrations. At the highest concentration tested (20 µg/mL), the drug conjugated nanoparticle has eradicated nearly 70% of the mature/ old polymicrobial biofilm structure while completely inhibited the construction of community. To ensure the suitability of the Chi-TY-AuNP's in treatment of non-healing wounds in diabetic patients, the cytocompatibility and wound healing was also undertaken [2].

Chi-TY-AuNP's treatment revealed good cytocompatibility and decreased ROS levels in NIH-3T3 cell lines. Developed 1% and 2% (w/w) Chi-TY-AuNP's-Carbopol®934 formulations exhibited excellent rheological properties suitable for topical applications, supported by the nontoxic nature of developed gels in the dermal toxicity study in experimental rats. Findings of *in-vivo* wound healing study conclusively demonstrated the re-epithelialization of the wounded tissues with no residual scar. Increased collagen and fibroblast depositions, normal cellular integrity and neoangiogenesis were evident by the H&E and Masson's trichrome stained histopathological micrographs of the treated animal's skin tissues. The wound-healing effect mediated by Chi-TY-AuNP's-Carbopol®934 can be attributed to the additive antimicrobial and anti-inflammatory effects of tyrosol and chitosan in lipid peroxidation and oxidative damage minimizing activity [2]. Also, tyrosol being a polyphenolic compound, may act as a regulator at multiple phases of the wound healing process via preventing inflammation and secondary infection and augmenting collagen synthesis [3]. Collectively, *in-vivo* wound healing study demonstrated the reepithelialization of the wounded tissues and increased proliferation of fibroblast cells, collagen fibers and neoangiogenesis [2,4].

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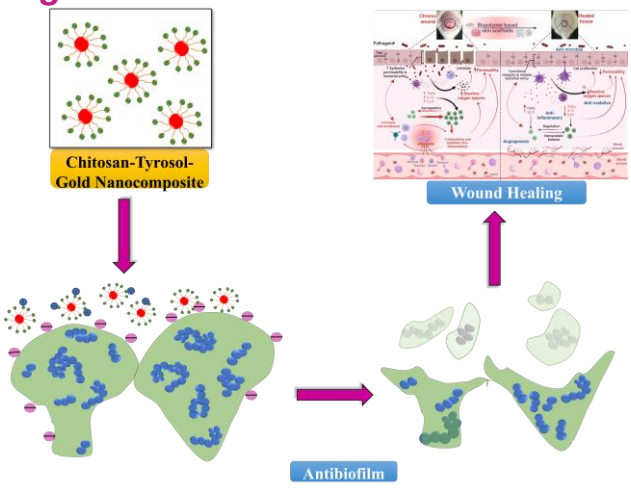

Figure 1. Chi-TY-AuNP's mediated Antibiofilm and Wound Healing activity.

Plasmonic Nanosensors for Optical Monitoring of Labile Zinc Inside Metastatic Cells

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Abstract

Triple-negative breast cancer (TNBC) accounts for 20% of breast cancer and tends to metastasize to the brain. For this reason, TNBC tumors have a higher rate of distant recurrence and with lower 5-year survival rate than other breast cancer. Zinc, an essential trace element and its aberrations involves closely with cancer progression and cellular dysfunction. In our work, we focus on sensing the potential relevance between zinc and TNBC metastasis. Based on surface enhanced Raman spectra (SERS) methodologies, zinc nano-sensor was designed. We chose the 2,2': 6'-2''-Terpyridine-4'-Thiol (TPY) as the sensing molecular and modified gold/silica nanocapsules (NCs) with TPY (NCs@TPY) to detect different amount of zinc in different metastatic TNBC cells. The tpy group can form stable complexes with Zn^{2+} and produced the relative SERS sensitive peak at 1034 cm^{-1}. For chemoselective SERS analysis, zinc was determined by the ratiometric increase of a Raman peak at 1034 cm^{-1}, relative to the main peak at 1021cm^{-1}. Our work compared the different concentrations of zinc in MDA-MB-231 cell, brain metastasis model BrM_2 cell and lung metastasis model LM_2 cell, we found out Zn^{2+} concentration increased a lot inside the metastatic cells than the other cancer cell under same amount of Zn^{2+} incubated conditions. Our sensor with the LOD of $10^{-11.72}$ M is much lower than the Zn^{2+} standard methods by Zinquin. Herein a highly sensitive monitoring nanosensor for in situ zinc analysis inside single cell is presented.

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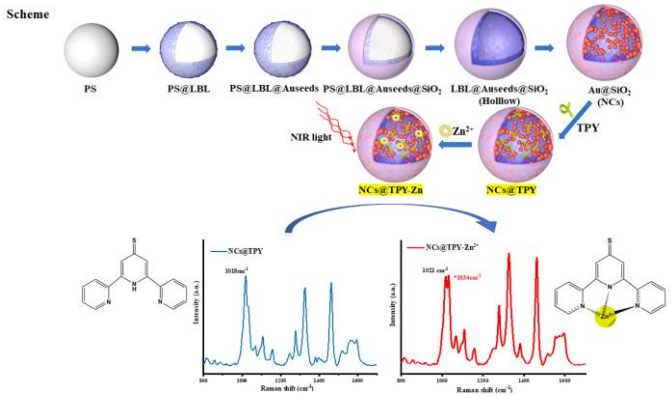

Figure 1. the scheme of zinc nanosensor. Based on the TPY characteristic molecule Raman signal, changes in intracellular zinc ion concentration are detected. After it combined with free zinc ions, the new Raman peak at 1034 cm^{-1} appeared, served 1034 cm^{-1} as the sensitive peak.

Polyallylamine functionalized with dextran for siRNA delivery.

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Background
Small interfering RNA (siRNA) is a small RNA molecule able to inhibit the expression of a target protein via degradation of its messenger RNA, impairing its translation. siRNA technologies have a great potential not only in basic research but also in gene therapy [1].
Cationic polyelectrolytes can complex with nucleic acids and deliver them inside cells. Upon endocytosis and liberation of the cargo polycation with protonable amines induce an osmotic swelling in the acidic environment of the endosomes that facilitates siRNA translocation into cytoplasm where must act on mRNA. [2].
Polyallylamine (PAH) is an interesting system for intracellular delivery of siRNA. Its positive charges can efficiently interact with both nucleic acids (cargo) and cell membrane (target). Also, it responds to pH variations allowing for the liberation of the complexed siRNA in the cytoplasm upon acidification of the endosome. However, positive charges induce toxicological endpoints, limiting the application of complexes of PAH *in vivo* [3, 4]. Besides complexing nucleid acids, PAH forms nanoparticles in presence of phosphate buffer. These nanoparticles have an interesting pH response, being stable in a narrow pH range between 6 and 8 and dissolving in a reversible way at lower and higher pHs.

Aim
Given the toxicity of the positive charges of PAH, we are testing PAH functionalized with dextran. Dextran is a non charged biocompatible molecule, and we hypothesize that after complexation of dextran functionalized PAH with siRNA a protective dextran layer will form around the nanoparticle, shielding positive charges, reducing toxicity, and/or favouring other endocytic pathways. We have explored the formation of nanoparticles of dextran modified PAH in phosphate buffer and the complexation of the polymers with siRNA.

Methods
PAH was substituted with different ratios of dextran chain/polymer chain. Dextran functionalized PAH was then complexed with siRNA and complexes characterized via dynamic light scattering and transmission electron microscopy. Gel retardation assay was used to confirm nucleic acids encapsulation.
The toxicity of the system was studied in two different cell lines: Jurkat, a model for suspension cells, and A549, lung carcinoma adherent cells.

Results
DLS studies revealed that the less-substituted polymers form nanoparticles in the tested buffers. Nanoparticle dimensions are dependent on ionic strength: overall, the higher the concentration of salts, the bigger the dimensions of nanoparticles. On the contrary, highly substituted polymers do not form nanoparticles,.
DLS studies also revealed that the range of pH stability of the nanoparticles varies with the degree of substitution.
Transmission electron microscopy (TEM) allowed for the visualization of the morphology of the assemblies. Depending on the number of dextran chains attached to PAH we observe the formation of nanoparticles or capsules, with an empty core.
Next, polymers were complexed with siRNA and complexes studied via DLS. Gel retardation assay proved the encapsulation of siRNA for different N/P ratios.
Nanoparticles in absence and presence of siRNA were studied in Jurkat cell line to evaluate their cytotoxicity profile. Toxicity was observed only at high concentrations, while the systems loaded with siRNA showed an excellent profile, with cell metabolic activity always above 80% when compared to the control. Similar results were obtained in A549.

Conclusions
We show here the formation of assemblies of dextran modified PAH in phosphate buffer and in presence of siRNA. The dextran modified PAH forms different structures through association with phosphate buffer depending on the number of dextran chains present. The polymer complexes siRNA efficiently and the presence of dextran reduces toxicological endpoints in vitro.

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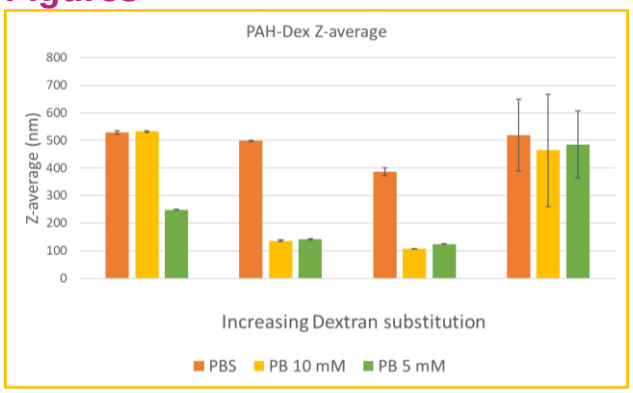

Figure 1. Dynamic light scattering of different dextran substituted polymers in different phosphate-containing buffers. Error bars represent standard deviation.

Safe and Sustainable by Design development of nanomaterials for green printed electronics.

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Sustain-a-Print (SaP)[1] project embraces the EU's Circular Economy Action Plan[2] to further advance the circularity potential of electronics and electronic equipment by the dire forecast for increased resource extraction and waste generation and their detrimental effects on climate and biodiversity (Figure 1).

Printed electronics (PE) is an additive manufacturing method characterized by its versatility, scalability, and low material usage[1], by printing conductive and dielectric inks on flexible/stretchable substrates opening new applications in the market. However, in traditional electronic production methods, the current life cycle for a PE product starts with materials (substrate, conductive and dielectric materials) obtained through mining of raw materials. The main goal of Sustain-a-Print (SaP) is to open new life-cycle routes and to design and implement sustainability into each step of the lifecycle (Figure 2).

The main objectives of the project are:

1. The **reuse and recycling** of valuable PE materials, thus contributing to a circular economy in the EU using **Safe and Sustainable by Design (SSbD)** methodologies.
2. Development of **sustainable materials and formulations** for PE, utilising either bio-based or recycled electronic waste sources, using **SSbD methodologies**.
3. Development of **sustainable formulations** for PE printing, using **SSbD methodologies**.
4. Development of **sustainable digital printing and assembly** of PE to address IEU specifications based on sustainable materials and formulations, using **SSbD methodologies.**

Nanomaterials have a crucial impact on printed electronics, providing multiple benefits and driving innovation in the development of **electronic devices**. Nanomaterials are pertinent in this context because of their distinctive characteristics, which augment the **efficacy and adaptability** of printed electronic devices[3].

The main ways of impact of nanomaterials on printed electronics are: (1) in their use as **conductive** nanoparticles, with nanomaterials like silver, gold, copper, and graphene, (2) **their flexibility and stretchability** with nanomaterials like Carbon Nanotubes, crucial on applications like wearable electronics and flexible displays, (3) their **enhanced performance**, offering superior electrical, thermal and mechanical properties compared to their bulk counterparts, (4) its s**mall size and high aspect ratio**, which enables their miniaturisation and possibility to create smaller and more compact devices, (5) nanomaterials can be **cost-effective** and allow the reduction of material usage while maintaining high performance, (6) their **compatibility with various printing techniques**, such as inkjet printing, screen printing and roll-to-roll printing[4].

The **potentially harmful effects of nanoparticles** on **human health and the environment** are a matter of great concern and are being extensively studied. Nanomaterials possess distinct physical and chemical characteristics due to their diminutive size and extensive surface area, potentially resulting in diverse **toxicological consequences** when compared to their larger counterparts.

Toxicity and ecotoxicity of nanomaterials depend on their Size, Surface Chemistry, Biological Interactions, and Bioavailability among others. Thus, standardized methods for risk assessment need to be developed, for toxicity studies, but also, for risk assessment and for establishing safe exposure limits[5].

The **Safe and Sustainable by Design strategy** adopted by the project will allow us to mitigate risks and potential hazards in the products developed within the project. These strategies will be based on the properties of the materials and processes under study in the project to reduce their impact on health and the environment. The strategies will be divided into **Safe by Product Design**, which will be focused on the mitigation of the potential hazards of new nanotechnology products to improve biocompatibility and **Safe by Process Design**, based on containment approaches and engineering techniques to remove ultrafine particles released at relevant stages in the life cycle, from the synthesis to the EoL[6].

These approaches and methodologies will be demonstrated through the development of a green and safe electrochemical enzymatic lactate sensor. Analytical figures including sensitivity, repeatability, limit of detection and robustness will be compared with artificial and real samples. The consortium of the project will also develop and validate an electronic Membrane Switch made of sustainable materials, conductive and dielectric inks and substrates for the production of digitally printed prototypes.

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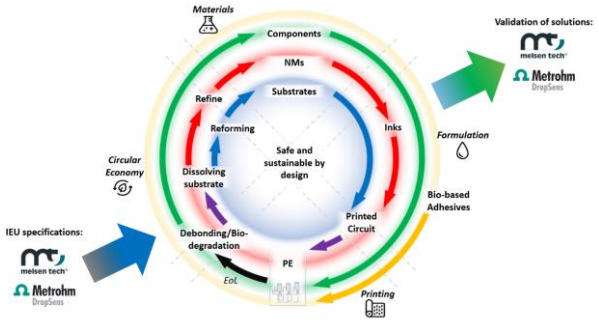

Figure 1: The SaP concept for sustainable PE production based on recycling/biodegradation of NMs and substrates, and reusability of components. IEU specifications will be addressed by applying the SaP concept as a foundation for the SaP methodology guided by SSbD activities.[1]

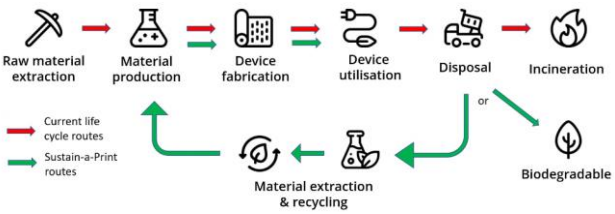

Figure 2: Illustration of current life-cycle routes for PE based on processes and the sustainable alternatives that are considered in SaP.[1]

Anti-inflammatory activity of polyarginine nanocapsules loaded with astaxanthin

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Astaxanthin (AST) is a carotenoid obtained from natural sources that has been reported to have an extremely strong antioxidant, anti-inflammatory, and neuroprotective activity [1]. Therefore, AST might have protective effects in neuroinflammatory diseases such as multiple sclerosis, an inflammatory immune-mediated disease of the CNS characterized by demyelination and neurodegeneration. However, the therapeutic application of AST is hindered by its low aqueous solubility and poor stability [2]. In order to improve AST stability and bioavailability as well as its potential application in neuroinflammation, we have developed polyarginine (PARG) nanocapsules (NCs) loaded with AST. PARG is a cationic polymer that has been reported to enhance interaction and transport through cell membranes [3]. The NCs were synthesized using a modified solvent displacement method described previously by Lollo (2017) [4]. PARG was tested at different concentrations (0.06 - 0.25 mg/mL). This strategy allowed us to obtain stable PARG-NCs in the range of 157 - 204 nm, with PDI values (0.1 - 0.2), and positive zeta potential between 33.8 and 57.5 mV, confirming the adherence of polyarginine on the oily droplet´s surface. The positive Zeta potential is relevant since the NCs are designed to be mucoadhesive and to promote absorption of AST through cell membranes. To determine cytotoxicity of PARG-NCs, rat astrocytes were incubated with NCs synthetized with different concentration of PARG (0.06, 0.125, and 0.25 mg/ml) and at volumes of 5-25% of cell culture medium volume. After 48 hours, cell viability was assessed by flow cytometry. The results showed that cell viability was higher than 90% in the presence of PARG-NCs at concentrations of 0,06 mg/mL and 0,125 mg/mL PARG and at volume lower than 15% (Figure 1). To evaluate the *in vitro* anti-inflammatory activity of AST-PARG-NCs, interferon (IFN)-gamma activated rat astrocytes were treated with PARG-NCs loaded with 20 µM AST and the expression of the glial fibrillary acidic protein (GFAP), a commonly used marker of astroglial activation, was determined by flow cytometry. The preliminary results suggest that activated astrocytes treated with PARG-NCs containing 20 µM AST exhibited lower expression of GFAP than control cells (Figure 2). Taking together, these results indicate that PARG-NCs are safe and that they might be useful as nanosystem for AST delivery with potential anti-inflammatory application in neuroinflammatory diseases. To the best of our knowledge, this is the first time that anti-inflammatory activity of PARG-NCs loaded with AST is reported using astrocytes.

