nano bio & med 2024

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nanoBioMed2024 Foreword

On behalf of the Organising Committee, we take great pleasure in welcoming you to Barcelona (Spain) for the nanoBio&Med2024 International Conference. This event, after successful editions organised within ImagineNano in Bilbao 2011 & 2013, and in Barcelona from 2014 to 2023 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&Med2024 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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Nanoreactors for real time monitoring of the biomolecular markers in clinical diagnostics

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Basic vital parameters of the patient such as body temperature, pulse and respiratory rate, blood pressure can be continuously monitored at the patient's bedside. This is ensured by a series of physical sensors with an electronic readout. In contrast, real-time monitoring of the biomolecular markers still cannot be realized due to the lack of instrumentation that enables stable and continuous detection of biochemical processes with the sufficient time resolution. In acute and postoperative patients, the lack of real-time monitoring of biomarkers may delay adjustment of medical treatment in the event of complications and worsen prognosis.

We propose the system of nanoliter reactors fabricated using the droplet-based microfluidic approach as a tool to solve the aforementioned problem. The liquid can be divided into nanoliter compartments that contain the reagents for detecting different analytes in the sample, e.g. glucose, lactate or other protein-based species. Next, nanoliter compartments are transported towards detector and read-out one-by one, enabling time-resolved monitoring of the biomolecular markers. To enable real-time bedside examination of postoperative patients, we developed a portable, droplet-based fluid device. The clinical validation of the device is carried out using two models: the analysis of α-amylase levels in drainage secretions after abdominal surgery (1) and the lactate levels in blood and interstitial fluid in frames of animal studies (2). The presented droplet-based platform can be applied for analysis of different body fluids, diseases, and towards a broader range of biomarkers, including lipase, bilirubin, lactate, inflammation, or liquid biopsy markers, paving the way towards new standards in postoperative patient monitoring.

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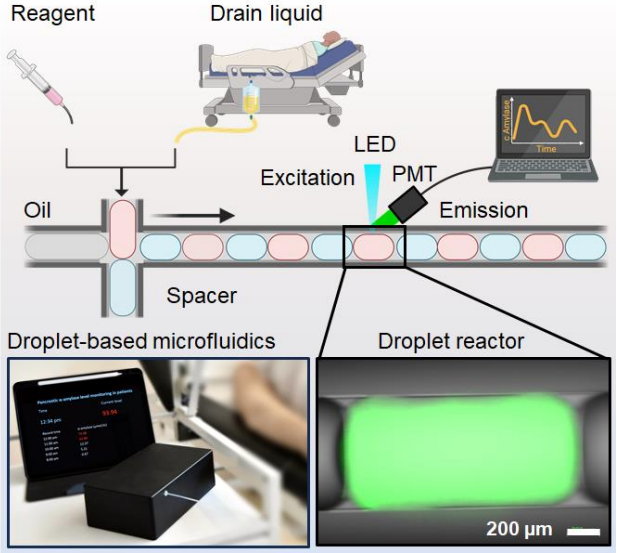

Figure 1. Detection concept: illustration of biomarker concentration detection using a droplet-based detector.

Nanoneedles for Cell and Gene Therapy

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Nanoneedle arrays, consisting of high-aspect-ratio nanostructures on nanotextured surfaces, have emerged as powerful tools for probing and manipulating the intracellular environment. Their unique design allows them to interact with cells with minimal perturbation, making them highly effective in delivering a variety of therapeutic agents—including small molecules, nucleic acids, proteins, and nanoparticles—into cells without inducing toxicity. Notably, nanoneedles have shown high efficiency in targeting cells that are traditionally difficult to transfect, such as primary human cells, stem cells, immune cells, and neurons. This capability is unlocking new possibilities for the ex vivo genetic engineering of primary cells with unprecedented efficiency. Moreover, the application of nanoneedles in vivo, particularly on the skin and eyes of live animals, is paving the way for novel topical therapies involving nucleic acids and biologics. These advances underscore the potential of nanoneedles in developing advanced therapy medicinal products (ATMPs), including cell and gene therapies, as well as CRISPR-based gene editing.

This talk outlines our progress in developing nanoneedles for topical gene therapy and for gene editing of cells used for autologous cell therapies. It will highlight fabrication methods for incorporating nanoneedles within a broad range of medical devices, including bandages, contact lenses, catheters and hydrogel. It will demonstrate the application of nanoneedles to skin wounds in epidermolysis bullosa for Collagen Type VII gene editing and gene therapy, and nucleic acid therapy for the topical treatment of corneal endothelial dysfunction. Nanoneedles also mediate the base editing of primary skin fibroblasts and the introduction of chimeric antigen receptor genes in regulatory T cells for cell-based therapies in epidermolysis bullosa and graft-vs-host disease.

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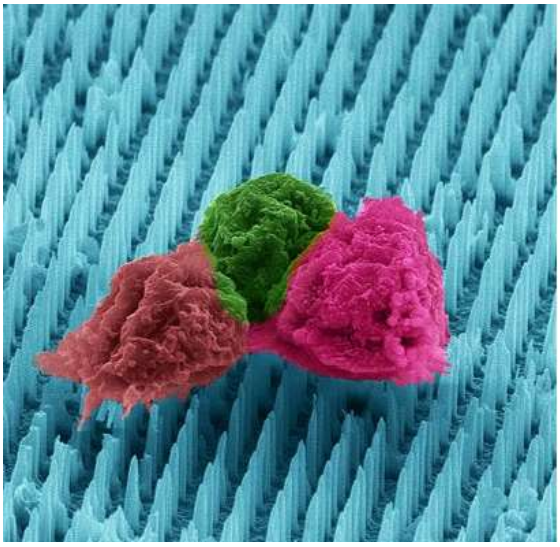

Figure 1: Scanning Electron Micrograph of three cells on nanoneedles.

Hybrid nano-delivery systems for micrornas in cardiac regenerative medicine: design, characterisation and preclinical testing in *in vitro* 2d and 3d tissue models

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Cardiovascular diseases are the leading cause of death. Among them, myocardial infarction (MI) causes the irreversible loss of cardiomyocytes, replaced by a dysfunctional scar tissue, mainly populated by cardiac fibroblasts, which often leads to heart failure [1]. Recently, specific microRNAs (miRNAs) have been identified for post-MI treatment, being able either to induce the proliferation of cardiomyocytes, or to trigger the direct reprogramming of cardiac scar fibroblasts into cardiomyocytes, or to reduce fibrotic response [2-4]. However, gold-standard lipid nanoparticles (LNPs), clinically approved for different RNA therapies, such as the vaccination against Covid-19 and the treatment of liver-related diseases, suffer from poor biological half-life and main accumulation in non-target organs, mainly the liver [4]. Hence, safe, precise and efficient nanoparticles for the *in vivo* delivery of miRNAs to cardiac tissues are currently missing and needed for future therapeutic applications of miRNAs.

To this purpose, firstly we have developed new lipoplexes, based on [2-(2,3-didodecyloxypropyl)-hydroxyethyl] ammonium bromide (DE) and L-alpha-dioleoylphosphatidylethanolamine (DOPE), showing improved biocompatibility and ability for intracellular miRNA delivery respect to commercial transfection agents [4]. Then, for *in vivo* applications, we have designed and patented novel ligand-functionalized polymer-lipid hybrid nanoparticles (f-HNPs/miRNAs), having: (i) a lipidic core for high miRNA loading; (ii) a synthetic polymer shell, improving biological half-life and allowing controlled and sustained miRNA release; (iii) specific selected ligands, grafted on the nanoparticle surface, for active cell targeting. The f-HNPs/miRNAs showed higher miRNA encapsulation efficiency (99% vs 65%) and biocompatibility (80-100% vs. 50-60%) and similar transfection ability than commercial transfection agents in 2D cell models. In parallel, reliable *in vitro* models replicating human cardiac scar tissue were engineered for more reliable preclinical validation of nanomedicine approaches. To reproduce cardiac fibrotic tissue hallmarks (extracellular matrix architecture, composition, and stiffness), electrospun scaffolds with biomimetic architecture, biochemical properties and surface stiffness were prepared, based on randomly-oriented poly(ε-caprolactone) PCL nanofiber mats, surface grafted with human type I collagen (C1) and fibronectin (F) [5]. Human cardiac fibroblasts (CFs) underwent phenotype switch into myofibroblasts upon 7 days culture on the biomimetic scaffolds, without the need for transforming growth factor-beta (TGF-β). Model reliability and predictivity was validated upon antifibrotic drug treatment [5], showing the downregulation of fibrotic markers (α-SMA expression, and cell-produced C1 and F). The model was then exploited for the study of biocompatibility, internalization ability and efficacy of f-HNPs/miRNAs, loaded with negmiR, siRNA-Cy5 and a set of four reprogramming miRNAs, respectively. Design of complex models including other cells, e.g., cardiomyocytes, is ongoing to assess precision cell delivery. Finally, *in vivo* preclinical trials in a mouse model of chronic MI showed significantly improved heart biodistribution of f-HNPs/siRNA-Cy5 than control unfunctionalized HNPs/siRNA-Cy5, minimizing liver, spleen and kidney accumulation. *In vivo* acute toxicity tests in mouse models showed the safety of f-HNPs/miRNA. Cardiac ejection fraction was enhanced 30 days post-MI treatment with reprogramming miRNAs by f-HNPs.

Hence, f-HNPs/miRNA showed safety and target efficiency in 2D and 3D human fibrotic cardiac tissue models and in an *in vivo* mouse model of MI. By changing miRNAs and surface functionalization, other applications of f-HNPs are under exploration, such as the induction of cardiomyocyte proliferation.

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Biomedical microdevices enabled by microfluidic technology

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Microfluidics is the science and technology of manipulating and analyzing fluid flow at small scales, typically from millimeters down to micrometers. At these scales, fluid flow is almost always laminar which enables excellent control over the flow. Another special feature at these scales is the dominance of surface tension, which provides the possibility to exploit capillary effects to effectively manipulate fluids. Microfluidic devices can be made using a range of microfabrication approaches and materials, and these enable to integrate tailored electronic or mechanical functions.
These unique properties of microfluidic technologies enable a range of new biomedical applications. In this lecture, I will show three microfluidics-technology-enabled biomedical microdevices we are developing in our lab. (1) A cancer-on-chip device in which can create a breast duct and a blood vessel to mimic and study the process of cancer cell invasion, migration, and intravasation. (2) A wearable sweat sensing device that is clamped on the finger of hospitalized patients so that critical biomarkers like lactate, cortisol, and glucose can be continuously monitored. (3) A smart glaucoma eye implant in which a magnetic microvalve is integrated that, after implantation, can be switched using an external magnet to keep the eye pressure within safe limits..