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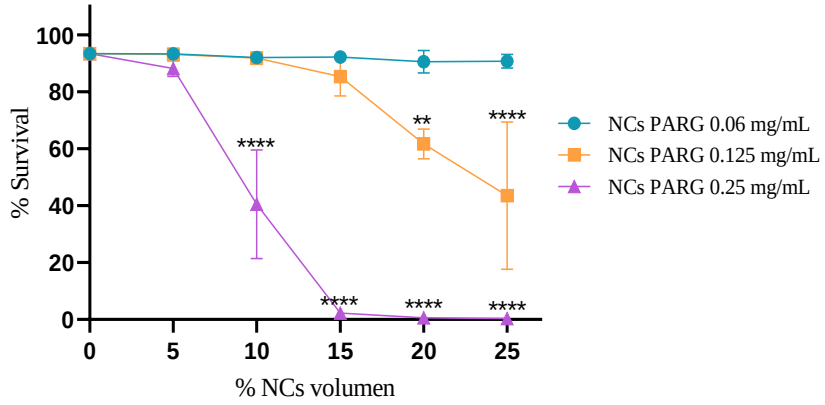

Figure 1. Survival percentage of astrocytes incubated for 48 h with NCs PARG (0.06, 0.125, 0.25 mg/mL of PARG) at volumes of 5 to 25% in relation to cell culture medium volume. **** $p < 0.0001$ vs NCs with 0.06 mg/mL PARG. The results are shown as mean of n = 3

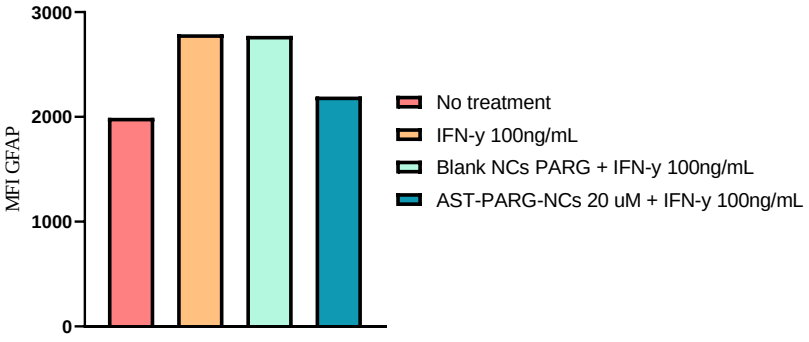

Figure 2. GFAP expression in rat astrocytes after inflammatory activation with 100 ng/mL IFN-gamma and treated with Blank NCs PARG (vehicle) or AST-PARG-NCs 20 µM.

Fine-Tuning Neuroblastoma Differentiation and Extracellular Vesicle Secretion through Electrical Stimulation Time Components

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Introduction: Recent advancements in electrobiology research have unveiled the potential of low voltage electrical stimulation (ES) in the range of endogenous electric field as a modulatory tool for promoting pro-regenerative cellular responses, including neural differentiation[1]. Previously we showed that direct ES trigger regenerative responses, such as altering stem cell fate, motility and function[2]. Accordingly, ES of mesenchymal stem cells has been shown to upregulate the expression of several regenerative, neuroprotective, neurodegenerative and angiogenic markers (such as VEGF, BDNF, NGF, etc.)[3].

One of the challenges in bioelectrical stimulation is the estimation and optimization of the effective parameters. Amplitude of the voltage and/or current, signal shape, frequency, and duty cycle play important role in communicating effective signal to the cells.

In this study, we maintained a constant field amplitude of 25 mV/mm, which represents a relevant endogenous field. Our primary focus was to investigate the impact of temporal factors, such as frequency and duty cycle, on neural differentiation within a human neuroblastoma (SH-SY5Y) model. The impact of ES, in the absence of additional essential neurotrophic factor, i.e., brain-derived neurotrophic factor (BDNF), has been compared across different time components and equated with the group stimulated by BDNF.

Moreover, we studied the effect of ES parameters on modulation of extracellular vesicles (EVs) secretion. EVs are the insoluble fraction of the cell secretome, which consists of membraned micro/nano particles (50-150 nm for exosomes and 100-1000 nm for microvesicles). These vesicles facilitate the transport of bioactive lipids, proteins, various RNA and DNA subtypes, thus serving a vital role in cell-cell communication. Additionally, recent discoveries have highlighted their potential benefits in tissue regeneration[4]. In this study we explored the effect of electrical stimulation parameter on production and cargo of the EVs from SH cells and assess the size and concentration of particles as well as protein content.

Methodology: SH-SY5Y cell were expanded in growing medium (DMEM/F12 + 10% FBS + 1% Antibiotics). Cells were seeded at the density of $3x10^4/cm^2$ in ES chambers developed in our lab (8-well pate setup; each well with surface area of 10 cm^2, 3 plates used per condition per experiment). The day after seeding, cells were exposed to pre-differentiation medium consist of Neurobasal, B27, glutamate and retinoic acid for 5 days. The cells were subjected to either pulsed electrical stimulation of 25 mV/mm (ES1: 1Hz, 500µs (0.05% duty cycle); ES2: 50Hz, 500µs (2.5% duty cycle); ES3: 1Hz, 250 ms (25% duty cycle)), or 5ng/ml BDNF treatment.

One day post treatment, cells were fixed and assessed for the expression of neural markers (MAP-2 and Tau-5) using the immunofluorescent technique (IF). Metabolic activity of the cells was assessed using the alamar blue assay. Finally, the cell secretome was purified through centrifugation at 300xg and 2000xg to eliminate dead cell debris and apoptotic bodies. The purified secretome was then subjected to ultracentrifugation at 100,000xg using a 30% sucrose in PBS cushion. EVs were obtained and purified through an additional washing step with PBS and ultracentrifugation, resuspended in PBS and stored at -80°C. EVs were assessed using Nanoparticle Tracing Assay to determine their size and concentration. The morphological quality is assessed with transmission electron microscopy. The protein content is assessed by means of mass spectroscopy.

Results and Discussion: It is observed that low-intensity electrical stimulation (25 mV/mm) induces neural differentiation in neuroblastoma cells, even in the absence of BDNF, and this effect is comparable to the gold standard chemical stimulation using BDNF. Figure 1 A shows expression of Tau-5 in all ES and BDNF stimulated groups. Morphological evaluation of neurite out growth and complexity of the network reviled that there is a significant difference between cell treated with ES protocols and BDNF. Our comprehensive assessment (results are not shown) showed that although BDNF treatment might benefit the number of the primary neurite, electrical stimulation, significantly increases the number of branching, nodes and secondary and tertiary neurite. There are meaningful morphological differences between the cells in the stimulation groups corresponding to the temporal component of the stimulation protocol. As the duty cycle increases, the complexity of the neurite network also grows.
Furthermore, our observations revealed that the secretion of EVs is influenced by the frequency and duty cycle of the stimulation regimen. In summary, the particle count of EVs generated through electrical stimulation conditioning (ES-EVs) significantly exceeded that of the control group (No ES). Importantly, ES-EVs exhibited morphological

similarities to natural EVs, suggesting that electrical stimulation does not induce any abnormal effects on EVs. The proteomic assessment of EVs (results are not shown) indicated that all EVs isolated in this study express more than 90% of EVs signature proteins indexed in Exocarta. These results confirm that EVs from electrically stimulated groups contain large amount of pure exosomal fraction and do not express abnormalities. More importantly, we identified the significant abundance of 30 unique proteins that are only expressed in the ES-EVs: proteins in involved in cellular transport and membrane dynamics (RAB1B, TSPAN14, ATP2B4, RAP1A, TMED9); proteins involved in cytoskeletal regulation (TPM4, DYNLL2, RP2); proteins involved in RNA processing and transport: (STAU1, SNRPD3, PRPF19, ILF2, HNRNPH3); proteins associated with extracellular matrix and cell adhesion (ALCAM, HAPLN3, PCOLCE, SCPEP1); proteins with enzymatic functions (TPT1, PTP4A2, HACL, GBA3); proteins associated with neuronal function (DPYSL3, SUPT16H); proteins involved in signaling and cell regulation (IGFBP5, SSB, TPP1); Proteins with roles in metal ion transport (SLC39A14, ALAD). There are also significant differences in the abundance of some of these proteins between the ES conditions that are under analysis.

Conclusion: Pulsed electrical stimulation at 25 mV/mm emerges as a potent physical approach for inducing neural differentiation in neuroblastoma cells, even in the absence of the critical growth factor BDNF. Our study demonstrates that the effectiveness of this method is dose-dependent, with the frequency and duty cycle significantly influencing the morphological expression of neurite outgrowth. Moreover, these parameters also exhibit a meaningful impact on the production yield of EVs and their protein cargo. In summary, our findings underscore the viability of fine-tuning of cell behavior and cell secretion through the regulation of electrical stimulation parameters.

Acknowledgments

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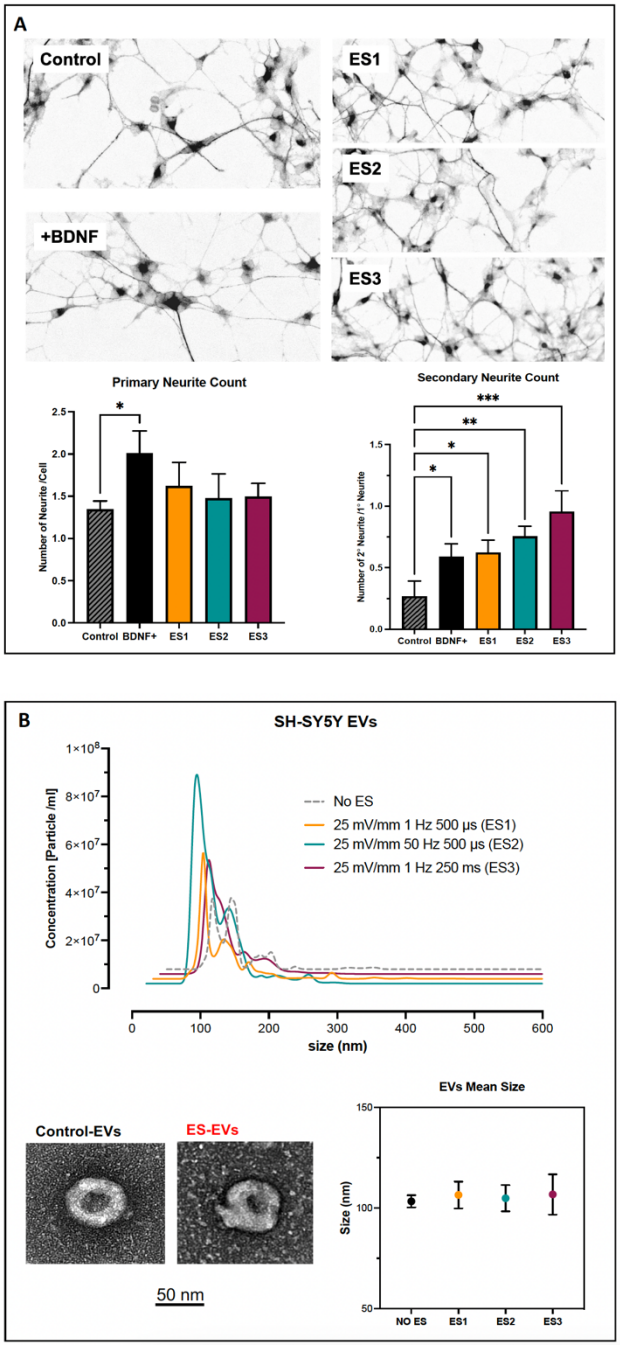

Figure 1. A) Influence of electrical stimulation on neural differentiation in SH-SY5Y cells. Immunofluorescent images depict the expression of Tau-5 (an axonal marker) in Control, BDNF+, ES1, ES2, and ES3 conditions. Quantification of primary and secondary neurites is based on three independent experiments (n=3). B) Impact of electrical stimulation on the secretion of extracellular vesicles from SH-SY5Y cells. Relative changes are illustrated through NTA analysis (top and bottom right) and TEM images (bottom left).

Drug-loaded Urease-powered nanomotors for the potential treatment of bladder cancer

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In recent years, enormous research efforts have been made to minimize the side effects of drugs and to increase their therapeutic efficiency in the treatment of cancer. Bladder cancer, for example, is the ninth most common cancer worldwide for which current therapies prolong patient survival, but also show high relapse rates, making it urgent to improve existing therapies. Using the catalytic reaction of enzymes that consume bioavailable fuels to propel micro- and nanoparticles (nanomotors) has expanded their potential applicability in nanomedicine and might provide a platform to overcome drug delivery challenges. Here, we present urease-powered nanomotors based on mesoporous silica nanoparticles (MSNP) loaded with clinically relevant drugs (Mitomycin, Erdafitinib) for the potential treatment of bladder cancer. The procedure of MSNP synthesis to obtain homogeneous particle size distributions and ensure proper pore opening for subsequent drug loading has been optimized. Furthermore, swarming behaviour in ionic and proteinaceous media has been tested in presence of different concentrations of urea (0 mM up to 300 mM). In addition, spectral flow cytometry as a novel tool to analyse particle delivery efficiency has been carried out with mouse bladder carcinoma cells (MB-49) after incubation (1 h, 4 h) with active (MSNP-Urease) and passive (MSNP-BSA) FITC-labelled nanoparticles at different urea concentrations. When the nanoparticles were incubated with MB-49 cells, active nanomotors showed a 3.2-fold increase of the delivery efficiency in presence of 100 mM urea compared to passive particles after only 1 h of incubation. The drug loading results, biocompatibility tests and enhanced delivery efficiency of active nanomotors to MB-49 cells may proof their potential to be used in future nanomedical applications for the treatment of bladder cancer.

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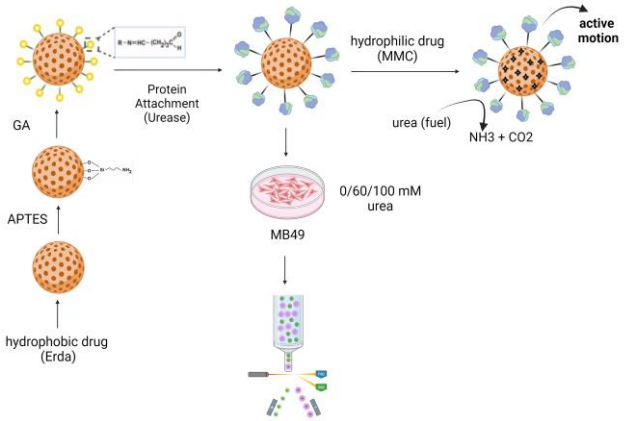

Figure 1. Graphical abstract of drug-loading strategy of Urease-nanomotors. Cell internalization experiments were carried out with non-loaded nanomotors using spectral flow cytometry to determine the delivery efficiency to MB49 cells in presence and absence of fuel.

Magnetic navigation of swarms of enzyme-powered nanomotors with photothermal properties for immunogenic cell death

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Immunogenic cell death (ICD) is a process where damage-associated molecular patterns (DAMPs), such as ATP and calreticulin (CLRT), are released or exposed at the cell's surface. ICD has emerged as a promising strategy for enhancing the efficacy of cancer immunotherapy. Recent studies have demonstrated that ICD can be induced by means of light-triggered effects without the need for chemotherapeutics with potential side effects.1 Light-responsive nanomaterials could improve ICD induction further if they could collectively displace and penetrate more efficiently into tumors. Advanced nanomaterials able to convert chemical energy into motion or nanomotors (NMs) are being actively explored due to their ability to overcome different biological barriers and their capability to collectively displace in the form of swarms.2 Among these, urease-powered nanomotors have gained significant attention due to their biocompatibility, biodegradability, and the possibility of using urea as fuel at physiological concentrations to power their motion.3 In addition, the use of iron oxide as chassis of these motors allows to combine the motion capabilities with photothermal and magnetic properties to offer an additional advantage for therapies.4

In this work we investigated the magnetic navigation capabilities of swarms of urease-powered iron oxide nanomotors (IONMs), guided by external magnetic fields, for enhanced and selective displacement and accumulation in desired regions of 3D-printed phantom models. In addition to their navigational abilities, we evaluated the photothermal properties of the iron oxide nanoparticles for induction of vapor nanobubbles (VNBs) formation upon irradiation with a pulsed laser to induce selective cell killing. As light-triggered cell death by means of VNBs holds the potential for generating DAMPs crucial to activating anti-tumor immune responses, we proceed to characterize the release of ATP and CLRT exposure in treated samples.
The combination of magnetic navigation and photothermal properties of IONMs proved to have clear potential for the selective displacement and accumulation of NMs and to induce the release and exposure of ICD hallmarks upon irradiation.

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Confocal Imaging of Label-Free Nanoparticles in Cells and Biological Tissues

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Noble metal nanoparticles (NPs), particularly gold (Au) and silver (Ag) NPs, have recognized relevance in chemistry, physics, and biology because of their outstanding optical, electrical, and photothermal properties. Optical properties, as localized surface plasmon resonance, are easily measurable signatures indicative of their morphology (size and shape), composition, surface chemistry, aggregation state and physical environment that can be used to identify molecular targets and chemical transformation processes. [1] Due to the growing interest in the use of metal NPs in medicine and biology, detailed cellular studies are required before their application in vivo for treatment or diagnostic purposes. [2] Herein, we present the observation of unlabelled Au NPs on Confocal Laser Scanning Microscopy (CLSM), by using the light reflectance instead of the commonly used fluorescence mode. The NPs size resolution limits for CLSM observation is studied experimentally. Theoretical calculations, of the size-dependent optical properties are also presented to support this argument. The Au NPs used were synthesized using the seeded growth citrate reduction method [3], and the NPs sizes range from 15nm to 150nm. Full characterization of the produced NPs was also performed by Transmission Electron Microscopy (TEM), UV-Vis spectroscopy and Dynamic Light Scattering (DLS). Further, the intracellular observation of different sizes of Au NPs using the reflectance mode is also presented in cultured cells and tissue sections. This work reveals as a method to observe NPs in living systems in real-time and non-invasive way, which can be extended to other inorganic

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Nanostructured Electrodes for In Vitro Electrical Stimulation Platforms

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Introduction: The significant burden associated with neural disorders requires urgent measures to expedite research and the development of new therapeutics. Electrical stimulation is a promising but still relatively underexplored treatment[1]. We believe that There is a lack of suitable tools for conducting high-throughput studies of neural electrical stimulation, encompassing cell anatomy and electrophysiological aspects[2]. Moreover, it remains unclear which combination of parameters (stimulation regimen) is effective in various applications. This lack of clarity arises from an incomplete understanding of the biophysical mechanisms governing the transfer of electrical signals to the biological environment.

To address these challenges, we have developed a testing platform that combines nanostructured platinum electrodes with a microgrooved-compartmentalized cell culture module. Additionally, we have fabricated and characterized the electrochemical properties of the nanostructured pt electrodes[3]. We studied the influence of nanostructuration on the electrical double layer that dictates the signal attenuation, and charge injection. Finally, we measured the influence of implementing nano-roughness on charge injection capacity of Pt electrodes.

Methodology: Nanocolumnar Pt electrodes (NC) obtained by electron beam evaporation of Pt in glancing angle (tilted) configuration on custom cut glass substrates. The growth rate will be fixed at 0.8 Å/s and the tilt angle at 80°. The morphology of nanostructures will be assessed by means of scanning electron microscopy, Figure 1 (top, left). Electrical impedance spectroscopy (EIS) measured using Metrohm Autolab Potentiostat AUT204.FRA32M, using a dummy set up. We used culture medium (DMEM w/o phenol red w/o serum w/o pyruvate) as electrolytes. A miniature Ag/AgCl electrode were used as the reference electrode in 3-electrode measurements. 10 mV was used as applied voltage, in a frequency range of 1 to 10^5 Hz, and Nyquist and Bode plots were obtained to extract impedance data. Using computational simulation, the corresponding equivalent circuit, was extracted to be used for simulation and calculation of signal attenuation. Cyclic voltammetry (CV) was done using the same setup, applying a voltage range of -0.5 to +0.9 V at a scan rate of 100 mV/s. The current/voltage plots were generated for each sample and charge injection capacity calculated using the area under the cathodic peak associated with the reduction reaction during the cathodic sweep.

The microgrooved-compartmentalized cell culture module was fabricated using standard UV and soft lithography techniques, Figure 2. Initially, metallic masks were prepared. A two-step photolithography process was employed to create a master mold in silicon (Si) for defining microgrooves measuring 500 µm in length, 10 µm in width (step 1) as well as compartments (step 2). In the first step, a 2-inch Si wafer was spin-coated with SU-8 resist in the appropriate formulation to achieve a layer thickness of approximately 15 µm for the grooves. After a soft bake following the manufacturer's instructions, the resist was exposed to UV light using the mask. Subsequent development and hard baking revealed the channel array mold on the Si wafer. Subsequently, a second layer of SU-8 resist with different properties was applied to achieve a thickness of around 100 µm, which was suitable for defining the pads, through the same UV lithography protocol. Those pads used as a guide to place silicone cell culture inserts. The final chamber was created from polydimethylsiloxane (PDMS, Sylgard 184 Dow Corning) by casting and curing the elastomer around silicone cell culture inserts.

Results and Discussions: We demonstrated that Z is significantly smaller at lower frequencies for NC electrodes compared to the Pt thin films (TF), Figure 1 (top, right). The NC effective capacitance (~ 536 µF) is also larger than that of TF (~ 5.5 µF) due to its increased electrochemically active area. We developed a model based on equivalent circuit obtained from the EIS and tested several stimulation protocols, compared the delivered signal distortion in NC and TF. Figure1 (bottom right), demonstrates an example of such simulation; an anodic pulse of 1 ms and 800 mV amplitude applied and the corresponding current delivered by NC and TF was measured. The delivered current signal was significantly attenuated when TF is used due to small double lyre capacitance, compared to NC. Charge storage capacity of Pt increased at least 3 time due to nanostructuration, Figure 1 (bottom, left).

Conclusions: We have developed a platform that combines nanostructured Pt electrodes with a microgrooved-compartmentalized cell culture module, for electrical stimulation of neural cells. We demonstrated that nanostructuration significantly improves electrochemical properties of the bioelectrodes, including charge storage capacity. Moreover, nanostructuration has positive effect on conserving the delivered signal. We also, established a methodology for simulating stimualtion conditions before experimental work. Future work will be focused on culturing neural cells in this platform, to expand their axonal compartment within the grooves, and testing the effect of electrical stimulation on growth and survival of the axons.

Acknowledgments

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Figures

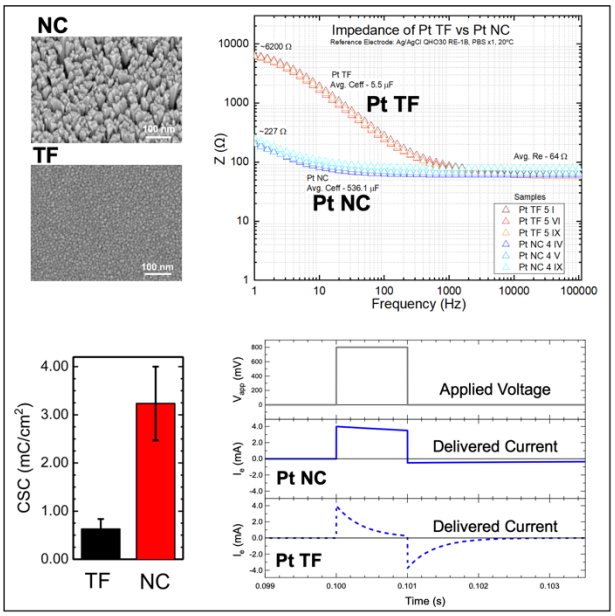

Figure 1. Electrodes and electrochemical characterizations. Top, left: Scanning electron microscopy images of nanocolumnar (NC) and thin film (TF) Pt electrodes. Top, right: Bode plot of Pt TF and Pt NC demonstrating the electrical impedance. Bottom, left: charge injection capacity. Bottom right: Simulation the delivered current by NC and TF in result of a 1 ms pulse at 800 mV applied voltage.

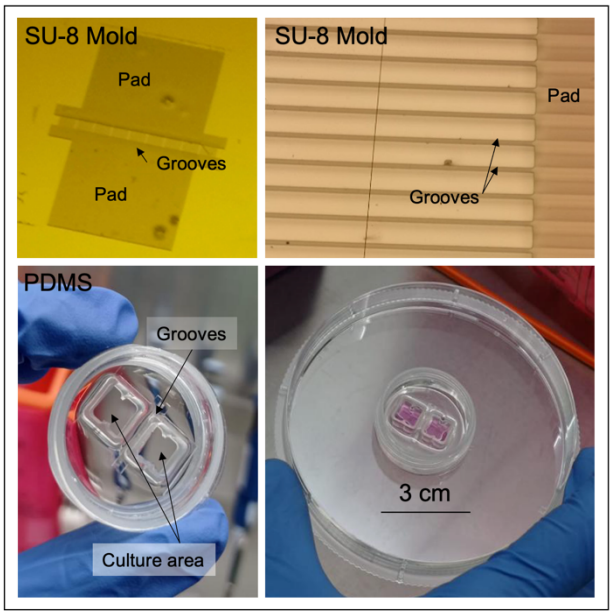

Figure 2. The microgrooved-compartmentalized cell culture module. Top, SU 8 mold. Bottom, PDMS final platform contain culture area and microgrooves.

Development of stratified porous scaffolds based on polyesters as supports for indirect cell co-culture

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One of the main problems in the field of biomaterials and drug development is the use of animals in experimentation, which is an ethical issue that concerns all the society.[1] In this sense, tissue engineering is working to find alternatives. One of these alternatives is the development of *in vitro* models based on polymer supports for *in vitro* cell growth. These structures, moreover, could be used not only in drug testing or *in vitro* research to reduce the use of animals in experimentations, but also for tissue regeneration, simulating from simple tissues in which there is a single cell type, to more complex tissues from cell co-culture.[2]

Thus, a three-dimensional system with a stratified porous structure have been developed to allow both indirect cell co-culture and drug release assays (**Figure 1**). For this purpose, porous supports (scaffolds) have been obtained by means of the solvent-casting particle-leaching technique using salt as porogen, whose pore size allows cells to be housed in its interior, on which electrospun membranes have been arranged, forming a sandwich structure. These membranes form a structure of cross-linked fibers, leaving spaces between them that are much smaller than the cell size, so that they allow the passage of nutrients and molecules through them, but act as a barrier to the cells, preventing their migration to other areas of the three-dimensional system.[3]

Different polyesters were used to adjust the hydrophilicity, biodegradability, drug release kinetics and biological behavior, as desired. Moreover, fiber diameter was stablished at 1.8 µm and membranes with and without curcumin as model drug were obtained by modifying the electrospinning parameters.[4] Curcumin was introduced by two methods: blend and coaxial electrospinning, so different delivery profiles were observed due to in blend electrospinning curcumin is placed in the whole fiber, while in coaxial electrospinning forms the core.[5] For that reason, in coaxial electrospinning the release depends strongly on the degradation, that is higher for polylactic acid and poly(lactic-co-glycolic) than poly(ε-caprolactone).

Because of the thickness limit of electrospinning, the 3D structure was obtained by using a sacrifice membrane. [6]

Finally, its use in indirect cell co-culture was tested by seeding fibroblast L929 in the scaffold, showing that scaffold allow cells to proliferate inside them, preventing an outside migration, so that indirect cell co-culture can be achieved by means of these structures.

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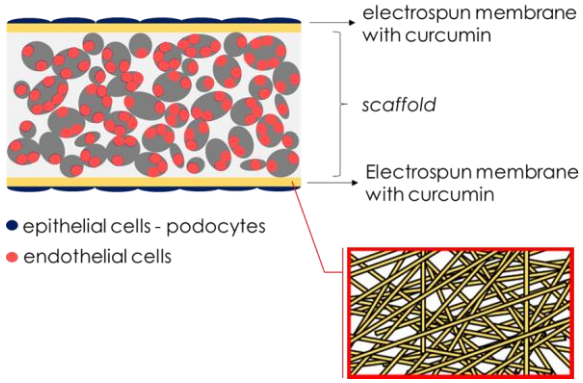

Figure 1. Scheme of the three-dimensional structure

Correlating the physicochemical properties of nanocapsules with their targeting efficiency

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In this work, I am synthesizing nanoparticles of different sizes and two compositions, PS, PAH which in turn affects their colloidal stability. I prepared conjugates of different sizes (508nm, 98nm, 20nm) of nanoparticles with varying densities of antibodies and do characterization of conjugates by using Fluoresence microscopy, UV-Vis, fluorimetry and DLS. Now I will take advantage of previous results of the lab showing that ZIP4 is a valid target to deliver nanocarriers and I will modify the synthesized nanoparticles with anti-ZIP4. The group has synthesized 2 types of monoclonal antibodies thus enabling be to test which one shows a better targeting efficiency. Furthermore, I will also change the density of the antibodies and check the targeting efficiency. With this work I expect to correlate two systems, (i) the physicochemical parameters of nanoparticles (size, composition, colloidal stability) with (ii) antibodies parameters (specificity, density) to define which is the best geometry to enhance ZIP4 targeting. As a measurable outcome, I will measure the targeting efficiency of the synthesized systems and I will validate the best performing geometry *in vitro* and *in vivo* using previous models developed in the lab.

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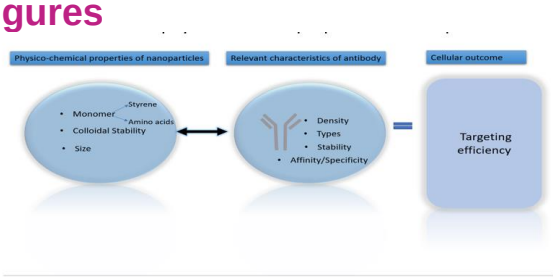

Figure 1. correlation of the physicochemical properties of nanoparticles to their targeting efficiency

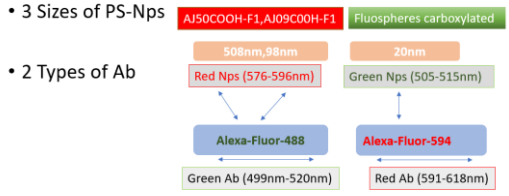

Figure 2. Synthesizing nanocapsules of different sizes of PS with varying densities of antibody

Antimicrobial Photodynamic Therapy Using Encapsulated Protoporphyrin IX for the Treatment of Bacterial Pathogens

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In Antimicrobial Photodynamic Therapy (APDT), photosensitizers induce light-driven local photochemical reactions that generate reactive oxygen species (ROS) responsible for pathogen inactivation [1]. APDT offers precise spatio-temporal control and clinical utility against a wide range of microbial infections. However, challenges include limited light tissue penetration and potential photosensitizer photobleaching [2] [3].To address these issues, photosensitizer encapsulation within nanoparticles enhances antimicrobial efficacy. In this study, we investigate the photodynamic antimicrobial efficacy of protoporphyrin IX, a clinically approved photosensitizer, both in its free form and loaded into PLGA nanoparticles, against Staphylococcus aureus. We also assess the reduction in its cytotoxicity when encapsulated compared to equivalent doses of its free form in mammalian cell cultures.