Figures

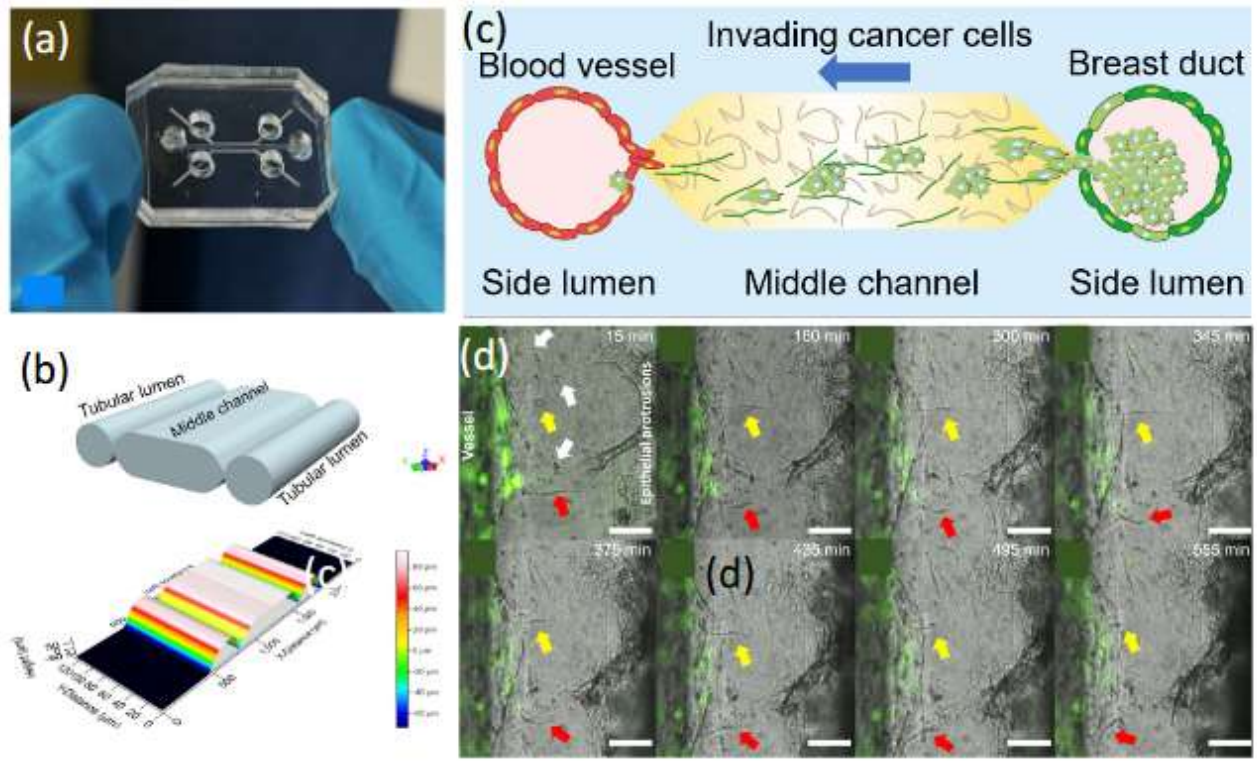

Figure 1. Cancer-on-chip device to study tumor cell invasion, migration, and intravasation. (a) Photo of the device, made in glass using femtosecond laser machining. (b) The channel structure consisting of two lateral tubular channels and a middle compartment; The micro-gaps connecting the lateral tubular channels to the middle compartment are fully open along the main axes of the channels. (c) Schematic representation of the cross section of the chip, depicting cancer cell invasion from a breast duct epithelium into the collagen I
matrix,
and further intravasation into the vessel. (d) Images of cancer cell invasion and intravasation progression at several time frames up to 555 min after 6 days in culture (triculture of MCF10a, MDA-MB-231, and HUVECs). Arrows show the cancer cells in the collagen I between the epithelium (right) and endothelium (left); yellow and red arrows in each point to an individual cancer cell migrating towards and intravasating into the vessel. HUVECs (green). Scale bar, 100 μm.

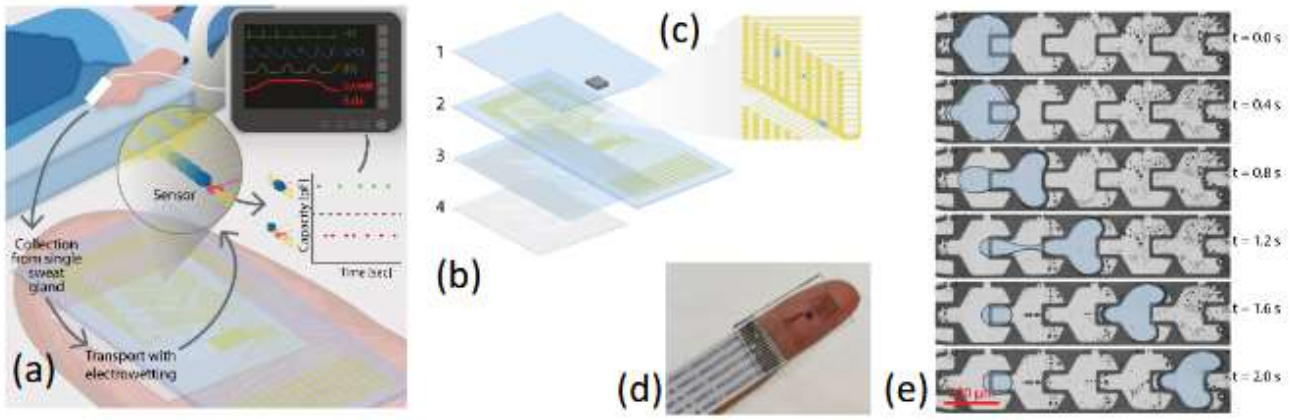

Figure 2. Wearable sweat sensing device**.** (a) The sweat sensor device is clamped on the patient's finger, continuously samples sweat, and determines biomarker content. (b) Exploded view of the device with different device layers: (4) patterned medical tape, (3) glass collection plate, (2) electrowetting layers, (1) biosensor location. (c) Top view of one electrowetting structure section. (d) The actual device on a fingertip. (e) Demonstration of electrowetting transporting sub-nanoliter sweat droplets within the device

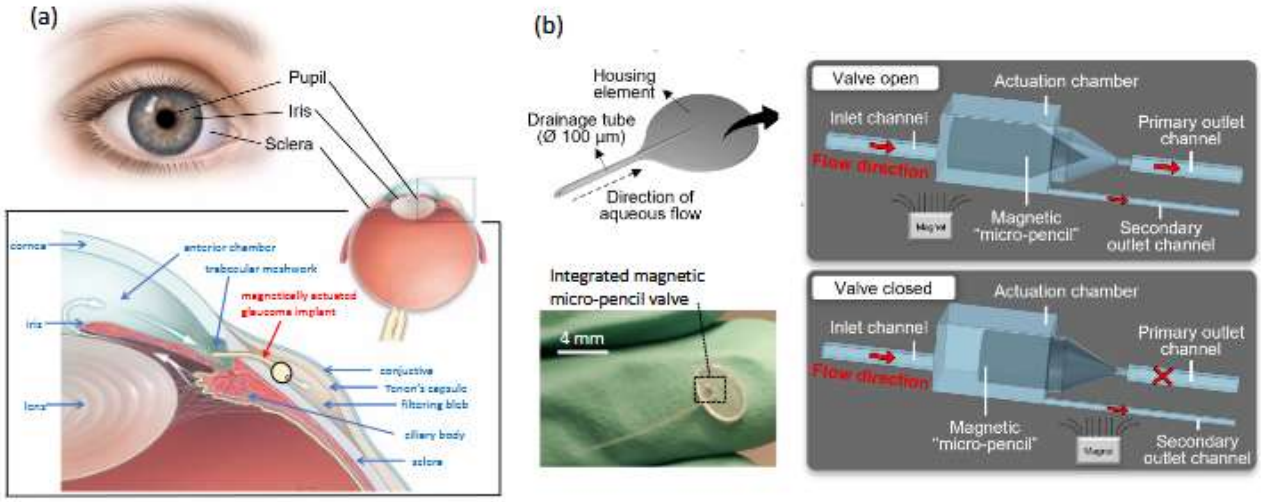

Figure 3. Smart glaucoma drainage device. (a) Anatomy of the eye and placement of the magnetically actuated glaucoma implant in the eye. (b) Schematic depiction of the magnetically adjustable glaucoma implant design, a photo of the actual device, and the actuation mechanism of the integrated micro-pencil valve; the total length and largest diameter of the magnetic micro-pencil are 1 mm and 350 µm.

Engineering Protein-Nanomaterial Composites: Advanced Tools in Nanomedicine

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The treatment and diagnosis of many diseases still remain a challenge. Inspired by nature, we explore biomolecules and their derivatives as novel therapeutic/diagnostic agents. Among biomolecules, proteins rise huge interest due to their high versatility, biocompatibility, and biodegradability. In particular, we use a class of engineered repeat proteins, the consensus tetratricopeptide repeat (CTPR) proteins due to their stability and robustness as a base scaffold that can be easily tailored to endow desired functions to the protein. For example, the introduction of metal-binding residues (e.g., histidines, cysteines) drives the coordination of metal ions and the subsequent formation of nanomaterials.[1,2] Additionally, new binding capabilities can be encoded within the CTPR unit or this can be conjugated with other peptides/proteins.[3] These properties allow the development of protein-nanomaterial composites.[2,3,4] Generally, the fusion of two distinct materials exploits the best properties of each, however, in protein-nanomaterial composites, the fusion takes on a new dimension as new properties arise.

These composites have ushered the use of protein-based nanomaterials as biopharmaceuticals beyond their original therapeutic scope and paved the way for their use as theranostic agents. In this context, engineered proteins have emerged as promising scaffolds to hold simultaneously therapeutic and diagnostic functions, as has been recently demonstrated in our pioneering in vitro and in vivo examples.[3,4,5]

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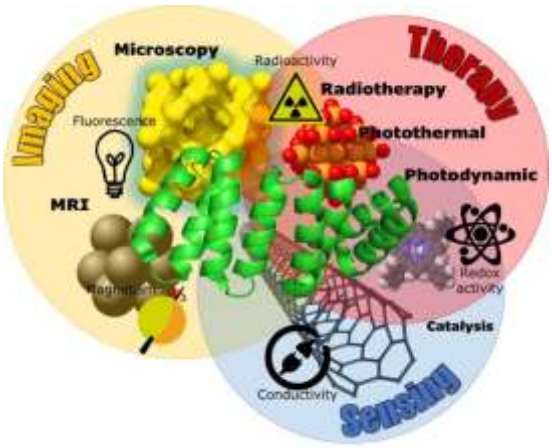

Figure 1. Scheme of engineered protein-nanomaterial composites and potential biomedical fields of application.