For the selection of the photosensitizer, DHR123 probe quantified ROS production upon photodynamic activation of ICG (indocyanine green) and PpIX (Protoporphyrin IX). For ICG ROS analysis, samples were measured before and after 3-minute irradiation with an 808 nm diode laser (1 or 0,5 W/cm²). For PpIX ROS evaluation, PpIX in DMSO mixed with MilliQ water was irradiated with a 532 nm diode laser at 0,5 W/cm². Control measurements ensured ROS generation only when irradiating light, and control experiments ruled out temperature-induced ROS. Photosensitizer photobleaching was examined by recording UV-Vis spectra before and after 5 minutes of irradiation (808 nm for ICG, 532 nm for PpIX) at 0,5 W/cm² irradiance. PpIX-NPs were synthesized using the emulsification-solvent evaporation method, with protoporphyrin IX in the core. Transmission electron microscopy (TEM) assessed particle morphology and size. Nanoparticle tracking analysis (NTA) determined NPs size and distribution and zeta potential of the nanoparticles was measured by Dynamic Light Scattering (DLS). UV-Vis spectrophotometry measured drug loading by quantifying the encapsulated photosensitizer in the NPs. Release from PpIX-NPs was evaluated in PBS with 2% Tween® 20 (v/v) and quantified by UV-Vis spectrophotometry. Free PpIX antibacterial activity against S. aureus was tested at concentrations (0,5-10 ppm) in 2% DMSO. Positive controls included untreated S. aureus and 2% DMSO. After a 1-hour incubation, samples were exposed to a 532 nm laser (0,5 W/cm2, 5 minutes). Viable bacteria were quantified via serial dilution. To assess the antimicrobial efficacy of PpIX-NPs, the previous doses of the free photosensitizer were tested considering the drug loading (0,5-10 ppm). Positive controls included untreated bacteria and samples with empty (PpIX-free) nanoparticles. NPs cytotoxicity in fibroblasts was determined using the Blue Cell Viability Assay after 24 hours of incubation with different concentrations (0,5 - 2 ppm) of PpIX released from PpIX-NPs or the free drug at the same concentrations.

ICG and PpIX were assessed for antimicrobial photodynamic therapy based on ROS production and photobleaching. PpIX displayed superior ROS production, and photostability, making it the preferred choice for further antimicrobial studies. Then, PpIX was encapsulated obtaining PpIX-NPs. They had a spherical morphology, with a slightly larger size (33,6 ± 9 nm) than empty NPs. Empty and PpIX loaded NPs showed electrokinetic potentials of -11,9 ± 0.6 and -12,2 ± 1 mV, respectively at neutral pH. PpIX release reached 54 wt.% in 1 hour for a potential rapid application. Encapsulation efficiency was 13,7 ± 1,7 wt.%, and PpIX loading was 0,14 ± 0,09 wt.%.

Protoporphyrin IX-loaded PLGA nanoparticles demonstrate high aqueous solubility, photostability, and retained antimicrobial activity upon light irradiation when compared to equivalent doses of the free photosensitizer.

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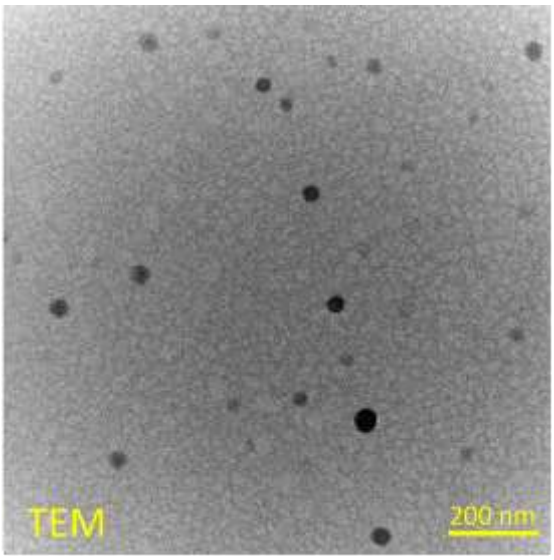

Figure 1. TEM image of PpIX-NPs.

Impedance cytometry as a tool for prognostic analysis in prostate cancer treated with radiotherapy

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Introduction:

Metastatic prostate cancer (PCa) is challenging to treat due to a limited understanding of the mechanisms driving metastasis development and the absence of reliable prognostic markers [1]. Liquid biopsy analysis is a clinically approved minimal blood test based on the enumeration of circulating tumor cells (CTC) and used to assess early patient prognosis and response to the treatment, including radiotherapy [2]. Due to the high CTC heterogeneity and plasticity, the currently approved tests need further improvement in terms of accuracy and reliability [3]. Recent studies suggest that the electro-physical properties of cancer cells, such as conductivity (σ) and permittivity (ε), may be utilized as potential prognostic markers [4-6]. Despite the high clinical demand, a method for distinguishing primary tumor and metastatic PCa cells based on their electrical properties has yet to be reported.
In this study, we will employ nano- and microfabrication techniques to develop an impedance cytometry approach in conjunction with various biological models to train our system. Thanks to the collaboration between biological and engineering research groups, we will have the opportunity to test advanced biological models, including cell culture (*in vitro*), synergetic mouse models, and patient samples. The increased complexity of these models has the potential to significantly improve the training process of machine learning data analysis.

Materials and methods:

To create the electronic sensing structures, we are utilizing both electron beam lithography (EBL) and ultraviolet (UV) lithography. The interdigitated sensing structures are formed through metal deposition. Additionally, we employ soft lithography to imprint cytometrical microchannels into polydimethylsiloxane (PDMS).

Results:

We are currently developing a detection system that will be used to assess the electro-physical properties of primary tumors, metastases, and CTCs.

Outlook:

In the future, this impedance cytometry system may be used to develop reliable prognostic tests based on the detection and enumeration of CTCs with metastatic properties in patients' samples. Additionally, analyzing circulating immune cell populations during radiotherapy may help characterize the immune status of patients and predict tumor immune evasion.

Conclusions:

The development of a label-free, sensitive, and reliable non-invasive diagnostic test based on the electric properties of tumor cells is expected to improve the sensitivity and reproducibility of traditional liquid biopsy-based diagnostic approaches and make them more time and labor-efficient.

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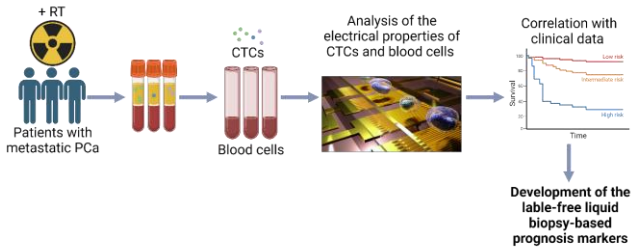

Figure 1. For prostate cancer (PCa) patients undergoing radiotherapy (RT), we are developing a non-invasive prognostic method that combines Liquid Biopsy and electrical impedance cytometry. This approach involves enumerating circulating tumor cells (CTCs) from blood samples and scrutinizing their electrical properties using an impedance cytometer. Our journey toward establishing a dependable platform for prognostic marker analysis encompasses preclinical development, from *in vitro* to *in vivo* experiments to examining patient-derived samples.

p-4-Vinyl Pyridine-co-Cannabidiol Polymer: application in therapy

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Abstract
Lung cancer is the leading cause of cancer death worldwide. Despite numerous therapeutic advances based on chemotherapy, surgery, radiotherapy and immunotherapy, they are not enough to improve the prognosis in advanced stages of the disease, being necessary new therapeutic strategies [1]. In this context, the use of nanoparticles for drug transport can reduce some limitations of the treatments, such as reduced bioavailability, drug toxicity in healthy cells, and the possibility of natural elimination of the drug by the human body. In this study we have evaluated the activity of cannabidiol (CBD), one of the main phytocannabinoids of Cannabis sativa L, loaded in a nanoformulation [2,3,4,5]. For this purpose, we have used 4-p-vinylpyridine (4PV) nanoparticles and co-polymeric nanoparticles with CBD covalently incorporated on the surface (solid@p4VP-co-CBD). The in vitro cell model used in the study was the A549 non-small cell lung cancer (NSCLC) cell line, in which was carried out antiproliferative, cell colony and wound-healing assays. The results didn't show toxicity of the new nanoformulation, being safe for therapeutic use. Moreover, the cytotoxicity and cell viability reduction of the drug was preserved in the new nanoparticle, but in a reduced form compared to its free form. Even so, the ability to inhibit cell migration in the encapsulated drug was completely nullified. In conclusion, this nanoparticle was able to maintain some of the antitumor properties of CBD, allowing its use in the development of a targeted therapy that can benefit from the advantages of nanoencapsulation.

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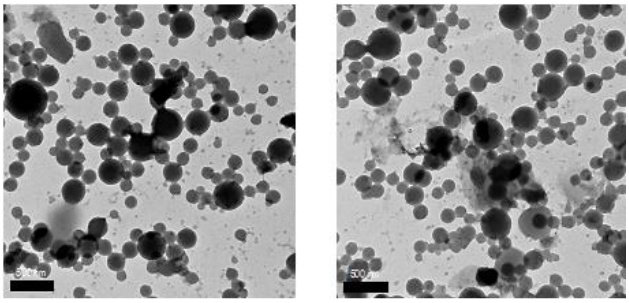

Figure 1. TEM images of solid@p4VP-co-CBD

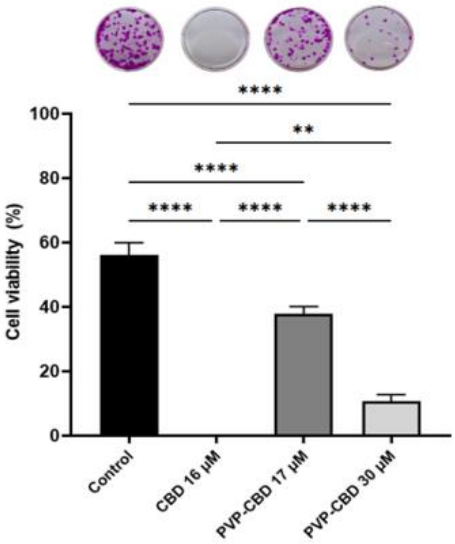

Figure 2. Percentage cell viability after 72 h incubation with CBD (IC50) and solid@p4VP-co-CBD (IC50 and 30 µM).

Gastrointestinal cancer: hyperthermia treatment

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Abstract
In recent years, unhealthy habits such as smoking, lack of physical activity and an unbalanced diet have contributed to the increase in the incidence and mortality of several types of gastrointestinal cancer, with colorectal cancer being the third in incidence and the second in mortality, followed by stomach and esophageal cancer [1]. Furthermore, current cancer treatments have many associated problems, so there is a need to look for new therapeutic options that can improve the quality of life of patients. Nanomedicine has emerged as a promising alternative to improve both the diagnosis and treatment of cancer. Nanoformulations can deliver drugs directly to the tumor region, improving the precision of treatment, increasing the amount of drug at the target site, and reducing side effects. Among these nanoformulations, magnetic nanoparticles [2,3] have unique physicochemical properties that allow them to be used in the diagnosis and treatment of cancer. They can be directed to the tumor region by applying a magnetic field and are used as contrast agents in various imaging techniques. Furthermore, magnetic nanoparticles have the ability to generate high temperatures in the tumor region after being exposed to an alternating external magnetic field. This characteristic is used to employ combined chemotherapy and hyperthermia treatments, which increases the antiproliferative activity of the treatment and improves effectiveness both in vitro and in vivo. Therefore, in the present work the most recent results obtained through the application of hyperthermia in the treatment of gastrointestinal cancers have been analyzed, highlighting the advantages of this emerging therapy.

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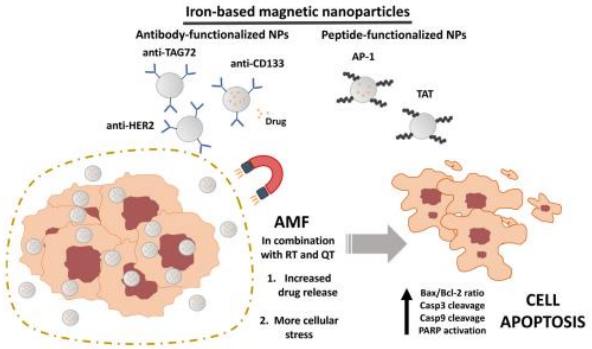

Figure 1. Use of magnetic nanoparticles in in vitro AMF hyperthermia application experiment.

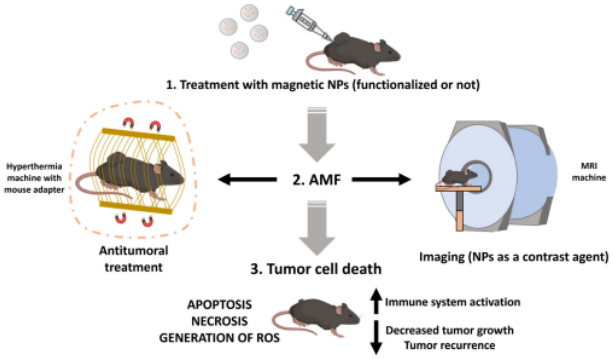

Figure 2. Use of magnetic hypothermia in in vivo experiments.

Antimicrobial Applications of Green Synthesized Bimetallic Nanoparticles from *Ocimum basilicum*

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Abstract

Antibiotic resistance is an important and emerging alarm for public health that requires the development of new potential antibacterial strategies. In recent years, nanoscale materials have emerged as an alternative way to fight pathogens. Many researchers have shown great interest in nanoparticles (NPs) using noble metals, such as silver, gold, and platinum, even though numerous nanomaterials have shown toxicity. To overcome the problem of toxicity, nanotechnology merged with green chemistry to synthesize nature-friendly nanoparticles from plants. Here, we describe the synthesis of NPs using silver (AgNPs) and platinum (PtNPs) alone or in combination (AgPtNPs) in the presence of *Ocimum basilicum* (*O. basilicum*) leaf extract. *O. basilicum* is a well-known medicinal plant with antibacterial compounds. A preliminary chemical–physical characterization of the extract was conducted. The size, shape, and elemental analysis were carried out using UV–visible spectroscopy, dynamic light scattering (DLS), and zeta potential. Transmission electron microscopy (TEM) confirmed polydisperse NPs with spherical shape. The size of the particles was approximately 59 nm, confirmed by DLS analysis, and the polydisperse index was 0.159. Fourier transform infrared (FTIR) demonstrated an effective and selective capping of the phytoconstituents on the NPs. The cytotoxic activities of AgNPs, PtNPs, and AgPtNPs were assessed on different epithelial cell models using the 3-[4.5-dimethylthiazol-2-yl]-2.5-diphenyltetrazolium bromide (MTT) cell proliferation assay. They discovered low toxicity, with a cell viability of 80%. The antibacterial potential of the NPs was evaluated against *Escherichia coli* (*E. coli*), *Enterococcus faecalis* (*E. faecalis*), *Klebsiella pneumonia* (*K. pneumoniae*), and *Staphylococcus aureus* (*S. aureus*) strains. Minimum inhibitory concentration (MIC) assays showed AgPtNP activity till the least concentration of NPs (3.15–1.56 µg/mL) against ATCC, MS, and MDR *E. coli, E. faecalis,* and *S. aureus* and the Kirby–Bauer method showed that AgPtNPs gave a zone of inhibition for Gram-positive and Gram-negative bacteria in a range of 9–25 mm. In addition, we obtained AgPtNP synergistic activity in combination with vancomycin or ampicillin antibiotics. Taken together, these results indicate that bimetallic nanoparticles, synthesized from *O. basilicum* leaf extract, could represent a natural, eco-friendly, cheap, and safe method to produce alternative antibacterial strategies with low cytotoxicity.

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Figures

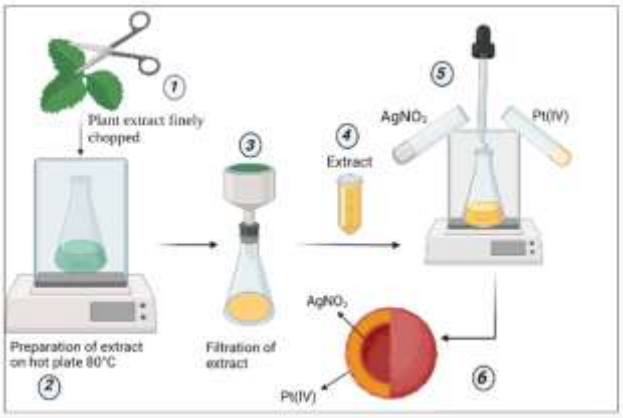

Figure 1. Schematic representation of synthesis of AgPtNPs from *O. basilicum*. (1) Fresh leaves of *O. basilicum* were chopped finely (2) Then, the finely chopped leaves were used for the preparation of the exact on a hot plate at 80 °C (3) The prepared extract was then filtered through a Whatman filter paper No.1 (4) This extract was used for the preparation of the bimetallic NPs (5) At 80 °C, and under continuous shaking conditions in the presence of two noble metals, namely Silver nitrate and Potassium tetrachloroplatinate (II), synthesis of the NPs was carried out (6). The hypothesis was that the NPs formed would possess an inner core of silver and an outer cover of platinum.