Nanoactuators for Therapy and Diagnosis

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In the last decades, inorganic nanoparticles have been steadily gaining more attention from scientists from a wide variety of fields such as material science, engineering, physics, or chemistry. The very different properties compared to that of the respective bulk, and thus intriguing characteristics of materials in the nanometer scale, have driven nanoscience to be the center of many basic and applied research topics. Moreover, a wide variety of recently developed methodologies for their surface functionalization provide these materials with very specific properties such as drug delivery and circulating cancer biomarkers detection. In this talk we describe the synthesis and functionalization of magnetic and gold nanoparticles as therapeutic and diagnosis tools against cancer. Gold nanoprisms (NPRs) have been functionalized with PEG, glucose, cell penetrating peptides, antibodies and/or fluorescent dyes, aiming to enhance NPRs stability, cellular uptake, and imaging capabilities, respectively. Cellular uptake and impact were assayed by a multiparametric investigation on the impact of surface modified NPRs on mice and human primary and transform cell lines. Under NIR illumination, these nanoprobes can cause apoptosis. Moreover, these nanoparticles have also been used for optoacoustic imaging, as well as for tumoral marker detection using a novel type of thermal ELISA and LFIA nanobiosensor using a thermosensitive support.

Developing optical structures based on nanoporous anodic alumina for biomedical applications

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Nanoporous anodic alumina (NAA) is a popular porous material obtained by the electrochemical anodization of aluminum. NAA is based on a cost-effective technology and their structure presents a self-ordering defined by a close-packed hexagonal array of well-defined cylindrical nanopores. NAA's versatility and potential are demonstrated by its impressive set of properties, which include nanopores with high aspect ratio, optical properties, chemical resistance, thermal stability, and intrinsic photoluminescence [1-3].

Their geometric characteristics such as pore diameter and length and interpore distance can be precisely determined by the anodization conditions such as voltage and time of anodization, temperature, and electrolyte and by post-anodization treatments (etching and annealing) [4-5]. The highly effective surface area (hundreds of m^2/cm^3) makes of NAA an interesting platform for sensing and loading and releasing of active agents [6-7]. Recently, different anodizing approaches have been proposed to create new structures and pore geometries. The application of periodic variations of current or voltage (square, sinusoidal, Gaussian waves) during anodization transfers to the material as a periodic variation of the pore diameter, and consequently, it is possible to design 3D structures and photonic structures with stop bands tunable within the UV-VIS-NIR range [7-9].

This work will focus on recent advances in the design and fabrication of NAA and their photonic and optic applications as optical biosensing. We will analyze different technological parameters and its effect on the structure and present examples of biosensing based in reflectometric interference spectroscopy, surface plasmon resonance, Tamm plasmon resonance, photoluminescent spectroscopy and surface-enhanced Raman scattering [10-11]

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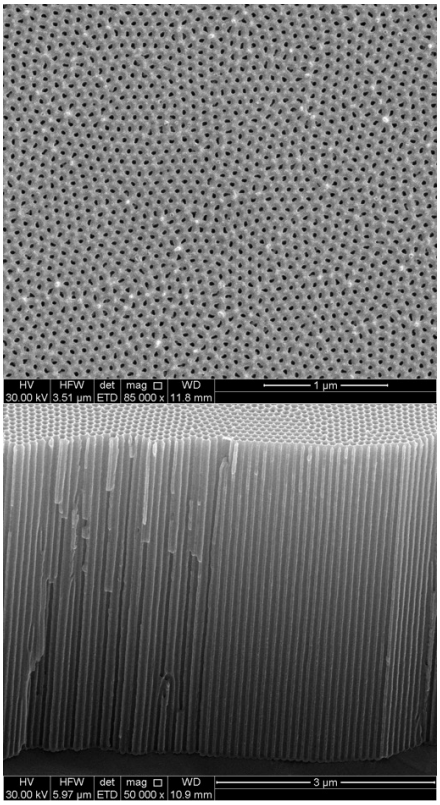

Figure 1. Field Emission Scanning Electron Image of a top view (up) and cross section view (bottom) of a NAA structure.

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Multifunctional neural interfaces with tapered optical fibers

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The adoption of multimodal optical fibers to access deep brain regions has empowered the neurophotonic community to pioneer new frontiers in optically interfacing with the mammalian brain. These advancements not only enhance optical control and monitoring of neural activity but also integrate multiple capabilities into a single device, including electrophysiological and neurochemical detection. Furthermore, they accelerate the exploration of emerging optical neural interface paradigms, such as surface plasmon resonances, which are still in their early stages of development.

Within this framework, this presentation will delve into neural interfaces that leverage the synergistic combination of microfabrication, nanotechnologies and modal properties of tapered optical fibers (TFs) [1-5]. We will discuss how the wide surface area of the fiber taper enables wide-volume optical neural interfacing with deep brain regions, with depth resolution.

Leveraging the broadband nature of the optical neural implant, we will describe how visible spectral range can be employed for control and monitoring of neural activity, while the near-infrared enables to gather spontaneous Raman spectroscopy to extract relevant information on the cytoarchitecture of the mouse brain. This allows monitoring molecular alterations linked to circuit dysfunction as well as diagnostic markers of various pathologies.

Using an original two-photon lithography approach to pattern the surface of the taper, the edge of tapered fibers can be patterned to host microelectronic elements. These include electrodes for extracellular recording of neural activity, generating optrodes that can interact with the tissue with both electrical and optical signals. Resistive temperature sensors can also be integrated with the same technology, allowing to better study the effects of light radiation on neural tissue. This non-planar patterning can achieve resolutions down to a few nanometers through unconventional bottom-up nanofabrication, paving the way for the implementation of Surface Enhanced Raman Spectroscopy (SERS) and thermoplasmonics in neuroscience research.

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Figures

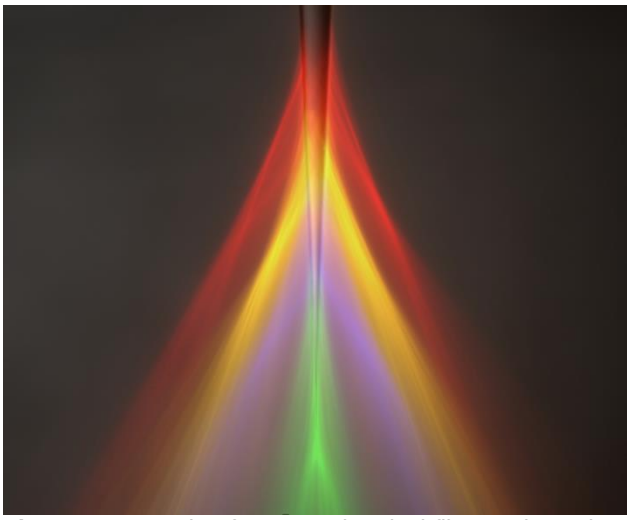

Figure 1. Example of a tapered optical fiber. False colors represent different modal subsets that can exchange energy with the surrounding environment at different section of the taper

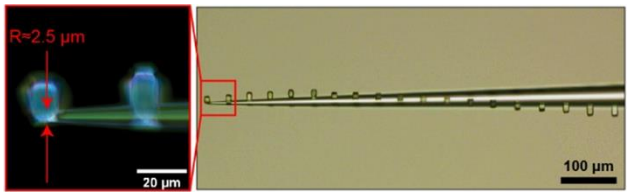

Figure 2. Example of two-photon patterning on the edge of tapered optical fibers

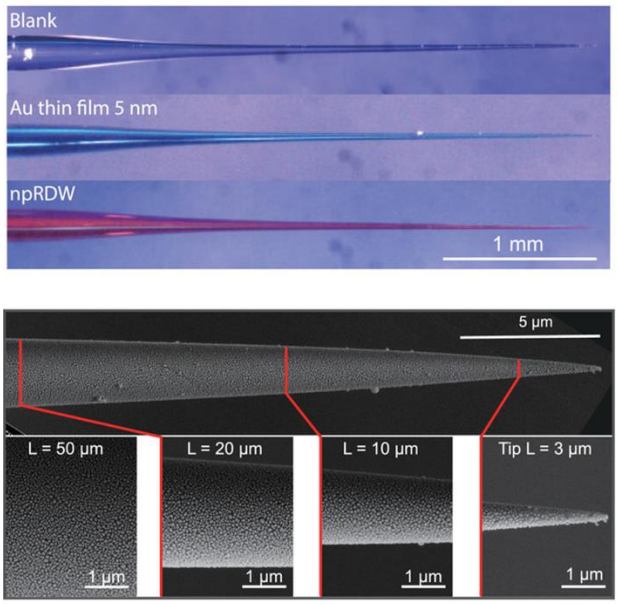

Figure 3. Nanoparticles nucleated on the tapered fiber's surface.

Molecular Electronics with DNA towards Detection of Nucleic Acids

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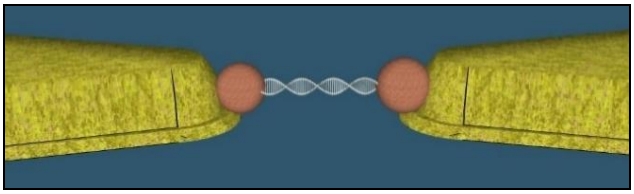

Abstract

The DNA double-strand recognition, as well as the ability to manipulate its structure open a multitude of ways to make DNA useful for molecular electronics. We recently reported a breakthrough in measuring charge transport in DNA (Nature Nanotechnology 2020) in a special configuration. This finding is of great importance by itself for understanding electricity in DNA in particular, and for molecular electronics in general. However, it also paves the way for the design of new ultra-sensitive detectors for DNA and RNA. Addressing these challenges is at the heart of early detection of cancer, pathogens, emergency medicine as well as for pandemics like the COVID-19.

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Cerium oxide Nanoparticles protecting tumoral cells and restoring immunosurveillance cure cancer

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An increased metabolic activity is correlated to cancer cell survival and proliferation. The increased metabolic activity of cancer cells results in an increase of oxidative stress, driving metabolic shifts that interfere with the immune response to malignant cells. A single injection of Reactive Oxygen Species (ROS) scavenging cerium oxide nanoparticles into an angioimmunoblastic T cell lymphoma mouse model showed preferential accumulation of the NPs in the spleen where an increased mitochondrial activity and increased ROS concentration was reverted to redoxtasis. In addition, in the treated models, T-CD4+-PD1high cells driving malignancy were significantly reduced accompanied by an antitumoral activation of previously exhausted T-CD8+ cells, all in all, dramatically increasing survival rates.

Plasmonic hollow nanocapsules as a versatile platform for cancer management

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We present a nanotechnological platform consisting of 3 main building blocks i.e., a cancer target, a monoclonal antibody towards this target, and a nanoformulation. The main working principle of this platform is based on tumor targeting. We specifically recognize Zip4, a tumor target presents only in the epithelium of tumoral cells. Based on this recognition, the platform can target and controlled the delivery of an active ingredient.
To validate the technology, we first proved in vitro and in vivo specific tumor targeting and now we are validating the efficiency of targeting the chemotherapeutics vs. its non-targeted form. One big advantage of this platform is its versatility since the 3 building blocks can be used all together or independently. Furthermore, the nanoformulation is itself composed of different building blocks that can be assembled differently yielding different properties to the nanomaterial. For example, in one configuration the nanomaterial has photothermal properties for melanoma treatment or for photodynamic therapy, while in other configuration it has SERS sensing properties. I will present this platform and examples for its biomedical applications.

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Beyond Intravenous Administration of Therapeutic Nanocarriers

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Nanocarriers specifically designed to improve pharmacokinetics and drug therapeutic outcomes have already demonstrated high potential for treating several complex pathologies. For that a rational nanocarrier design and an adequate administration are both necessary.

I will explain our advances proposing novel administration routes for nanomaterials to safely arrive at the targeted organs. The intravenous route still has many drawbacks, including side effects associated with systemic drug distribution and the high accumulation of most nanomaterials in the liver, kidneys, and spleen, which may cause chronic injuries or immune-mediated side effects such as infusion reactions.

The first example refers to endovascular brain delivery, targeting neuroregeneration after stroke [1]. The second one concerns nebulization to reach distal areas of the lungs for sepsis treatment.[2,3]

Figure

Figure 1. Nebulization of polymeric nanocarriers with commercial medical equipment.

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Biocompatible chemical nanobots and their applications in biomedicine

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Engineering medical nanomotors/nanobots will imply the use of biocompatible materials and bio-friendly propulsion mechanisms. Our strategy comprises the use of biocatalysts such enzymes for converting biologically available fuels, such as the urea contained in the urine, into a propulsive force. Moreover, nanoparticles' chassis are generally recognized as safe (GRAS) material, FDA or EMA approved materials.

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) providing swimming capabilities, biocompatibility, imaging, multifunctionality and actuation in vitro and in vivo. I will present some of the proof-of-concept applications of biocompatible nanobots such as the efficient transport of drugs into cancer cells and 3D spheroids (1), the imaging of swarms of nanobots in vivo in confined spaces like the bladder of living mice (2). Moreover, I will present our recent advances in the treatment of bladder cancer in mice using radionuclide-labelled nanobots (3) and crossing mucus layers present in the colon of mice (4).

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ENGINEERING AND CONTROLLING SYNTHETIC AND LIVING MICROROBOTS FOR BIOMEDICAL APPLICATIONS

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Current trends in healthcare are shifting from curative to predictive, personalized, and pre-emptive medicine. Medical microrobots have the potential to drive this transformation by enabling earlier diagnosis, patient stratification, improved treatment administration, and continuous monitorin [1].

In this talk, I will focus on the design and control of microbots to deliver drugs more effectively to targeted diseased sites—a long standing challenge in medicine. Transmitting magnetic fields to guide drug carriers to specific locations is promising, however, current methods often struggle with physiological barriers and are limited to accessible areas.
Here we propose the engineering of magnetic microrobots powered by scalable torque-based actuation via rotational magnetic fields to enhance drug delivery to deep-seated tumors. This approach is particularly effective for microrobots with high anisotropy, which can be boosted by both the shape and magnetocrystallinity of the magnetic materials used. We demonstrate biohybrid microrobots—live bacteria augmented with magnetic nanomaterials—that combine chemotaxis as autonomous navigation with external magnetic control. This hybrid strategy improves tumor targeting compared to unactuated controls [2,3].

Additionally, we have developed synthetic microrobots from biodegradable hydrogels engrafted with patterns of magnetite nanoparticles. By applying dynamic magnetic fields during microfluidic fabrication, we create anisotropic capsule-like microrobots with strings of nanoparticles [4]. These microrobots, actuated with rotational magnetic fields, effectively dissolve thrombi in vascular models and induce convection enhanced drug transport.

Furthermore, on the control side, I will introduce a method for spatially restricting rotating magnetic fields to enhance targeting precision and reduce side effects [5], along with a design integrating inductive feedback for real-time tracking under continuous actuation, enabling closed-loop control [6].

In summary, these advances promise to improve both the detection of diseases and the safety and efficacy of drug delivery, potentially paving the way for microrobots to be integrated into clinical practice.

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Figures

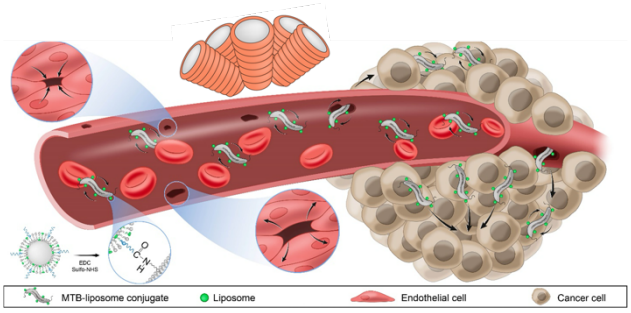

Figure 1. Concept of magnetically controlled biohybrid microrobots for enhanced drug delivery (from [2], Reprinted with permission from AAAS).

Exploring cancer cells through the lens of physics: the role of optomechanical technologies

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Historically, cancer has been regarded as a genetic disease, in which key mutations provide cells with the capability for uncontrolled growth and proliferation, the ability to avoid cell death in the primary tissue, and the potential to continuously mutate to achieve metastasis and drug resistance.
It is now recognized that tumorigenesis, tumor growth, and metastasis are closely linked to transitions in the mechanical properties and interactions of cancerous cells and their microenvironment [1].
Here, we will focus on two essential mechanobiological hallmarks that cancer cells undergo: i) continuous and dynamic cytoskeleton remodeling adapted to cancer progression, and ii) increased mechanical power linked to dysregulated metabolism in cancer.
These hallmarks are studied using a set of optomechanical technologies, including atomic force microscopy [2] , quantitative phase imaging [3] , and micromechanical sensing devices (**Fig. 1**). These techniques have been developed with distinguishing theoretical and computational methods, including denoising algorithms, image processing, and inverse problem methods. These methods enable the study of the mechanical properties of cancerous cells with enhanced specificity, visualize intracellular fluctuations with unprecedented sensitivity, and provide the first evidence of the existence of vibration modes in complex human living cells under physiological conditions.

The results shed new light on the roles of cytoskeleton remodeling and altered metabolism in cancer. Moreover, the technologies are well-suited to determine the functional impact of genetic variants in cancer, which is an unmet need in precision oncology. Additionally, the discovery of mechanical resonances in living cells opens fascinating avenues for mechanobiological therapies.

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Figures

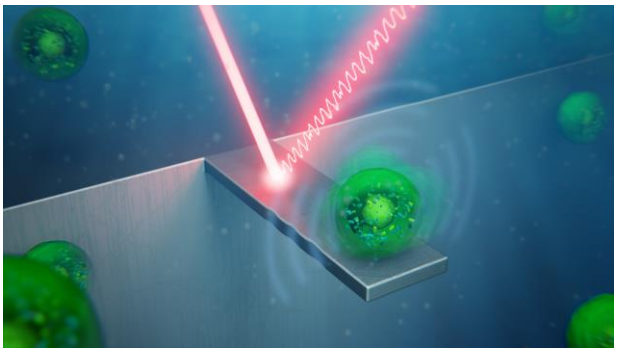

Figure 1. Schematic cartoon of a living cell attached to small microcantilever resonator.

Laser Nanostructured Reduced Graphene Oxide Films: A Cost-Effective and Scalable Approach for Biosensing Applications

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In this presentation, we introduce a laser nanostructuration technique for the fabrication of reduced graphene oxide (rGO) films embedded with metal nanoparticles (MNPs), offering a cost-effective, scalable, and versatile approach to biosensor development. This single-step process combines the simultaneous reduction of graphene oxide and deposition of metal nanoparticles (such as gold, silver, and platinum) to create conductive, nanostructured surfaces with enhanced electrochemical properties. The method is notable for its simplicity and affordability, requiring minimal equipment and facilitating high-throughput production, making it suitable for point-of-care (PoC) diagnostics.
We will highlight the application of this technology in various biosensor formats, including capacitive immunosensing for the detection of clinical biomarkers like CA-19-9 glycoprotein, as well as in enzyme-free real-time monitoring of hydrogen peroxide released from live cancer cells. Additionally, we will explore the integration of rGO electrodes into lateral flow assays, improving their sensitivity and providing a promising platform for advanced PoC diagnostic tools. These examples illustrate the potential of laser nanostructured rGO films to address the need for affordable, reliable, and scalable biosensors for diverse diagnostic and monitoring applications.

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Acknowledgements

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ERC for Nanosciences

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The European Research Council (ERC), set up by the European Commission in 2007, is the premier European funding organisation for excellent frontier research. The ERC's mission is to encourage the highest quality research in Europe through competitive funding and to support investigator-driven frontier research across all fields, based on scientific excellence.

Through its long-term grants, it provides scientific freedom and financial stability to its grantees and the reputation of excellent research. ERC projects have led to more than 200’000 articles published in scientific journals, over 2’200 patents and other IPR applications, and ERC grantees founded or co-founded over 400 start-ups so far.

Based on my fourteen years of experience working at the ERCEA, I will attempt to demonstrate how ERC and its projects contributed to the progress of the various nano-research fields through data and successful project examples. I will present the available funding options and the peer-review evaluation process, and I will give some tips for those who intend to apply for an ERC grant in the future.