Synthesis, characterization and evaluation of hyaluronic acid-functionalized biomimetic-magnetoliposomes as drug delivery systems

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Nanotechnology has become very attractive for its applications in several fields, comprising biology, medicine, especially oncology offering new therapeutic approaches that could overcome the limits of the current conventional treatments.
In this context, the concept of drug delivery systems (DS), which exploit the peculiar properties of tumor cells or their milieu, is highly attractive, as it recapitulates the advantages of topical treatments.
Nanoparticles (NPs) have become very attractive, as they represent the realization of the old “magic bullet” concept. This being to selectively carry a therapeutic molecule to a specific target (tumor cells), while sparing the healthy cells, reducing the systemic exposure and adverse effects.
Besides magnetic nanoparticles (MNPs), the most used are the ones composed of iron oxide and they can be obtained by synthetic chemistry, but they are also produced in nature as magnetosomes by magnetotactic bacteria. Unfortunately, culturing these bacteria is cumbersome and difficult to be scaled up so, a possible solution is to synthetize inorganically the MNPs with magnetosome membrane associated proteins (MAPs) which play a crucial role in the mineralization process of the nanocrystals[1]. One of these MAPs is MamC protein that directs the *in vitro* nucleation and growth of MNPs, eliminating the requirement of bacterial cultures and improving the production of magnetosome-like biomimetic magnetic NPs (BMNPs) with high yields[2].
Recently, BMNPs synthetized in presence of MamC protein from *Magnetococcus marinus MC-1* and covered by a lipid layer of 1,2-distearoyl-sn-glycero-3-phosphocholine [LP(BMNPs)] have been used as DS and become particularly interesting[3]. Indeed, MamC protein also confers a negative charge to BMNPs that can be functionalized by electrostatic interactions with chemotherapeutic drug as doxorubicin (DOXO)[4]. Moreover, exploiting their inner properties, BMNPs can be manipulated by an external gradient magnetic field (GMF), besides being multifunctional highly biocompatible platforms[5].
Among targeting agent such as monoclonal antibodies directed against tumor associated markers, hyaluronic acid (HA) is an anionic glycosaminoglycan that has gained special attention since it interacts with CD44 receptor that is overexpressed on a variety of solid cancers (colon, ovarian, breast, lung, and pancreatic)[6] and its aberrant expression and dysregulation contributes to tumor initiation and progression. So, the pleiotropic roles of CD44 in carcinoma potentially offer new molecular target for therapeutic intervention[7]. In this context, 14800 Da HA has been linked to an aminated phospholipid (1,2-dipalmitoyl-sn-glycero-3-phosphoethanolamine, DPPE) by reductive amination[8] and used in the synthesis of HA-LP(BMNPs) nanoformulations.
Herein, BMNPs were synthetized, functionalized with DOXO, and then encapsulated in +/-HA conjugated liposomes obtaining +/-HA-LP[(+/-DOXO)-BMNPs].
The obtained nanocomplexes were extensively characterized by transmission electron microscopy analysis (TEM), hydrodynamic radius, ζ-potential, Fourier-transform infrared (FT-IR), and colloidal stability. The crystals inside the nanoformulations showed a rhombic shape, with a size lower than 200 nm, a negative ζ-potential value and their effective functionalization with the different moieties was confirmed. *In vitro* biological tests were performed in red blood cells (hemolysis test) and with several cell lines of human breast cancer (MDA-MB-231 and MCF7) and human ovarian cancer cell line (A2780). Biocompatibility was analyzed by ROS production and MTT assay and +/-HA-LP(BMNPs) were not found to have cell toxicity.
The targeting ability of HA-LP(BMNPs) was evaluated on three cancer cell lines with high (MDA-MB-231), middle (MCF7) and low (A2780) CD44 expression and compared to that of LP(BMNPs). To this aim, the cellular interactions of the nanocomplexes were evaluated by iron quantification and Prussian Blue staining. The results showed that the uptake of HA-LP(BMNPs) was significantly higher in MDA-MB-231 cells, suggesting that the entry of HA-conjugated formulations is likely receptor-mediated.
Moreover, when LP(DOXO-BMNPs) were incubated with different cell lines, the nanocomplexes exerted cytotoxic activity which increased when HA was conjugated to the nanoformulations.
These promising results show the suitability of the HA-LP(DOXO-BMNPs) as magnetic nanocarriers for local targeted chemotherapy and for future agents for hyperthermia and photothermia paving the way for the development of powerful new approaches for cancer therapy suggesting a tumor multiple attack by combined strategies.

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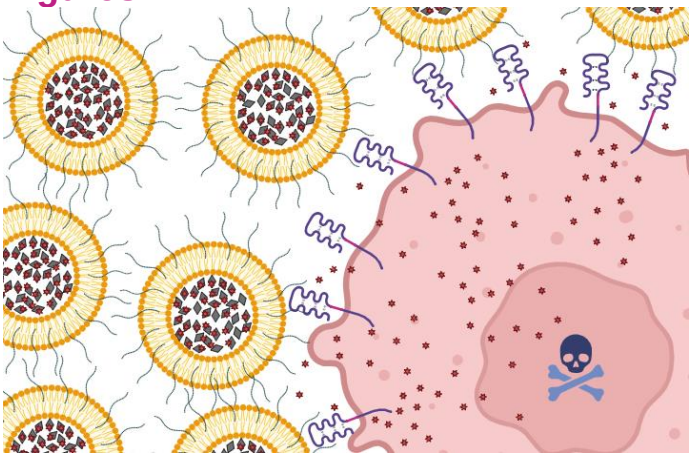

Figure 1. Schematic model of interactions of the nanocomplexes with cells: HA-LP(DOXO-BMNPs) nanoformulations interact with cancer cells overexpressing CD44 receptor where DOXO is delivered acting as cytotoxic agent.

Enhancing Therapeutic Efficacy in Osteoarthritis Through the Formulation of Resveratrol and Phloretin Loaded Poly (Lactic-Co-Glycolic Acid) (PLGA) Nanoparticles

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Osteoarthritis (OA) is a chronic joint disease characterized by cartilage degradation, alterations in bone formation or subchondral bone remodeling and progressive synovial inflammation [1]. Natural products are among the frequently utilized pharmaceutical components intended to target and treat a number of emerging and developing disorders. These compounds exhibit diverse pharmacological properties, including anticancer, anti-inflammatory, antibacterial, anti-oxidative, immunosuppressive activity, among others. Nevertheless, their use is limited by their poor water solubility, low bioavailability, low stability, and rapid metabolism [2]. Nanotechnological approaches and specifically nanomaterials as drug delivery vectors can solve some of major drawbacks of existing pharmacological strategies [1].Different nanocarriers such as polylactic co-glycolic acid (PLGA) nanoparticles (NPs) have been developed as effective methods for sustained drug release in OA treatment due to their stability and in vivo cell uptake [3,4,5,6]. For this reason, the goal of this study was to encapsulate the therapeutic compounds resveratrol (Resv) and phloretin (Phl) within PLGA nanoparticles and to study their cytotoxic and anti-inflammatory effects related to OA on a chondrogenic cell line (ATDC-5). PLGA NPs were synthesized using the simple emulsification-solvent evaporation method, with resveratrol or phloretin loaded into the inner core of the emulsion. Scanning electron microscopy (SEM) was used to assess the morphology and size of the particles. NP size, size distribution and electrokinetic potential were determined by Dynamic Light Scattering (DLS). The drug content of Resv in NPs was determined directly by measuring the encapsulated resveratrol amount in PLGA NPs after dissolution using UV-Visible. The drug content of Phloretin was quantitatively determined by UPLC. The release of Resv and Phl from prepared NPs was assessed in PBS media (pH 7.4) containing SDS 0.3% (w/v) and quantified by UV-Visible and UPLC method, respectively. The cytotoxicity of PLGA nanosystems in ATDC-5 cell line was evaluated by the Blue Cell Viability Assay, after 24h of incubation with different concentrations (0.1 -2 mg/ml) of PLGA NPs or loaded with Resv (Resv@PLGA-NPs) or Phl (Phl@PLGA-NPs) and with the equivalent doses of free drug. To test the anti-inflammatory activity, ATDC-5 cells were treated with 250 ng/mL of lipopolysaccharide (LPS) for 24h after being pre-incubated with the subcytotoxic concentration of Resv@PLGA- NPs, Ph@PLGA-NPs and the equivalent doses of free drugs for 4h. The Griess reaction was used to assess the nitrite accumulation in culture media as a hallmark of inflammation. Both Resv@PLGA-NPs and Phl@PLGA-NPs exhibited spherical morphology with an average size of 117 ± 26 nm and 132 ± 33 nm, respectively, and a negative charge of -27.65 mV and -25.02 mV, respectively (Figure 1 A,B). Both Resv@PLGA-NPs and Phl@PLGA-NPs exhibited remarkable encapsulation efficiency (EE%) (36.48 and 7.57 %) and drug loading (DL%) (17.37 and 3.41 %), respectively. The obtained Resv@PLGA-NPs and Phl@PLGA-NPs showed an initial burst release followed by a sustained release. On the other hand, the viability results demonstrated the cytocompatibility of both Resv@PLGA-NPs and Phl@PLGA-NPs within the tested concentration range, with percentages exceeding 70% in most cases, complying with ISO 10993-5 recommendations. The experiments suggested that resveratrol and phloretin could be successfully incorporated into polymeric nanoparticles, as demonstrated by their absence of negative effects on ATDC-5 cells and effective encapsulation in PLGA. Moreover, these nanoformulations exhibited significant anti-inflammatory effects while maintaining the activity of the loaded therapeutic molecules, and therefore, are a potential candidates for OA treatment.

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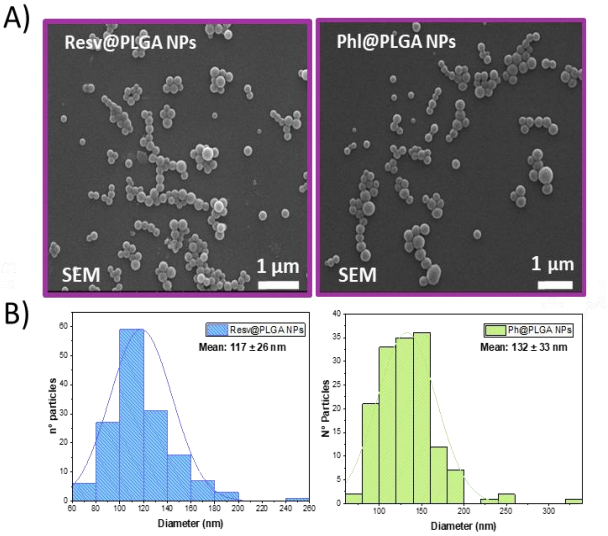

Figure 1.SEM images of Resv@PLGA and Phl@PLGA nanoparticles display a monodisperse population of NPs (A). Particle size distribution histogram derived from the analysis of 150 NPs from SEM images (B).

Graphene Oxide pegylated as antimicrobial against skin infections

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Since the discovery of penicillin, medicine has been developed around the availability of antibiotics to fight infections [1]. However, the adaptive capacity of bacteria and their rapid multiplication have led to the emergence of antimicrobial resistance (AMR) as a survival mechanism, which represents the greatest health challenge nowadays. A study published in 2018 by Upreti *et al*. revealed that 56.9% of skin infections were caused by Staphylococcus aureus [2]. *Staphylococcus aureus* is a clear example of the result of decades of indiscriminate antibiotic use and represents the connection between the treatment of wounds or skin lesions with antibiotics and nosocomial infections associated with long-stay hospital patients [3]. This bacterium can enter the bloodstream leading to circulatory, respiratory, and even bone infections, which is not only a skin problem but a potential systemic problem.

Therapeutic strategies against bacteria, such as the use of nanomaterials, are currently being investigated. In this work graphene oxide (GO) is used. This nanomaterial has a two-dimensional structure with carbon atoms distributed hexagonally, and contains several oxidized functional groups, which improves its dispersion and stability. Several studies have demonstrated some antimicrobial activity of carbon-based nanomaterials against both Gram-positive and Gram-negative bacteria, due to the physical and chemical interactions that occur when GO layers come into direct contact with bacterial cells. On the other hand, polyethylene glycol (PEG) increases the biocompatibility and water solubility of nanomaterials, so its use in biomedicine is very common. The main objective of this study is to determine the efficacy of two different combinations of carbon-based nanomaterials, GO and GO-PEG, against *Staphylococcus aureus*, one of the bacteria species with the highest rate of multidrug resistance worldwide [4].

A complete characterization of these nanomaterials was performed before analyzing their efficacy against *S. aureus*. The techniques used for the complete characterization were Atomic Force Microscopy (AFM); Transmission Electron Microscopy (TEM); Scanning Electron Microscopy (SEM); Ultraviolet-Visible Spectroscopy (UV-Vis); Zeta Potential; and Fourier Transform Infrared Spectroscopy (FTIR) [5]. Electron microscopy provides 2D and 3D images of the synthesized nanomaterials (Figure 1 shows SEM images of GO and GO-PEG). AFM supplies images of the surface and information on its roughness. The Zetasizer Nano-ZS equipment was used to measure the zeta potential, which provides information on the stability of the nanoparticles (Figure 2 reveals the zeta potential at different pH values). By means of FTIR and UV-Vis is possible to characterize the functional groups present in the nanomaterial. These techniques have great importance mainly after pegylation to know how the polyethylene glycol has bonded to the surface of the carbon-based nanomaterial.

Finally, the antimicrobial potential of GO and GO-PEG against S. aureus is assessed regarding the Müeller Hinton broth microdilution method, one of the most widely used quantitative methods for defining bacterial susceptibility to antimicrobials. This standardized method allows the calculation of the minimum inhibitory concentration (MIC) of antibiotic, in this case GO and GO-PEG, for the strain tested [6]. In this sense, four different concentrations of both nanomaterials are analyzed.

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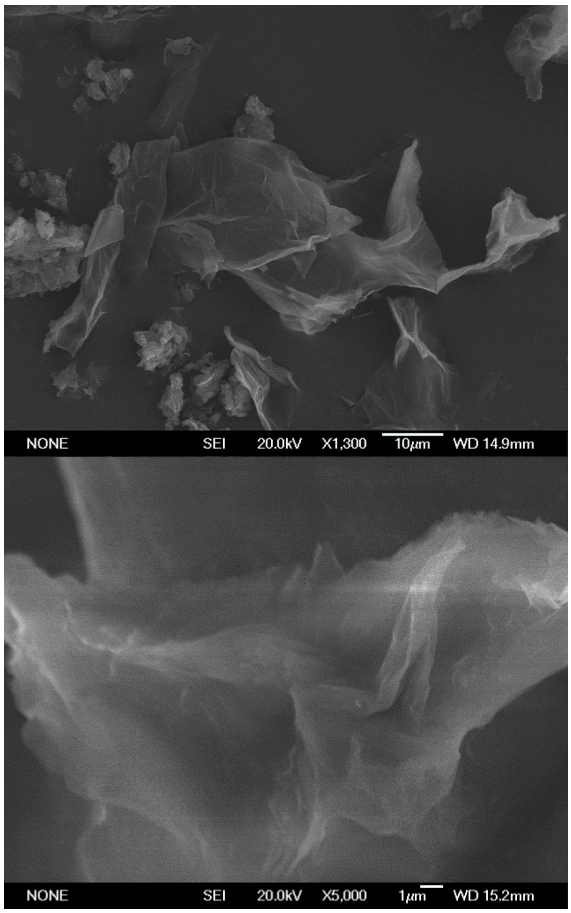

Figure 1. Scanning Electron Microscope images of the GO (top) and GO-PEG (down).

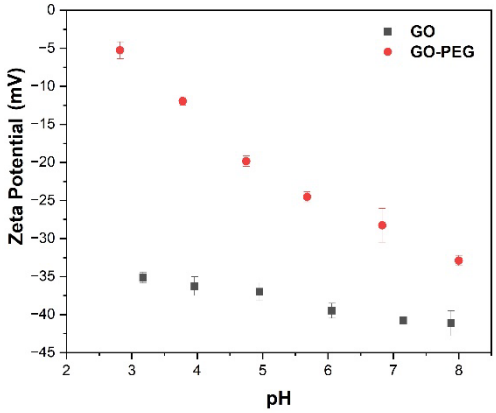

Figure 2. Zeta potential of GO and GO-PEG in aqueous dispersions at 0.1 mg/mL versus pH.