Figures

Photoporation as a Versatile Tool Against Cancer: From Gentle Immune Cell Transfection to a Powerful Approach for Inducing Anti-Tumor Immunity

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Photoporation is a technique based on the use of photothermal nanoparticles (NPs) and pulsed lasers in which a tremendous amount of light is absorbed in such a way that the water of the surrounding environment evaporates to form vapor nanobubbles (VNBs) emerging around the surface of the NP.[1] VNBs will first expand and then collapse, thereby generating fluid jets and high-pressure shockwaves, which can mechanically damage nearby biological structures. Photoporation have been successfully applied for delivery of a plethora of molecules to a wide variety of cell types,[2-4], and for disruption of biological barriers ex vivo,[5,6] and even in vivo for tissues easily accessible for laser irradiation.[7] Recent studies have demonstrated that photoporation from melanosomes could be used to induce immunogenic cell death (ICD), a process where damage-associated molecular patterns (DAMPs), such as ATP and calreticulin (CALR), are released or exposed at the cell's surface.[8] this novel finding positioned photporation as a promising strategy not only for direct cytosolic delivery but also as a therapeutic strategy for enhancing ant-tumor immunity.

In this talk, I will present our recent work focused on the use of photoporation for genetic engineering of NK cells. Our results demonstrated the successful transfection of NK cells with eGFP mRNA and gene editing by delivery of Cas9 RNPs for knock-out of the inhibitory receptor NKG2A. Importantly, no alterations to the phenotype of the cells (e.g. expression of surface markers and release of cytokines) could be detected, nor was the proliferation or cytolytic capacity of the cells influenced by either of the treatments. In addition, I will present our last results on the use of photoporation cell killing and exposure of ICD hallmarks upon irradiation.

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Figures

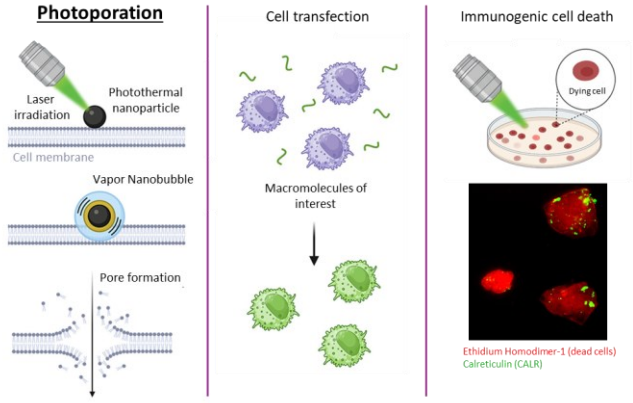

Figure 1. Photoporation as a versatile tool in immunology against cancer: (i) photomechanical effects upon pulsed laser irradiation of photothermal nanoparticles, as a consequence of the formation and collapse VNBs are exploited for (ii) transfection of immune cells and (iii) ICD induction.

Plasmonic hydrogels; enabling 3d sensing and imaging in breast cancer models

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The transition from traditional two-dimensional (2D) cell cultures to three-dimensional (3D) cell models has emerged as a pivotal bridge between in vitro research outcomes and their clinical applications. 3D cell models more accurately mimic the complex in vivo cellular microenvironments, offering valuable insights into cell behavior, disease monitoring or drug testing.

However, the adoption of 3D cell models has brought forth the need for improved imaging and sensing techniques capable of monitoring cellular events in these intricate 3D environments [1]. Traditional imaging techniques designed for 2D cell cultures often fall short when applied to 3D cell models due to their increased complexity and spatial arrangement. One promising solution is Surface Enhanced Raman Scattering (SERS), that allows for the spatiotemporal detection of biologically relevant molecules, the study of drug diffusion profiles or 3D imaging [2,3,4]. This technique utilizes the remarkable optical properties of noble metal nanoparticles (NP), which exhibit Localized Surface Plasmon Resonances (LSPR) that enable them to absorb and scatter light at specific wavelengths, generating high local electric fields on the surface [5]. These electric fields enhance the Raman scattering of molecules near the metal surface, permit extremely low detection limits, and multiplex detection ability. Additionally, the excitation wavelength can be tuned to the near infrared range (NIR) that corresponds to the biological transparency window (650-1350 nm), enhancing light penetration into tissues [6].

In this talk, different 3D models using biopolymers, synthetic polymers or decellularized extracellular matrix-based hydrogels containing plasmonic NPs for in vitro sensing and imaging will be presented. For sensing applications, bare gold NPs were employed for detection of cell-secreted metabolites whereas SERS imaging was performed using gold NPs decorated with SERS tags that can be also internalized by cells. Different material compositions, model configurations and NP combinations were explored in order to perform sensing as well as multiplex imaging of different cell populations in a 3D fashion.

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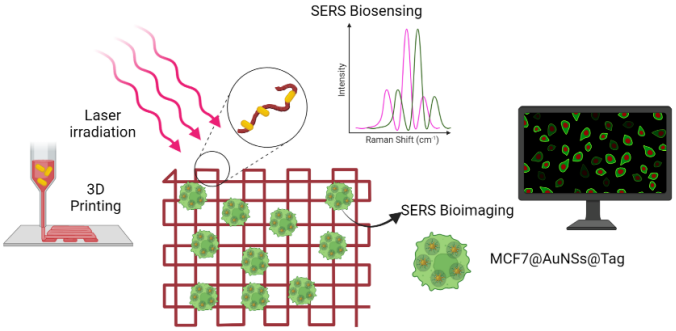

Figure 1. Schematic of 3D printed plasmonic scaffolds for label-free SERS sensing or containing SERS-tag decorated NP for SERS imaging of 3D breast cancer cell cultures.

Mecwins AVAC, a window opened on the world of proteins: from invading pathogens to fighter cytokines

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To be able to detect and quantify low-abundance proteins in biofluids for applications such as early disease diagnostics, highly sensitive detection methods are required. Mecwins main objective is to bring to the market its technology as a reference technique for medical testing through ultrasensitive detection of protein biomarkers, to help scientists and physicians reach the unreachable in the diagnostic field.

Mecwins uses its own revolutionary AVAC technology[1,2] based on single molecule digital counting to deliver ultra-high sensitivity, broad dynamic range, multiplexing capability, and a remarkable throughput. Mecwins' AVAC assay consists of two components: the AVAC cartridge to perform the sandwich immunoassay that relies in highly specific oriented-anchored antibodies bound to gold nanoparticles (GNPs) and to a dielectric surface (Figure 1), and the AVAC platform that functions as an optical reader and a particle counter (Figure 2).

Robustness of the AVAC technology has been demonstrated by developing several biological assays on oncology, cardiovascular, inflammatory and infectious diseases. In particular, the analytical validation of the p24 assay and the IL6 assay, that are aimed to detect early markers of HIV infection and of sepsis respectively, has demonstrated that the AVAC assays are capable to perform with a sensitivity several orders order of magnitude better than conventional assays, with good accuracy, and with excellent correlation with gold standard assays.

These results endorse Mecwins' AVAC technology as a key tool to open new options in the diagnosis and treatment, and to contribute to improve the quality of healthcare of those sections of the population particularly at risk.

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Figures

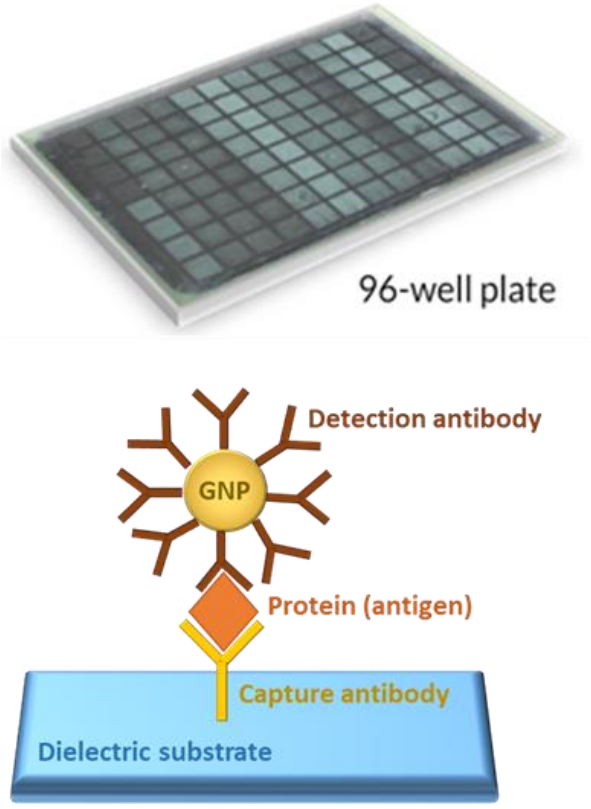

Figure 1. AVAC cartridge and sandwich immunoassay scheme.

Figure 2. AVAC platform.

Implementing user-friendly electrochemical nanobiosensors to improve the management of malaria

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Malaria is one of the most widespread parasitic diseases and ranks among the top three deadliest infectious diseases globally, alongside HIV and tuberculosis. This ranking is due to its high mortality rate and broad prevalence. In 2022 alone, malaria claimed over 600,000 lives, predominantly affecting children, pregnant women, and populations in non-endemic areas. Despite significant reductions in malaria cases due to aggressive public health initiatives and the advent of new vaccines, malaria continues to be a leading cause of death worldwide. This ongoing threat underscores the World Health Organization's emphasis on developing reliable point-of-care (PoC) diagnostic tests, which are crucial for guiding clinical decisions and assessing the impact of epidemiological interventions.[1]

Current diagnostic methods for malaria rely primarily on microscopic techniques and polymerase chain reaction. Although these methods are sensitive and specific, they are impractical for widespread screening due to their dependence on specialized personnel and equipment. Lateral flow assays (LFAs) have become the preferred diagnostic tools in low- and middle-income countries thanks to their affordability and ease of use. However, despite offering clinical sensitivities and specificities above 95%, the performance of LFAs has been compromised by the loss of the histidine-rich protein-2 (HRP2) antigen, leading to increased false-negative results, especially in cases of Plasmodium falciparum infection. To mitigate this issue, some LFAs now include additional biomarkers, such as lactate dehydrogenase (LDH). Nonetheless, LFAs still suffer from significant drawbacks, including subjective result interpretation and a lack of quantitative output, which hinders their ability to assess disease severity. This highlights the pressing need for innovative biosensors that can provide rapid, reliable, and quantitative diagnostics to enhance malaria management, prognosis, and the allocation of healthcare resources.[2]

Electrochemical nanobiosensors, particularly aptamer-based electrochemical sensors, are becoming increasingly recognized as viable solutions for diagnosing diseases in settings with limited resources. These sensors are user-friendly, cost-effective, and robust, making them ideal for widespread deployment. They function by detecting binding-induced conformational changes in the aptamer upon encountering a target molecule, which then generates a quantifiable electrochemical signal.[3] This innovative mechanism enables rapid, sensitive, and precise analysis, thus empowering healthcare workers to make well-informed decisions directly at the point of care. A key advantage of these sensors is their capability to accurately quantify both parasite and host biomarkers. The levels of these biomarkers can be directly correlated with the severity of the disease, enabling healthcare providers to effectively triage patients based on the risk of developing severe symptoms.