Designing Enzymatically-Powered PLGA Nanobots and Exploring its Swarming Behavior and Oil Interface Intercation

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Two main objectives have been considered in this study. Firstly, the design and development of enzymatic nanobots based on organic materials, which will improve its biocompatibility and biodegradability. Secondly, the study of the swarming behavior of these new enzymatic nanobots and their interaction with oil interfaces.

Nanobots have been widely investigated as the next generation of vehicles for drug delivery. Active motion, and especially their collective behavior (swarming), have shown an enormous advantage in terms of movement in complex medias,[1] overcoming biological barriers,[2] drug delivery[3] and tumor penetration.[4] Not only that, but also *in vivo* therapeutics outcomes have been observed.[5] Nevertheless, there is a general concern about the composition and simplicity of the different designs used, which may hinder their clinical applications. There is still the need to develop a simple nanobot based on organic materials which would be more appealing for industry and clinicians. Here, we present and compare the synthesis and characterization of two new urease-nanobots designs. Both are based on an organic biocompatible and biodegradable chassis of poly(lactic-co-glycolic acid), PLGA (FDA approved material and already used in clinics).[6] For their functionalization with urease, amine groups must be incorporated in the chassis surface. As PLGA is negatively charged, two different strategies have been developed. In one case, the core is combined with Chitosan (during synthesis), a natural positive polymer used as a dietary supplement. On the other hand, polyethylenimine (PEI) is used for providing a positive layer around the PLGA core (after synthesis). Moreover, the versatility of the chassis synthesis allowed us to encapsulate with high-efficiency hydrophobic (oil in water emulsion, OW) and hydrophilic (water in oil in water emulsion, WOW) standard drugs.

Studying its collective behavior, it was demonstrated how in the presence of the fuel (urea), nanobots experiment a sudden expansion that allows them to explore and reach further areas, if compared with passive controls. In parallel, we have seen how an oil surface acts as a barrier for nanobots. However, in the presence of the fuel, their catalytic reaction provokes the mixing of the oil/aqueous interface, displacing upwards the remaining oil phase. That allows the nanobots to cross this barrier. This phenomenon could be explored for common diseases such as acne, which is characterized by overproduction and accumulation of sebum (protective skin oil).

With all, PLGA has been demonstrated to be an efficient candidate for developing enzymatic nanobots. Further studies are required to understand how these new approaches will behave in front of biological systems, *in vitro* and *in vivo*.

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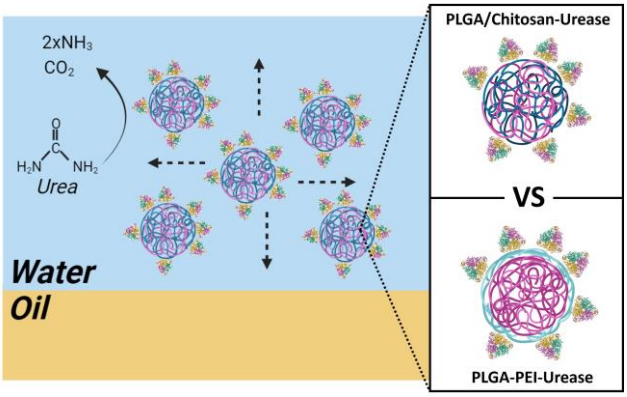

Figure 1. Graphical Abstract

Calcium phosphate nanoformulation for cancer treatment

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Abstract
Pancreatic cancer (PC) is a highly drug-resistant tumour, which makes it difficult to treat. In this context, poly-ADP ribose polymerase 1 (PARP1) is a relevant protein in chemoresistance in several types of cancer [1,2]. In this work, the synthesis of calcium phosphate nanoparticles (ACP) [3] encapsulating a PARP1 inhibitor (Olaparib, OLA) together with ascorbic acid (AA) has been performed. The nanoformulations were loaded with 13% OLA and 1% AA (NP-ACP-OLA-AA). Our results showed an interesting antitumour effect on three pancreatic cancer cell lines (PANC-1, Panc02 and MIA PaCa-2), matching or improving the effect of the free OLA. In addition, induction of tumours in immunocompromised SCID-NOD mice from the PANC-1 cell line showed that the mice group treated with NP-ACP-OLA-AA had lower tumour volume and longer survival compared to the free drug. This greater effect *in vitro* and *in vivo* is due to the gradual release of both compounds generated by their nanoencapsulation, protecting them from degradation and maintaining a controlled release of Olaparib for 72 hours. Analysis of *in vivo* samples shows that the NPs are able to efficiently reach tumours, generating an effective pro-apoptotic effect leading to cell death. Therefore, these NP-ACP-OLA-AA are shown to be a possible effective therapy, highly biocompatible and with great biodegradability compared to other alternative ways of administering OLA, producing a high induction of apoptosis and decreasing tumour growth-

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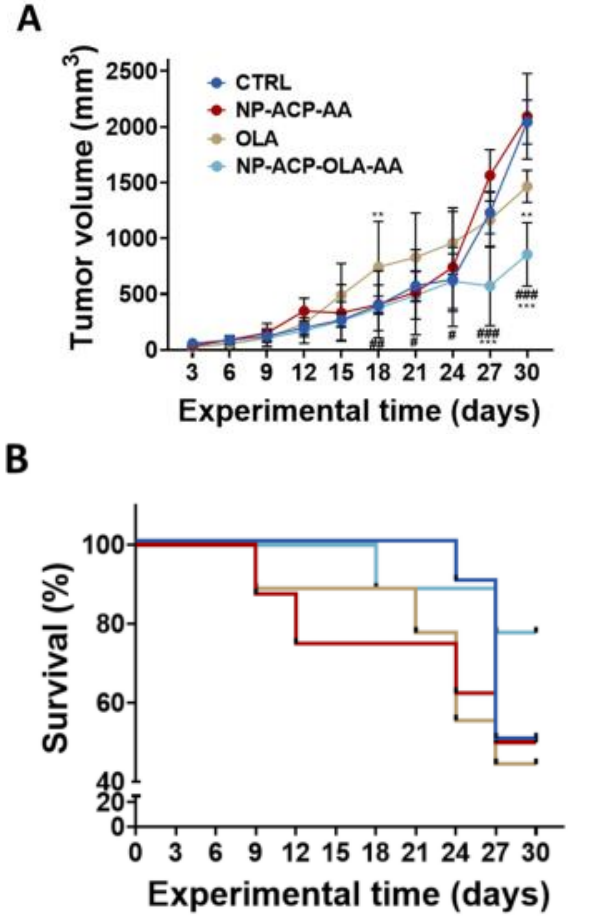

Figure 1. In vivo results in NSG mice with PANC-1 induced tumors. Tumor growth (A) and survival of mice (B) after CTRL, NP-ACP-AA, OLA, and NP-ACP-OLA-AA treatments

Colon cancer treatment: use of magnetoliposomes associated to LGR5

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Abstract

The lack of specificity of conventional chemotherapy is one of the main problems to be solved in cancer therapy. Biomimetic magnetoliposomes [1] are successful chemotherapy controlled-release systems, hyperthermia, and active targeting agents by functionalization of their surface with monoclonal antibodies. The membrane receptor Leucine-rich repeat-containing G-protein coupled receptor 5 (LGR5) [2] stands out as colorectal cancer (CRC) biomarker and appears to be related to treatment resistance and the development of metastasis [3]. The purpose of this study was to evaluate the effectiveness and safety of LGR5-targeted biomimetic magnetoliposomes loaded with oxaliplatin (OXA) or 5-fluorouracil (5-FU) in the selective therapy of CRC and their possible application in hyperthermia. Synthesis, characterization and determination of heating capacity of magnetoliposomes transporting OXA or 5-FU (with and without LGR5 functionalization) were conducted. *In vitro* antitumoral activity was assayed in multiple colorectal cell lines at different times of exposition. Besides this, cell internalization was studied by Prussian Blue staining, flow cytometry and fluorescence microscopy. *In vivo* acute toxicity of magnetoliposomes were performed to evaluate iron-related toxicity. OXA and 5-FU loaded magnetoliposomes functionalized with LGR5 antibody showed higher cellular uptake than non-targeted nanoformulation with a reduction of the percentage of proliferation in colon cancer cell lines up to 3.2-fold of the IC_{50} value compared to that of free drug. The differences between non-targeted and targeted nanoformulations were more evident after short exposure times (4 and 8 hours). Interestingly, assays in the MC38 transduced cells with reduced LGR5 expression (MC38-L(-)), showed lower cell internalization of LGR5-targeted magnetoliposomes compared to non-transduced MC38 cell line. In addition, magnetoliposomes showed an *in vitro* favorable heating response under magnetic excitation and great iron-related biocompatibility data *in vivo*. Drug-loaded magnetoliposomes functionalized with anti-LGR5 antibodies could be a promising CRC treatment strategy for LGR5+ targeted chemotherapy, magnetic hyperthermia, and both in combination.

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Figures

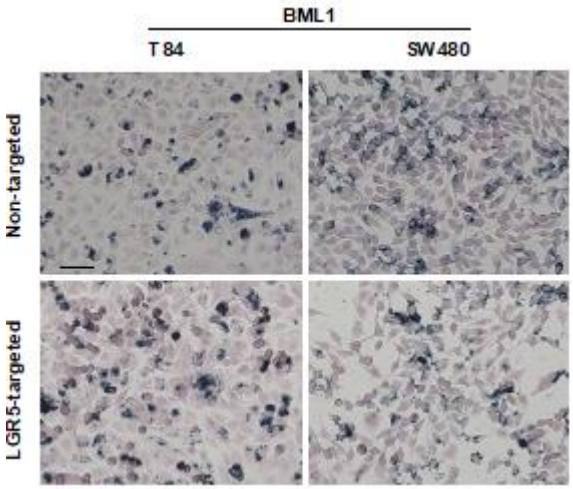

Figure 1. Internalization of magnetoliposomes with and without LGR5 functionalization at 24 hours of exposition in T84 and SW480 cell lines.

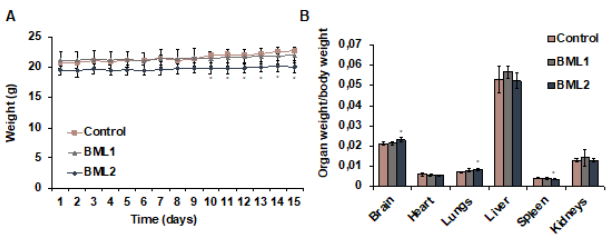

Figure 2. Acute toxicity of Fe in mice treated with blank magnetoliposomes BML1 and BML2 (A) Graphic representation of the weight variation in mice along the experiment. Data are presented as mean ± SD (n = 16). (B) Graphic representation the weight of the organs from sacrificed mice treated with BML1 and BML2.

Study of early effects of chitosan nanoparticles with glutathione in rats with osteoarthrosis

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Due to cartilage's limited capacity for regeneration, numerous studies have been conducted to find new drugs that modify osteoarthrosis's progression. Some evidence showed the capability of chitosan nanoparticles with glutathione (Np-GSH) to regulate the oxide-redox status in vitro in human chondrocytes. This work aimed to evaluate the capacity of Np-GSH in vivo, using Wistar rats with induced surgical osteoarthritis.

Radiographic, biochemical (GSH and TBARS quantification), histopathological, and immunohistochemical (Col-2 and MMP-13) analyses were performed to evaluate the progress of the osteoarthritic lesions after the administration of a single dose of Np-GSH. According to the results obtained, the GSH contained in the NPs could be vectored to chondrocytes and used by the cell to modulate the oxidative state reduction, decreasing the production of ROS and free radicals induced by agents oxidizing xenobiotics, increasing GSH levels, as well as the activity of GPx, and decreasing lipid peroxidation. These results are significant since the synthesis of GSH develops exclusively in the cell cytoplasm, and its quantity under an oxidation-reduction imbalance may be defective. Therefore, the results allow us to consider these nanostructures as a helpful study tool to reduce the damage associated with oxidative stress in various diseases such as

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Towards understanding the CeO_2NPs effect in an *in vitro* preeclampsia model

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Preeclampsia (PE) is a potentially lethal orphan-drug disease that affects 5-8% of pregnant women globally. PE is characterized by high blood pressure and endothelial damage, causing a systemic inflammation and oxidative stress with elevated levels of reactive oxygen species (ROS).

Trophoblasts are cells that develop as part of the placenta. They also provide nutrients and help the embryo adhere to the uterus. In this study, we employ immortalized trophoblasts (HTR8/SVneo) exposed to 1% O2 hypoxia to mimic a preeclampsia-like phenotype. Trophoblast under hypoxia tend to migrate aberrantly causing inflammation and ROS.

Our research focuses on the use of cerium oxide nanoparticles (CeO2NPs) known for their antiinflammatory and antioxidant properties as a possible treatment for PE. This effect could be achieved through its ROS scavenging capacity.

No changes in cell viability are observed when trophoblasts are exposed to 1% O2 (hypoxic conditions). CeO2NPs have no cytotoxic effect in trophoblasts exposed to 1% O2. An excess of trophoblast migration is associated with preeclampsia (hypoxic environment). CeO2NPs reduce migration in trophoblast exposed to 1% O2. Thus, CeO2NPs may be promising candidates for treating preeclampsia.

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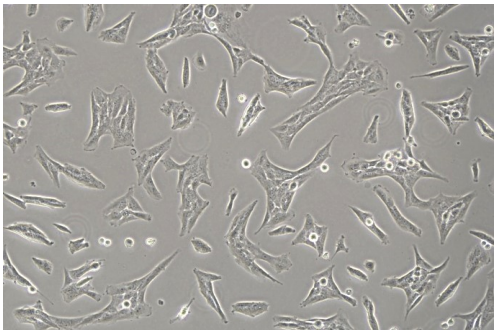
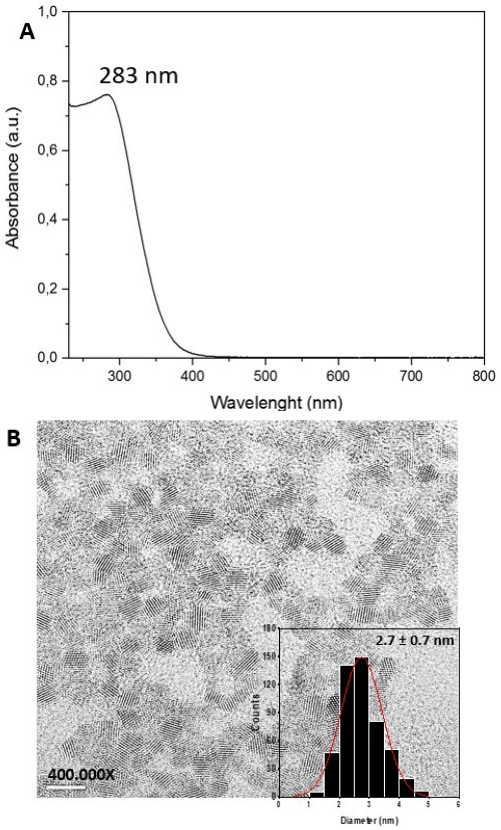


Figure 1. HTR-8/SVneo trophoblasts 40X



C

pH	8.3
Size by number (DLS)	4.2±0,9 nm
Z-pot	-17±3.4 mV

Figure 2. CeO2NPs UV-visible spectroscopy and size distribution (A). 2.5 nm CeO2NPs by high-resolution transmission electron microscopy (B). pH, size by dynamic light scattering (DLS), zeta potential (C).

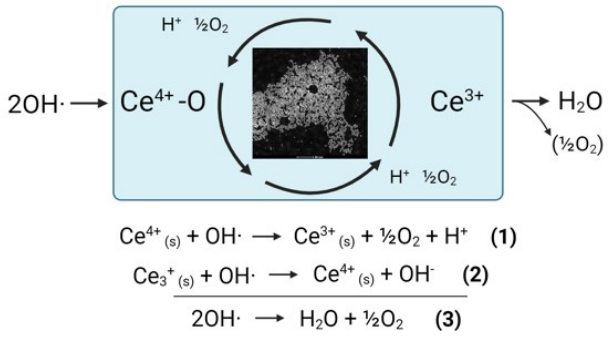


Figure 3. Schema of CeO2NPs possible bio-catalysis mechanism. Adapted from: Ernst LM, Puntes V. How Does Immunomodulatory Nanoceria Work? ROS and Immunometabolism. (doi: 10.3389/fimmu.2022.750175)

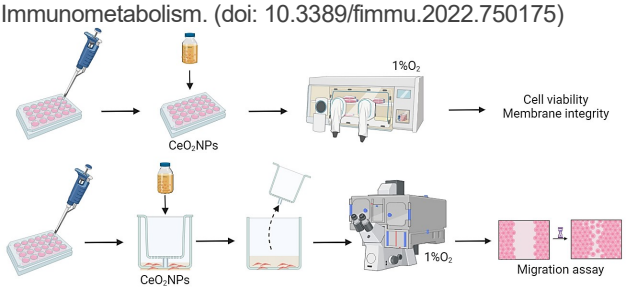


Figure 4. Schema of the methodology used to perform cell viability and membrane integrity assays (A), and the migration assay (B) under hypoxia conditions (1%O2).