In this presentation, we will first address the unique challenges associated with diagnosing malaria and, crucially, providing timely prognosis. We will then explore a range of available nanobiosensing technologies designed to overcome these challenges, with a specific focus on a recently developed electrochemical aptamer-based sensor that facilitates the quantification of the malaria biomarker LDH (Figure 1). Furthermore, we will discuss the future steps necessary for implementing these sensors into low-cost, graphene-based portable electrodes,[4] potentially transforming malaria diagnostics and contributing significantly to global health efforts.

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Figure

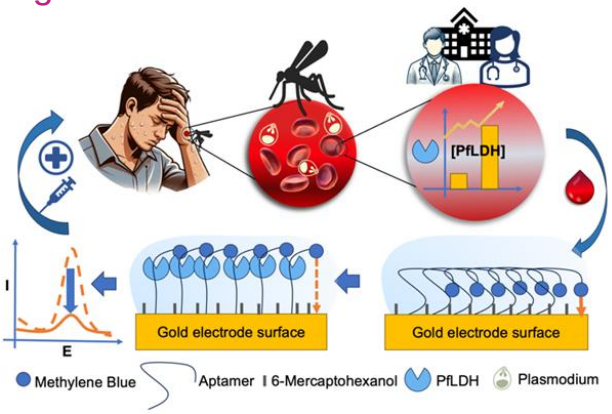

Figure 1. The detection of the malaria biomarker LDH may allow the prompt diagnosis and timely prognosis of malaria at the point of care.

Aggregation Dynamics of Misfolded Proteins in Water using Liquid Phase Transmission Electron Microscopy

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Liquid-phase transmission electron microscopy (LPTEM) offers exceptional capabilities for imaging time-resolved structures in their native liquid environment, eliminating artefacts associated with traditional drying or cryogenic treatments. One of the most promising applications of LPTEM is the investigation of molecular structures in cells, such as proteins.[1] The liquid nature of the sample allows access to previously unreachable protein states, as it permits the free movement of soft structures during imaging. This unique feature provides a significant advantage for structural biology research, allowing for real-time exploration of the protein structural landscape.

In our study, we used LPTEM to gain unprecedented insight into the dynamics of misfolded protein aggregation and its structural evolution over time, focusing on amyloid beta peptide (Aβ), which readily aggregates into amyloid fibrils. This process has been intensely studied due to its association with Alzheimer's disease (AD). However, directly observing the microscopic steps in the aggregation reaction and the characterization of intermediate oligomeric assemblies have been highly challenging. We employ LPTEM in combination with all-atom molecular dynamics simulations to enhance our understanding of protein dynamics.

Our findings provide the first visualisation of the dynamics of Aβ oligomers, the formation of Aβ protofibrils, and the interaction of Aβ oligomers with fibril surfaces. [2] This work demonstrates how LPTEM can be utilised to image key molecular events in the Aβ aggregation process, contributing to a deeper understanding of protein aggregation in solution, particularly in the context of Alzheimer's disease.

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RED BLOOD CELL ACTIVITY UNDER OPTICAL TWEEZERS

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Red blood cells (RBCs) possess unique mechanobiology that allows them to navigate efficiently through capillaries smaller than their own size. This ability is underpinned by the active biomechanics of the cells, which is driven by glycolytic ATP production and manifests as out-of-equilibrium fluctuations of the plasma membrane [1, 2]. These fluctuations, often seen as enhanced flickering, are propelled by motor proteins that mediate interactions between the spectrin skeleton and the lipid bilayer [3].

However, modulating this flickering in living RBCs without permanently altering their mechanical properties has been a significant challenge.

Our study aims to explore the effect of optical tweezers on RBC membrane flickering activity. We applied optical tweezers directly on individual RBC membrane. Our approach allows (i) for sensing the local force exerted by active kickers and (ii) for inducing a programmable trapping potential that can modulate flickering oscillations [4]. Our optical tweezers show negligible phototoxicity and a reversible manipulation of membrane flickering. We underscore the critical role of ATP in maintaining RBC membrane flexibility and dynamic behavior. Our findings provide insights into the mechanisms of RBC deformability and pathological conditions and set the basis for a novel approach to the optical control of biophysical forces.

Figures

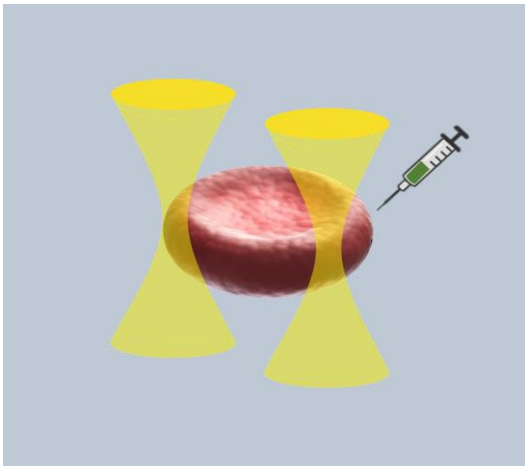

Figure 1. Schematics of a single red blood cell tested by multiple optical tweezers under different drug treatment.

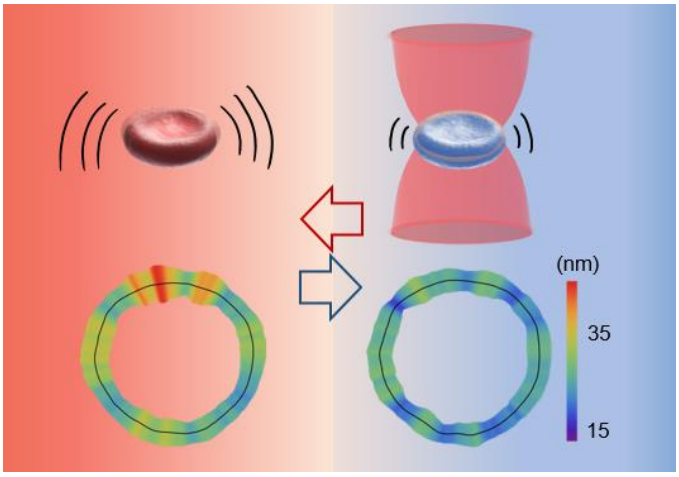

Figure 2. Free standing and active RBC (left panel) reversibly trapped by holographic optical tweezers (right panel), alongside the corresponding membrane deformation maps.

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PExM: polyplex expansion microscopy for cell trafficking studies

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Nanomedicine is a field at the intersection of nanotechnology and medicine, promising due to its potential to revolutionize healthcare [1]. Despite its long trajectory, there is still a long road ahead for its full development, and smart design of nanomedicines is still a challenge. Among other problems, this is due to the scarcity of tools available for the precise visualization and comprehension of nano–bio interactions, impeding progress towards the clinical phase [2]. One of the developed tools that stands out to be a strong nanoscopy technique for studying nano-delivery systems within cellular environments is expansion microscopy (ExM). This technique was used for tissue and cell expansion **(Figure 1)** and most recently for lipid molecule expansion inside cells [3]. Herein, we present for the first time polyplex expansion microscopy (PExM); a comprehensive examination of ExM as an already developed technique, but adapted for expanding polymer based nanocarriers, in particular polyplexes within cells, allowing the analysis of their trafficking **(Figure 2)**. With our method set up, PExM will be extensively used for the study of polyplex nanoparticle cell trafficking, becoming a high-resolution technique which can also be applied to primary amine containing polymeric nanoparticles without requiring expensive super-resolution microscopes.
This work has been published [4].

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Figures

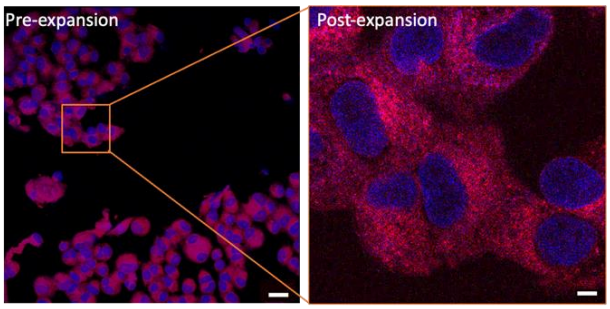

Figure 1. Isotropic expansion where pre-expanded and post-expanded cells images are shown. Scale bars are 5 µm for pre-expansion image and 23 µm for the post-expansion image.

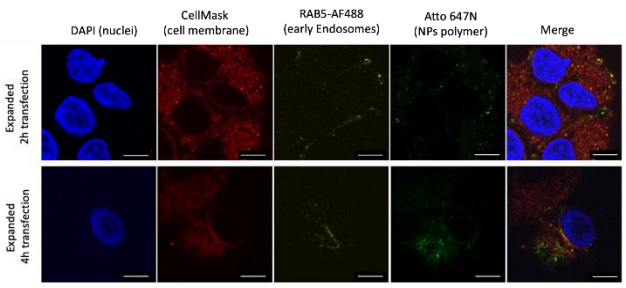

Figure 2. A549 cells expanded images with different pBAE NPs transfection timings. Nanoparticles were transfected for 2 and 4 hours and expanded with PExM protocol. Scale bar of 93 µm.