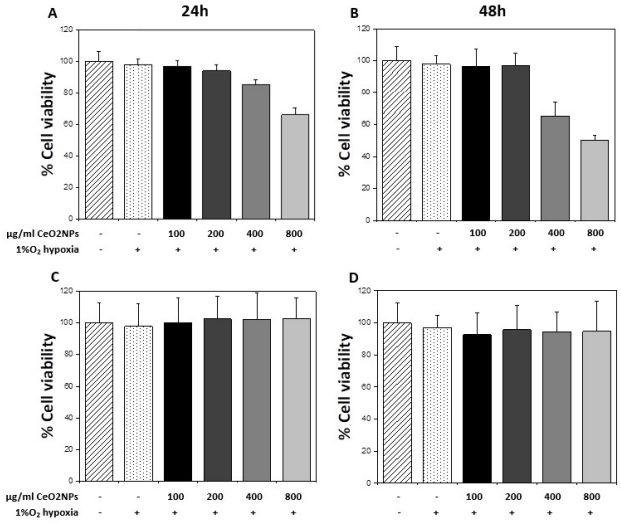


Figure 5. Cell viability by PrestoBlue assay (A,B) and membrane integrity by Lactate Dehydrogenase assay (C,D) in HTR-8/SVneo trophoblasts pre-treated with 0, 100, 200, 400 and 800 µg/ml CeO2NPs and exposed to 1% O2 for 24h and 48h. Data expressed as mean ± SD.

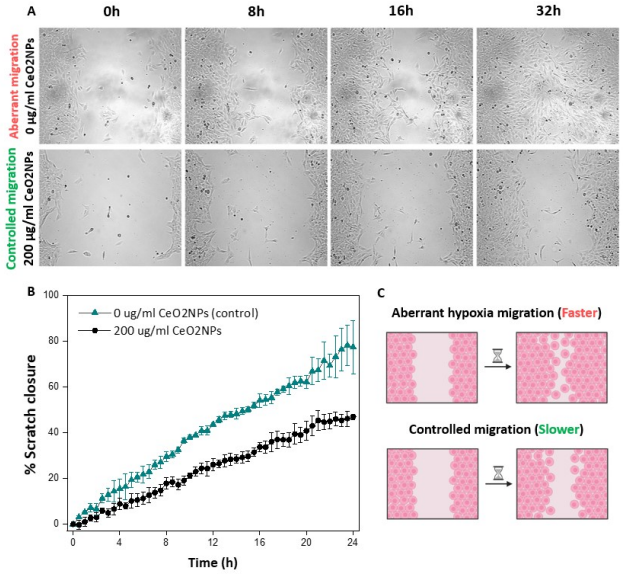


Figure 6. Migration assay time-lapse confocal microscopy images. Untreated (above panels) and 200 µg/ml CeO2NPs treated (below panels) HTR-8/SVneo trophoblasts exposed to 1% O2 at 0h, 8h, 16h and 32h after insert removal (A). Evaluation of scratch wound closure in % of untreated and 200 µg/ml CeO2NPs treated HTR-8/SVneo trophoblasts exposed to 1% O2. Measures performed continuously for 24h. Data expressed as mean ± SD (B). Schema of the aberrant migration that take place under hypoxia (PE in vitro mimic conditions) versus a normal controlled migration (C).

The side-chain chemistry of antifouling polymer brushes influences protein fouling and their hemocompatibility.

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Whenever an artificial surface comes into contact with blood, proteins are rapidly adsorbed onto its surface. This phenomenon, termed fouling, is then followed by a series of undesired reactions involving activation of complement or the coagulation cascade and adhesion of leukocytes and platelets leading to thrombus formation. Thus, considerable efforts are directed towards the preparation of fouling-resistant surfaces with the best possible hemocompatibility.[1]
We performed a comprehensive hemocompatibility study after heparinized blood contact with seven different polymer brushes prepared by surface-initiated atom transfer radical polymerization. We quantified the fouling resistance and analyzed thrombus formation and deposition of blood cellular components on the coatings. Moreover, we performed identification of the remaining fouled proteins via mass spectroscopy to elucidate their influence on the surface hemocompatibility (Figure 1). Compared with an unmodified glass surface, the grafting of polymer brushes minimizes the adhesion of platelets and leukocytes and prevents the thrombus formation. The fouling from undiluted blood plasma was reduced by up to 99%. Most of the identified proteins are connected with the initial events of foreign body reaction towards biomaterial (coagulation cascade proteins, complement component and inflammatory proteins). In addition, several proteins that were not previously linked with blood-biomaterial interaction were identified.[2]

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Figures

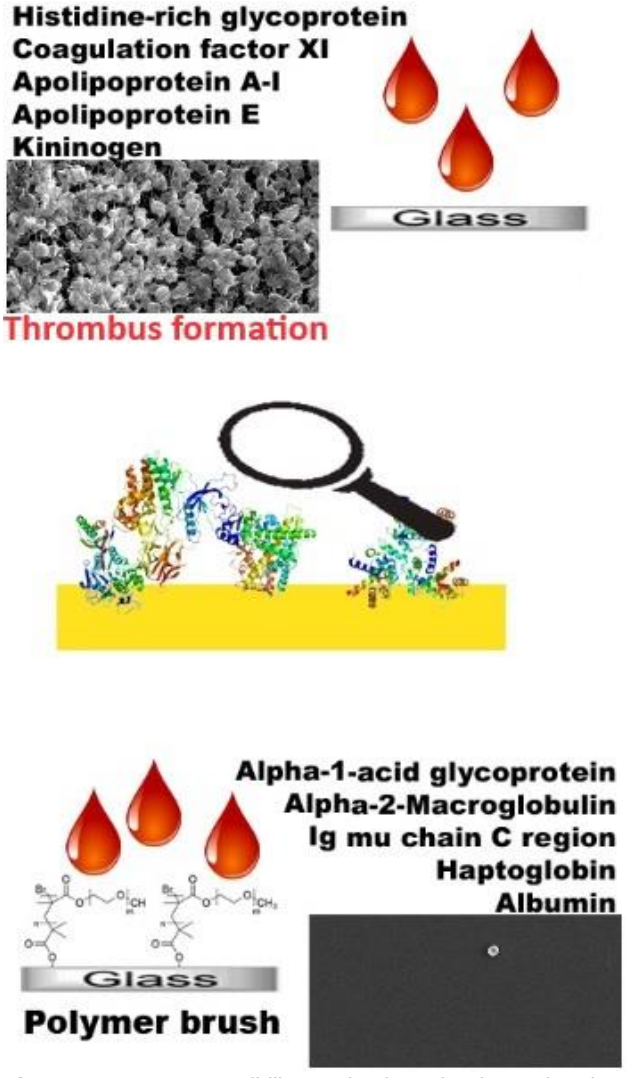

Figure 1. Hemocompatibility evaluation of polymer brushes and glass surface after heparinized blood contact and a following identification of the fouled proteins by LC-MS/MS.

Synergistic effect of swarms of enzyme-powered nanomotors for enhancing the diffusion of macromolecules

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In recent decades, nanotechnology has made significant progress in drug delivery systems. The goal is to improve therapy effectiveness by precisely releasing drugs to specific tissues. However, there are still challenges to overcome. One major challenge is the presence of biological barriers,[1] such as viscoelastic fluids like synovial fluid in joints, which mainly contain hyaluronic acid. The complex network of these fluids hinders the transportation of nanosystems, causing conventional particles to get trapped and limiting their ability to reach the target area.[2,3] Therefore, there is a need for innovative technologies that can enhance the delivery of therapeutic agents.

To overcome the obstacles presented by complex media, one promising approach is the development of "active" nanoparticles or nanomotors (NMs).[4–6] However, the exploration of enzyme-powered nanomotors capable of navigating and influencing viscous fluids is still in its early stages. These enzyme-powered nanomotors offer great potential, as their coordinated movement can be driven by enzymatic reactions, effectively utilizing the biofuels present in the human body. Furthermore, some of these enzymatic nanomotors can modify the characteristics of the extracellular matrix by reducing its viscosity, thus facilitating improved diffusion of therapeutic agents.

In this study, we introduce a nanotechnological strategy using two swarms of nanomotors, namely hyaluronidase NMs (HyaNMs, Troop 1) and urease NMs (UrNMs, Troop 2), which synergistically enhance the diffusion of macromolecules within the synovial fluid. Troop 1 demonstrates the capability to break down the intricate network of synovial fluid, both in vitro and ex vivo, thereby reducing its viscosity. This enables Troop 2 to navigate more effortlessly through the viscous media. Moreover, the collective movement of Troop 2 significantly enhances the diffusion of Dextran macromolecules. These findings offer promising prospects for utilizing enzyme-powered NMs in the treatment of joint injuries, augmenting therapeutic effectiveness, and facilitating faster and more efficient delivery of therapeutic agents compared to conventional approaches.

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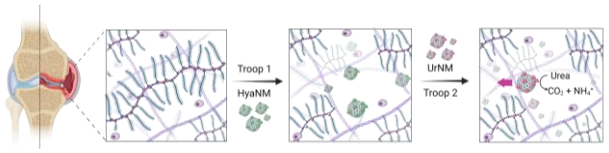

Figure 1. Conceptual idea of the novel approach using hyaluronidase NMs (HyaNMs) to interact with and reduce the viscosity of synovial fluid and urease NMs (UrNMs) for a more efficient transport of therapeutic agents in joints.

Enhancing nanomotor stability: the role of enzymatic protection and immunological safety

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In the last few decades, advancements in nanotechnology have paved the way for harnessing the power of enzymes through their integration with micro- nanoparticles, endowing them self-propulsion features[1–3]. Among these enzymes, **catalase (CAT)** has gained significant prominence due to its intrinsic properties, i.e., high turnover number and dismutation of hydrogen peroxide in water and oxygen bubbles which drive to enhanced motion properties by means of jet-like mechanism or buoyancy effect[4]. Recently they have been successfully applied in biomedical applications[5]. However, the presence of biomacromolecules with high potential to produce immune response hindering its application in clinic. In this regard, **single enzyme nanogles (SENs)** is an emerging technology which provides polymeric mantle around the enzyme protecting them from the media. It has been reported that this technology could increase the enzyme stability against temperature and organic solvents in addition to potential functionalities for further applications [6–8].

Here, we show the synthesis of catalase nanogels **(CAT@NGs)** functionalized with amine groups **(Figure 1)** and its immobilization covalently onto mesoporous silica nanoparticles **(MSNPs)** to fabricate for first time CAT@NGs-based nanomotors **(Figure 2)**. The preliminary results showcased not only the **preservation of catalytic properties** in the CAT@NGs but also upon immobilization we demonstrated its ability to exhibit enhanced self-propulsion at the **single particle** level and **collective behavior (swarm)**.

Furthermore, we propose a future experiment to demonstrate that the CAT@NGs-based nanomotors have the potential to evade immune responses, thereby safeguarding the organism from immunological overreactions.

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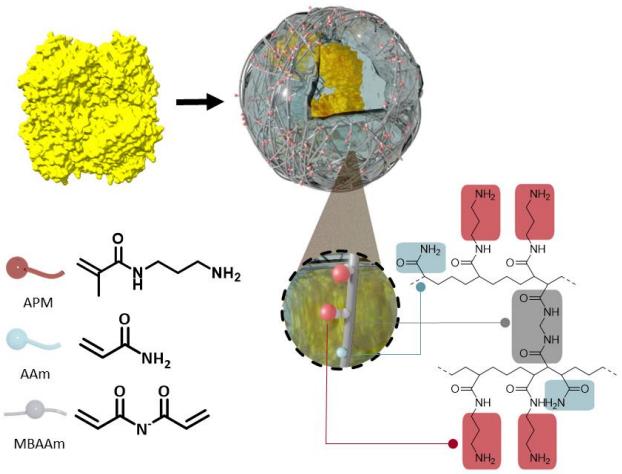

Figure 1. Schematic representation of CAT@NGs. Radical polymerization was performed with the monomers N-(3-aminopropyl)methacrylamide (APM), acrylamide (AAm), and N,N´-Methylenebis(acrylamide) (MBAAm) as a crosslinker to form catalase-based nanogels.

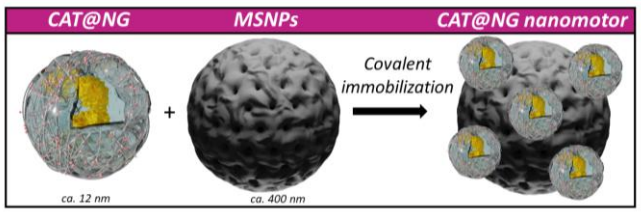

Figure 2. Schematic representation of CAT@NGs-based nanomotors manufacturing. The amines groups provided by CAT@NGs promote the covalent attachment onto MSNPs.

Exploring Monofloral Honey Incorporated to Double-Network Hydrogels for Innovative Cardiac Patch Applications

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Cardiovascular diseases, particularly those affecting the myocardium, remain a leading cause of morbidity and mortality worldwide. Novel approaches to address these issues are essential such as the use of double-network (DN) hydrogels, characterized by a dual brittle/flexible network structure, that have garnered considerable attention in tissue engineering. One of the most crucial factors in this field is achieving the necessary mechanical strength to replicate the mechanical characteristics of native tissue. In this context, DN hydrogels offer the mechanical robustness required for cardiac patches, simulating the native heart tissue's toughness. Another strategy is the use of natural bioactive compounds, such as honey due to antibacterial and angiogenic properties [1]. In particular, monofloral honeys are a well-known compound having nutritional benefits, and other biological activities [2], that could potentially contribute to the functionality of cardiac patches [3]. Moreover, it is known for its water retention capacity, able to improve the hydration and microenvironment within a hydrogel, facilitating a conducive milieu for cell growth and survival. Moreover, monofloral honey's potential antibacterial properties align with the need for maintaining a sterile environment in cardiac regeneration applications. In this work, a chitosan network, providing structural integrity, was mixed with polyvinyl alcohol (PVA), comprising toughness, for developing bioactive DN, [4]. To increase the bioactivity of the DN, an endemic monofloral honey from Chile, was incorporated as it is unique due to its monofloral composition. The rationale for combining DN hydrogels and the endemic honey lies in the potential synergy of their properties for biomedical applications.

Furthermore, to identify the botanical origin of the honey used, we conducted a melissopalynological study. Based on the microscopic analysis of pollen grains present in the sample, we classified the honey as monofloral honey, following the classification standards for Chilean kinds of honey [4]. The antimicrobial activity of the sample against *Escherichia coli*, *Staphylococcus aureus*, and *Salmonella enterica sv. Typhi* was also determined by analyzing the growth inhibition halo, using Penicillin G and Streptomycin antibiotics as controls. According to the laboratory analysis standards, and based on the bacterial inhibition result obtained, the analyzed honey sample is classified as "Active Honey." This classification is used to distinguish honeys that exhibit additional biological activity beyond their basic nutritional characteristics.

This research focused on investigating the impact of varying concentrations of honey (DN C-P-H_0.25 and DN C-P-H_0.50) on the mechanical properties under wet conditions. Figure 1 presents the stress-strain curves from which tensile strength, elastic modulus, and elongation at break were obtained for the hydrogels. As the data demonstrates, an increase in honey concentration within the hydrogels resulted in a reduction in both the elastic modulus and ultimate tensile strength. When considering the influence of stiffness on the DN hydrogels, the incorporation of 0.5% wt. honey led to a significant decrease of approximately 30% in Young's modulus. Conversely, when 0.25% wt. monofloral honey was incorporated, the reduction was more modest, around 8%. This reduction in stiffness may be attributed to honey's capacity to interact with polymer chains due to its hydroxyl groups, indicating a plasticizing effect [5]. Furthermore, this is reflected in the internal porosity of the hydrogels, which decreases from 2 µm to 0.8 µm and 0.6 µm upon incorporating 0.25% and 0.50% by weight of honey, respectively. This results in the formation of a nanoporous internal structure when cross-sectional analyses are conducted using scanning electron microscopy (SEM).

To provide a controlled and precise designed platform, we created 3D-printed scaffold structures from the DN hydrogels (Figure 2.a). This approach allows us to explore the cellular responses to these unique hydrogels under well-defined conditions. In order to evaluate the biocompatibility and cytotoxicity of the DN hydrogels and the effect of the two concentrations of monofloral honey on hydrogel bioactivity, the responses of the human umbilical vein endothelial cells (HUVECs) were measured in an in vitro study using Alamar Blue test on the 1st, 3rd, 7th and 14th days. The results illustrated in Figure 2. revealed that honey promotes cell proliferation during the days of the study. The rapid cell growth on honey-containing hydrogels suggests a beneficial effect, likely associated with the sugar content in the samples. However, over time, we observed a decline in proliferation, which could be due to the gradual dissolution of honey in the cell culture medium. Moreover, no adverse effects on cell viability were observed. Among the prepared hydrogels, C-P 0.5% wt honey exhibited the highest level of biocompatibility. The incorporation of honey into the hydrogel appears to enhance cell

proliferation and viability, potentially by supplying essential nutrients to the cells [6].

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Figures

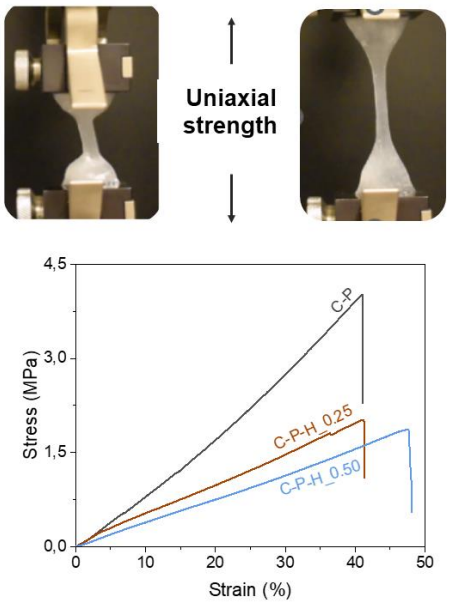

Figure 1. Graph of stress-strain and table of mechanical properties of the DN hydrogel samples.

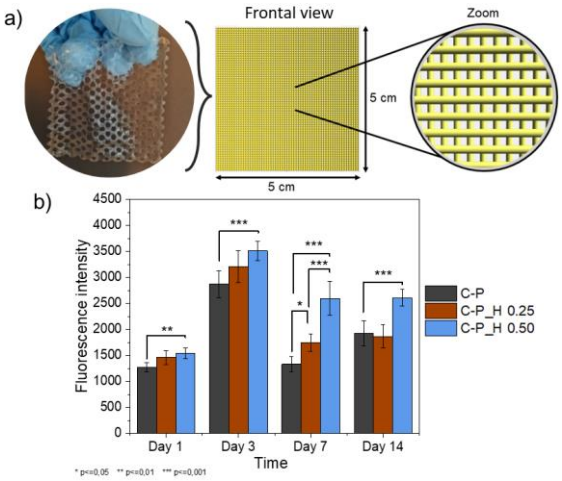

Figure 2. a) 3D-printed scaffolds and b) Cell proliferation analysis of HUVECs seeded on 3d printed scaffolds of DN hydrogel samples.

Radionuclide therapy with accumulated nanobots reduces bladder tumor size in vivo

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Enzyme-powered nanoparticles, known as nanobots, have emerged as a promising approach for performing tasks at the nanoscale, ranging from targeted drug delivery to precision medicine. Among these, urease-powered nanobots have shown improved diffusion and 3D navigation within biological environments[1] and drug delivery efficacy[2,3], compared to non-motile nanoparticles. The propulsion mechanism of these urease-powered nanobots, driven by urea (a readily available substance in the body), makes them particularly well-suited for potential applications in treating bladder cancer. Current treatments for this disease involve intravesical drug administration, which has shown good survival rates but limited therapeutic efficacy. Several factors, such as the sedimentation of therapeutic agents and the continuous addition of fresh urine, hinder the even diffusion of drugs throughout the entire bladder volume. Moreover, poor retention in the bladder and low penetration in the target site may leave certain subregions untreated, potentially leading to recurrence. To address these unresolved medical challenges, nanobots have emerged as a viable solution. In this context, our study demonstrates an enhanced accumulation of radiolabeled urease-powered nanobots within bladder tumors using an orthotopic murine model. Furthermore, we provide evidence that intravesically administered radio-iodinated nanobots exhibit a radionuclide therapeutic effect, resulting in significant tumor size reductions of approximately 90% when compared with non-treated mice. These promising results firmly position nanobots as highly efficient nanosystems for bladder cancer therapy.

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Figures

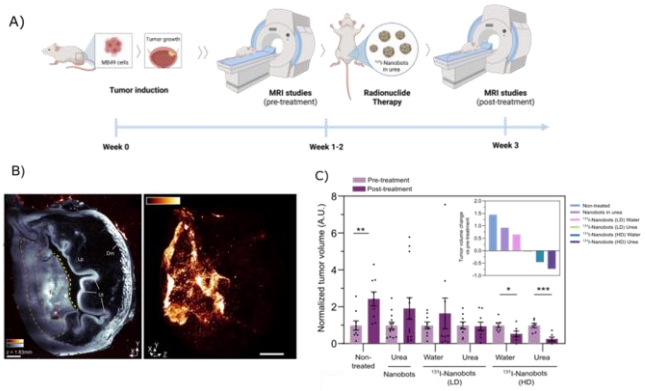

Figure 1. Nanobots penetrate and reduce bladder tumors size. A) Schematic representation of the radionuclide therapy studies. B) Left: Plane in the center of the bladder showing autofluorescence (grey) and scattered light-sheet (sLS) signal. Right: Maximum intensity projection of sLS signal inside the bladder. C) Normalized tumor volume obtained by MRI pre- and post-treatment. LD denotes low dose and HD high dose of ^{131}I.

Genomic SPR biosensor for the detection and monitoring of coronaviruses in wild and farm animals

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During the pasted two decades, coronaviruses (CoVs) have caused three epidemics and pandemics with a severe impact on global public health and the global economy. CoVs are sorted into four main genera based on their phylogenetic relationship and genomic structures: Alpha-, Beta-, Delta-, and Gamma-CoV. Alphacoronavirus and Betacoronavirus, also known as PanCoV, can infect a wide range of mammals, including humans, while Gammacoronavirus and Deltacoronavirus are mostly found in avian species[1]. In fact, the causal agent behind COVID-19, SARS-CoV-2, potentially originated from a coronavirus found in Chinese horseshoe bats, which initially crossed into an unidentified intermediate animal host.
Veterinary medicine has recorded several instances of emerging diseases resulting from coronaviruses crossing the species barrier, including the ongoing pandemic[2]. Due to this potential transmission risk from wild, farm, and domestic animals to humans, a better understanding of the CoVs circulation is needed to prevent and predict future epidemics.
However, current analytical methods, mainly based on Polymerase Chain Reaction (PCR) processes and other centralized laboratory techniques, are costly, laborious, and time-consuming, which greatly hampers an efficient routine screening of animal populations.

We propose a genomic Surface Plasmon Resonance (SPR) biosensor[3] for rapid detection, identification, and monitoring of CoV RNA (Alpha-, Beta-, Gamma-CoV) in samples from wild and farm animals. Our SPR biosensor is integrated in a small and portable device with a user-friendly operation, ideal for decentralized application at the point of need by non-specialized technicians.
We have designed and implemented a direct hybridization assay, using single-stranded DNA probes specific to the two viral RNA targets (panCoV and avianCoV). Sensor biofunctionalization was optimized to provide a stable probe immobilization with suitable grafting density to minimize possible steric hindrance effects. Also, hybridization conditions and parameters were studied to enhance the detection efficacy and ensure maximum specificity and selectivity. The limits of detection (LOD) and limits of quantification (LOQ) determined for the direct assay are in the pM-nM range, proving the high sensitivity of the biosensor. Furthermore, we have evaluated different signal amplification strategies, reaching detection limits for Alpha/Beta-coronaviruses and Gamma-coronaviruses were achieved at 129 pM and 1.49 nM, respectively. Finally, it is important to mention that the biosensor assay can be completed in less than 20 min sample-to-result turnaround time, and using a minimum volume of sample (100 – 150 μL), which is especially relevant for the screening of small animals.

Our genomic SPR biosensor introduces a rapid technique for identification of zoonotic coronavirus in different types of wild and farm animals, offering the opportunity to perform routine and cost-effective screening of the populations. The implementation of this type of biosensors in veterinary research and practice could aid in the control of viral infection transmission and in the prevention of future outbreaks.

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Figures

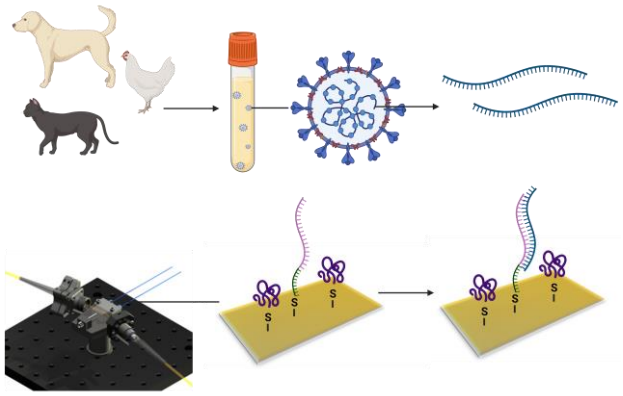

Figure 1. Biosensor for viral RNA detection in animal serum samples.

Novels drug delivery nanosystems to restore the anti-tumor activity of the immune system in cancer patients

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Background

Glioblastoma multiforme (GBM) is the most prevalent and challenging primary brain tumor to treat, known for its highly immunosuppressive tumor microenvironment (TME). This unique TME can be harnessed to develop innovative approaches for targeting specific cancer cell populations, particularly focusing on tumor-associated macrophages (TAMs), which play a central role in suppressing the anti-tumor immune response in GBM and contributing to a poor prognosis [1]. These TAMs consist of both resident microglia (MG) and macrophages originating from the bloodstream (bone marrow-derived macrophages, BMDMs). In 2019, our group has demonstrated that BMDMs are especially abundant in the core of GBM tumor masses, where they exhibit strong immunosuppressive activities [2].

Given these peculiar features of the TME, in this study we combined both the fields of nanomedicine and tumor immunology and proposed a novel approach to modulate the immune response in GBM patients by exploiting two controlled drug delivery nanosystems.

One is an Oil in Water (O/W) nanoemulsion that contains a potent inhibitor of heme oxygenase-1 (HO-1), Zinc protoporphyrin IX (ZnPPIX). We selected this inhibitor based on our recent research findings, which showed that the immunosuppressive activity of TAMs is primarily linked to their iron metabolism [3].

The other nanosystem is a polymeric nanoparticle loaded with an oxaliplatin derivate, diaminocyclohexane-platinum II (DACHPt) [4]. The use of oxaliplatin derivates trigger immunogenic cell death (ICD) within the TME of glioblastoma patients.

Methodolody

ZnPPIX loaded nanoemulsions (NEs) were produced via microfluidic technique, while DACHPt-loaded hyaluronic acid-polyarginine nanoparticles were prepared using the ionic gelation technique at LAGEPP laboratory, in Lyon [3,4].

Immunosuppressive activity of *in vitro*-derived macrophages was assessed by evaluating the proliferation of activated lymphocytes that were stained with CellTrace™ Violet Cell Proliferation Kit, activated with anti-CD3 and anti-CD28 and co-cultured in 96 plate with macrophages. After 4 days, proliferation of T cells was evaluated through the signal of CellTrace on $CD3^+$ cells, by flow cytometry. Cell viability assay were carried on GBM cell line (U87MG) to determine the targeting efficacy and safety of polymeric nanoparticles (NPs).

Results

We developed NEs loaded with ZnPPIX and invastigated their physico-chemical characteristics. Our results demostrated that NE-ZnPPIX presented a size of around 100 nm, a slightly negative zeta potential of -10 mV and an encapsulation efficiency (EE) of the drug of around 69% (figure 2). Immunesuppressive assay have demostrated that treatement with either ZnPPIX free drug or NE-ZnPPIX relief the immunesuppression activity of macrophages and promote the proliferation of the co-coltured lymphocytes (Figure 3, left panel).

In parallel we also developed DACHPt-loaded nanoparticles (NPs) that show a size of around 200 nm with a negative Zeta Ponteial (-20 mV). Also in this case the EE was high (around 70%).

Studies on GBM cell line showed a targeting specificity of DACHPt-loaded NPs as doses increased and blank NP did not interfere with cell viability at all the concentrations tested, confirming their biocompatibility and safety usage for a local and systemic treatment (figure 3,right panel).

Conclusions

Our results obtained through the *in vitro* models and our findings regarding the uptake of these nanosystems by cells present in the TME of glioblastoma (data not shown), suggest that the ZnPPIX loaded NEs could be used to target TAMs and induce their re-programming towards a more pro-inflammatory and anti-tumoral phenotype. In addition, our results indicate that the polymeric nanosystem could be used to target both tumor cells and myeloid immunosuppressive cells while inducing ICD (figure 4).

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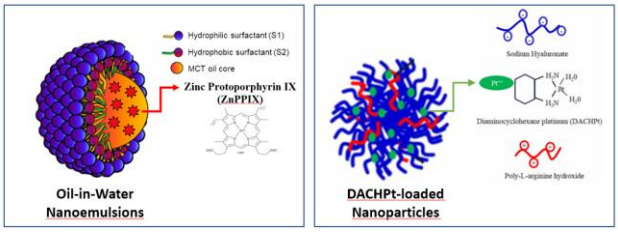

Figure 1. Schematic representation of the two nanosystem used for this study (*Adapted from [3,4]).*

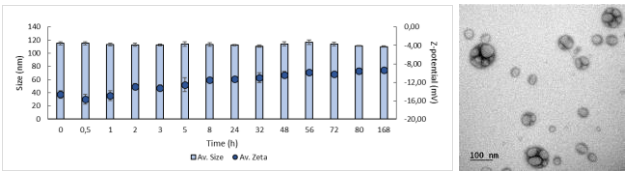

Figure 2. Characterization of NEs: Dynamic-Light-Scattering and Zeta-potential measurements indicate NEs average size around 110 µm, PDI < 0.2, zeta-potential values around -10 mV; TEM image showed a well-defined and homogeneous structures with spherical shape of NEs.

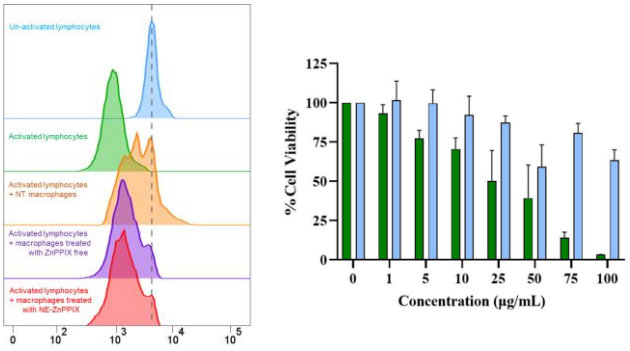

Figure 3. *Left panel:* Proliferation of activated T cells in the presence of untreat or drug-loaded NEs, a representative flow cytometry analysis of the immunosuppressive assay showing the efficacy of NE-ZnPPIX and that of the ZnPPIX free drug in restoring the proliferation of activated lymphocytes. *Right panel:* cell viability assay on U87MG cells in the presence of the polymeric nanosystem (empty NPs in light blue, DACHPt-loaded NPs in green).

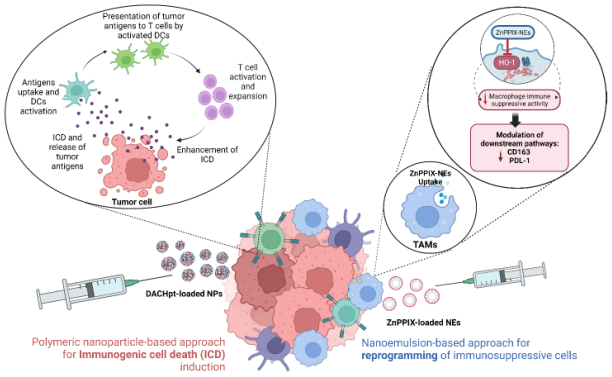

Figure 4. Proposed mechanisms of action of the two encapsulated nanosystems to restore the anti-tumour activity of the immune system in cancer.

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