NON-VIRAL NANOVECTORS TO ENGINEER T CELLS

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Nowadays, the use of engineered immune cells is one of the most promising approaches to treat cancer [1]. In particular, adoptive T cell therapies are widely used for haematological cancers and the principal way to deliver nucleic acids into cells is based on viral vectors [2, 3]. Notwithstanding the success of this approved treatment, the use of viral vectors faces with some obstacles such as their complex, highly specialized and expensive manufacture process, as well as limits in genetic cargo capacity, biocompatibility, variability of transduction efficiency between patients, and the risk of semirandom gene integration with potential oncogenic transformation/clonal expansion [4, 5]. In this context, we focused on the development of nanovector-based approach to genetically engineer T cells, which proved being challenging since primary T cells are refractory to transfection [6]. To achieve this aim, it is important to consider not only the physico-chemical properties of nanocarrier but also the phenotype and the status of T cells. Indeed, memory T with stem cell-like (T_{SCM}) phenotype creates a link between naïve and central memory cells entailing an increased proliferation capacity and a superior antitumor effector function [7]. In order, to investigate several non-viral vectors, T cells are isolated from healthy donors and activated for proliferation and expansion. We are investigating different interleukin concentrations (IL-2, IL-7, IL-15 and IL-21) to address a T_{SCM} phenotype by monitoring T cell activation marker and Wnt-B-catenin signalling pathway [8]. Two different approaches are currently under investigation to reach high transfection of T cells. The first relies on the employment of polymeric nanovectors characterized by high degree of biocompatibility and hemocompatibility; the second one embraces a biomimetic coating consisting of either membranes derived from red blood cell (RBC) functionalized or not with DOPE (1,2-Dioleoyl-sn-glycero-3-phosphoethanolamine), or a coating derived from peripheral blood mononuclear cell (PBMC) membranes. From preliminary results we obtained a good degree of balance between biocompatibility and transfection with a polymeric nanoparticle (around 13 %, EGFP as reporter gene). However, some nanovectors induce cytotoxicity despite single constituents are biocompatible (Fig.1), thus opening questions about the role of nanostructuration and its influence on T cells. The employment of membranes-coated nanovectors resulted in poor improvements on transfection efficiency. Interestingly, by exploring PBMCs-membranes coating from cells stimulated with a lipopolysaccharide to increase the superficial interaction receptors, we noticed that the autologous targeting of stimulated-PBMCs vehicles is higher than unstimulated PBMCs- and RBCs- membranes vehicles (Fig. 2).
In conclusion, the development and the assessment of T_{SCM} along with the design of biocompatible and selective non-viral nanovectors will allow us to pave the way for the generation of highly efficient nanovectors for nucleic acids delivery into immune cells.

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Figures

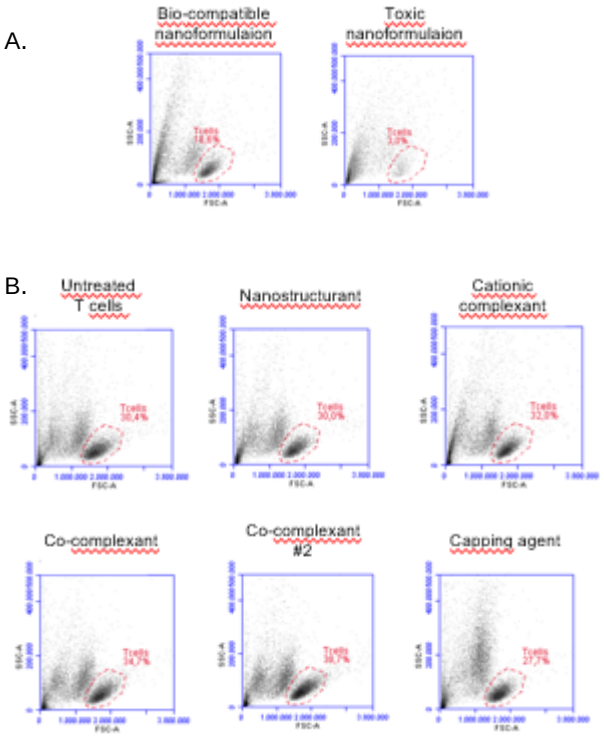

Figure 1. Primary T cells treatment with (A) two different polymeric nanostructured complexes and (B) complexes' single component.

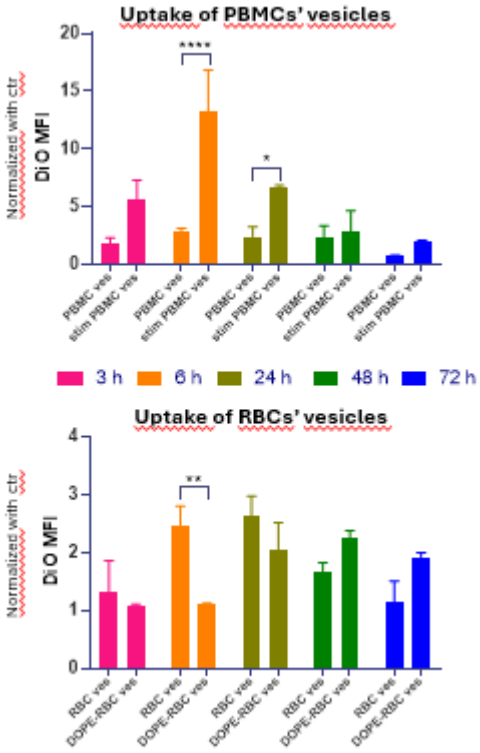

Figure 2. Uptake experiment to detect the targeting of PBMCs' vehicles (stimulated and not) and RBCs' vehicles (functionalized and not) at different time points.

TRIGGERED PROTEIN RELEASE FROM CALCIUM ALGINATE / CHITOSAN GASTRO-RESISTANT CAPSULES

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Therapeutic peptides and proteins are typically administered via parenteral routes due to their instability in the gastrointestinal tract, which causes degradation of these active components and limited absorption through the intestinal mucosa [1]. However, parenteral administration poses challenges when sustained therapeutic levels are needed or frequent dosing is required, often resulting in badly administered doses.

The oral route presents an alternative, offering benefits such as non-invasiveness, improved patient compliance, and ease of use, particularly for long-term treatments [2]. Despite these advantages, significant challenges remain in developing oral delivery systems for peptides and proteins, primarily due to the harsh environment of the gastrointestinal tract. Nonetheless, recent years have seen promising developments in oral delivery systems, with some advancing to clinical trials [3].

Following our previous studies [4], the aim of the present research was to investigate the encapsulation of BSA, a model protein, within calcium alginate and assess the potential of these capsules as a protein delivery system for the gastrointestinal tract. BSA was encapsulated in calcium alginate capsules, which were then coated with chitosan (Fig. 1).

The aqueous mixture of BSA and AlgLV resulted in the formation of coacervate droplets with a size of approximately 124 nm. Capsules were produced with dimensions of around 2.3 mm in width, 3.1 mm in length, and a water content of roughly 95.1 wt% [5]. A thin layer of chitosan was applied to coat the capsules, denoted as BSA-Alg/$CaCl_2$-CHT. These chitosan-coated capsules were evaluated for their potential as an oral delivery system, by investigating the release kinetics of BSA using an *in vitro* model that simulated both gastric and intestinal environments [5].

Release experiments indicated that these chitosan-coated capsules were highly effective at retaining most of the BSA within a simulated gastric fluid (SGF), showing minimal release at low pH. Interestingly, while the capsules remained stable in either SGF or simulated intestinal fluid (SIF) alone, they disintegrated rapidly when subjected to a two-step SGF-SIF treatment. BSA-Alg/$CaCl_2$ capsules, in the absence of chitosan, exhibited some swelling during the SGF stage and dissolved quickly in the subsequent SIF stage. In contrast, BSA-Alg/$CaCl_2$-CHT capsules, with chitosan, did not swell in SGF and took a longer time to disintegrate in SIF.

The results of the release experiments (Fig. 1), which simulated the digestion process, demonstrated that BSA remained encapsulated during the initial treatment with SGF. However, once the capsules were exposed to the SIF, all the BSA was released within less than one hour [5]. These findings indicated that the BSA-Alg/$CaCl_2$-CHT capsules are gastro-resistant and meet the in vitro requirements set by the European Pharmacopoeia. Consequently, Alg/$CaCl_2$-CHT capsules could be highly suitable for protecting proteins from the harsh conditions of the stomach and enabling their controlled release in the intestinal tract, showing promise as an oral delivery system for therapeutic proteins and peptides.

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Figures

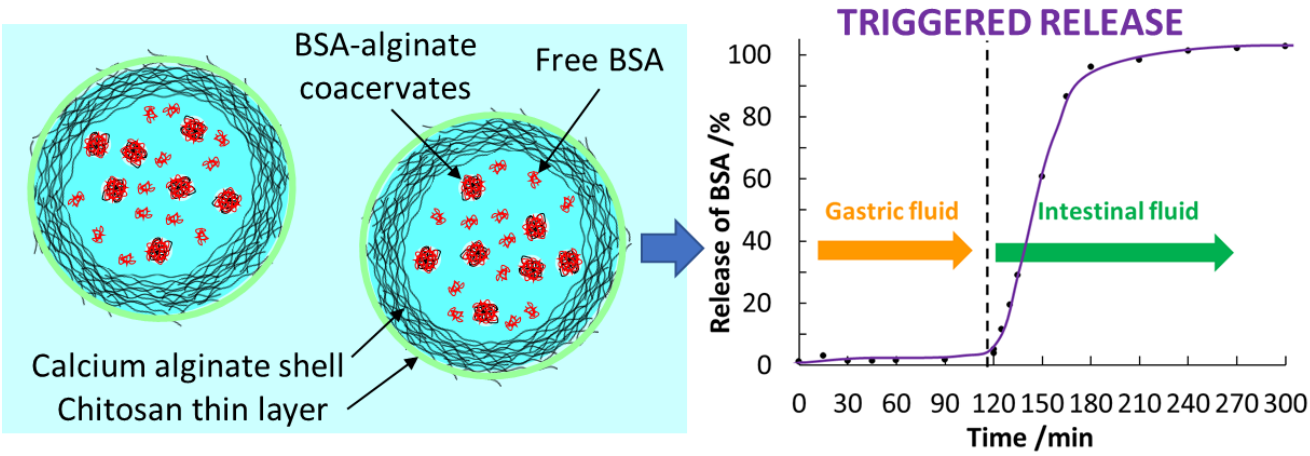

Figure 1. Structure of the BSA-Alg/$CaCl_2$-CHT capsules, and release of the BSA during the simulation of the digestion process (Reproduced from Ref. [5]).

Preclinical anti-tumor immune-mediated synergy of selenium nanoparticles and a nanovaccine against luminal B breast cancer

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Introduction: Selenium (Se) is an element crucial for human health, with anticancer properties. Although selenium nanoparticles (SeNPs) have shown lower toxicity and higher biocompatibility than the organic or inorganic Se compounds, bare SeNPs are unstable in aqueous solutions and prone to aggregation. [1] Furthermore, SeNPs have been demonstrated to induce apoptosis in cancer cells as well as to stimulate the immune system to target and destroy cancer cells. [2] Therefore, the aim of this work was to access the main immune cell populations responsible for SeNPs immunotherapy against luminal B breast cancer.

Methods:

SeNPs production and stability: 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.

Therapeutic Intervention Study Design of the Combination Treatment of BSA1-SeNPs and the $KRAS_{wt}$ Nanovaccine: 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. For intervention therapeutic study evaluating the antitumor efficacy of the combinational treatments of the $KRAS_{wt}$ nanovaccine and the BSA1-SeNPs, once the average volume of tumors reached ≈50–100 mm^3, mice were randomly divided into a control group and 4 treatment groups (n = 6 animals per group), as reported in **Figure 1**. $KRAS_{wt}$ nanovaccine were subcutaneously (s.c.) administered to mice via injection proximal to both left and right sides inguinal lymph nodes (50 µL per side containing 40 µg of MHC II-restricted $KRAS_{wt}$ peptide antigen, 20 µg of CpG-ODN and 40 µg of Poly(I:C)), on days 7 and 14 following tumor inoculation. BSA1-SeNPswere both intratumorally (i.t.) or i.v. administered at 1.25 mg kg^{-1} every 2 days. Tumors and spleens were collected from mice (n = 6 animals per group) after euthanasia and homogenized in a single-cell suspension in cold sterile PBS. Tumor single-cell suspensions were obtained by mechanical disruption and enzymatic digestion. pleens were also mechanically disrupted, and single-cell suspensions were depleted of erythrocytes using ACK lysing buffer for 5 minutes at 37 °C, being further filtered. Cells were seeded in 96-well plates, washed with PBS, and incubated with Ghost Dye Red 780. Afterwards, cells were stained with extracellular and intracellular fluorochrome-labeled anti-mouse antibodies, according to the manufacturer's instructions.

Results: SeNP were spherical, smaller than 50 nm, and with a narrow size distribution and stable in medium, plasma, and at physiologic pH, maintaining their size around 50-60 nm, for a prolonged period. Moreover, the combination of BSA1-SeNPs with a KRAS-loaded PLGA-mannose nanovaccine resulted in a strong reduction of tumor growth in an EO771-breast cancer mouse model. Indeed, the synergistic effect of $KRAS_{wt}$ nanovaccine combined with SeNPs was confirmed by the tumor growth inhibition of 62.2%, compared to 34% and 16.6% for SeNPs (i.t.) and $KRAS_{wt}$ nanovaccine, respectively (**Figure 2**). This synergistic anticancer effect of the combined treatment significantly increased the tumor infiltration of both B, NK, and CD8+ T effector cells. Furthermore, the tumor infiltration of Tregs and PD1-expressing T cells were decreased for the combined treatment with the SeNPs and the nanovaccine. (**Figure 3**).

Conclusion: Stable SeNPs at physiologic pH and plasma were produced. Also, SeNPs presented anticancer properties in EO771-bearing mice, presenting synergy with a $KRAS_{wt}$ nanovaccine. Therefore, this study offers valuable insights for the development of innovative combinatorial approaches using SeNPs to improve the outcomes of cancer immunotherapy.

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Figures

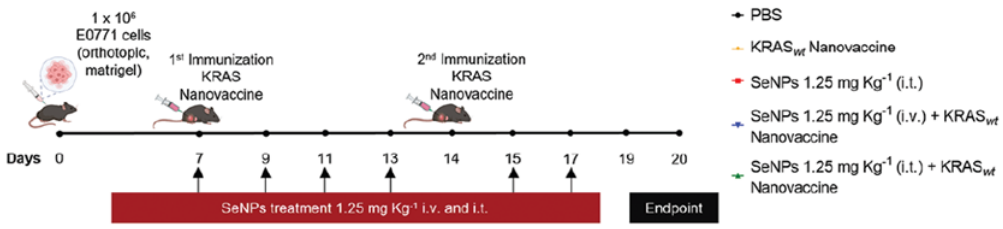

Figure 1. Experimental design of the *in vivo* study. C57BL/6J mice were orthotopically inoculated with 1 × 10^6 EO771 tumor cells and treated with BSA1-SeNPs every other day and $KRAS_{wt}$ nanovaccine on days 7 and 14

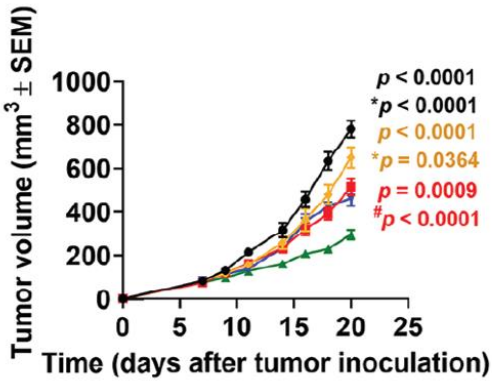

Figure 2. Effect of the individual and combined treatments of SeNPs and $KRAS_{wt}$ on EO771 tumour growth Data are presented as mean ± s.e.m of EO771-bearing mice (N = 2 *in vivo* assays, n = 6 animals per assay for all treatment groups except for SeNPs 1.25 mg Kg^{-1} (i.v.) + $KRAS_{wt}$ Nanovaccine (n = 6)). Statistical significance was analyzed by one-way ANOVA followed by Tukey multiple comparisons post-hoc test and p, p*, and p # values correspond to tumor volume at day 20 after tumor

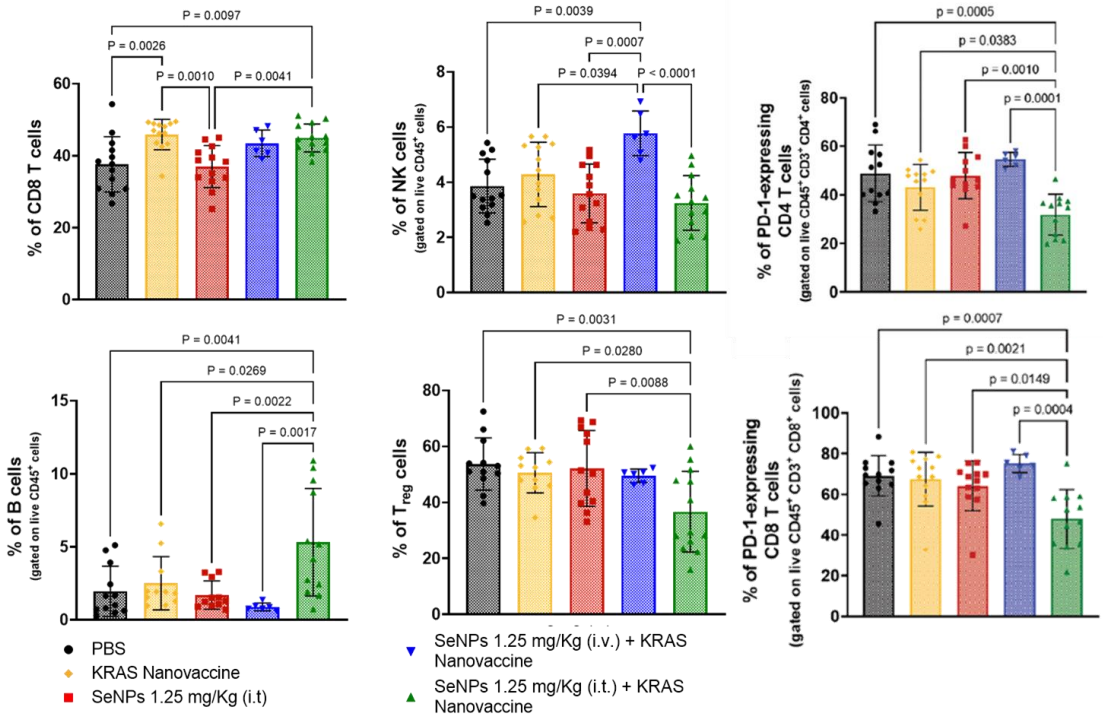

Figure 3. Effect of the individual and combined treatments of SeNPs and KRASwt on the immune cells' population in the tumour microenvironment. Highest infiltration of B and CD8+ T cells, and decreased Treg and PD1-expressing T cell levels for divalent combination of SeNPs intratumorally administered with $KRAS_{wt}$ nanovaccine was observed

Design, synthesis, and characterization of metallic nanoparticles as amplifiers for the optical readout of a novel SERS-based sensor system.

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Introduction

In the realm of biosensing, the quest for innovative platforms with unprecedented sensitivity and versatility has spurred the integration of diverse nanotechnologies, including functional nanoparticles (NPs) and DNA origami structures (Figure 1). This fusion of cutting-edge methodologies holds the promise of revolutionizing bioanalytical sensing capabilities, offering new avenues for rapid and precise detection across various applications. [1, 2]

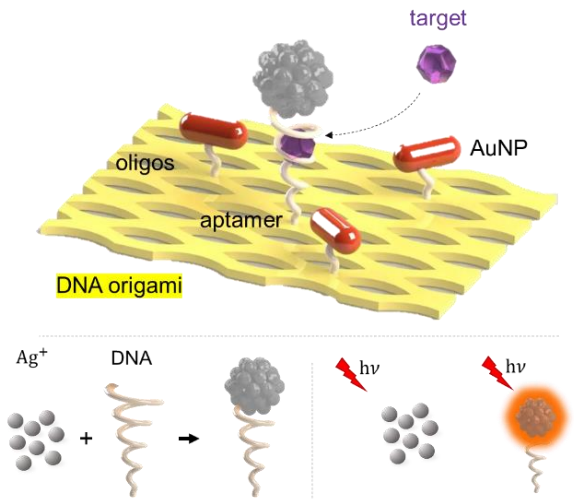


Figure 1: Schematic of DNA origami scaffolds for the arrangement of plasmonic ssDNA attached NP arrays for SERS signal amplification, with an AC-decorated DNA aptamer as bioRE. Bottom: Multifunctional ssDNA oligonucleotide including a sequence for the coordination of fluorescent AgACs.

At the vanguard of this transformative pursuit lies the DeDNAed project, a pioneering initiative poised to reshape the landscape of biosensing technology. Central to its mission is the strategic incorporation of surface enhanced raman spectroscopy (SERS) as a state-of-the-art optical analysis method, facilitating the rapid and precise detection of analytes with unparalleled sensitivity. [3]
Integral to the innovative architecture of the DeDNAed project is the strategic utilization of DNA origami as a versatile "nano-breadboard," providing a robust scaffold for the precise positioning of biorecognition elements (bioREs) in proximity to the plasmonic hotspots of functionalized nanoparticles (NPs).
This orchestrated arrangement promises to elevate the sensor platform to new heights of performance and efficacy. To achieve seamless integration and ensure utmost spatial precision, the project employs short oligonucleotide sequences as a well-established method for anchoring NPs and active bioREs onto the DNA origami scaffold. [4] Furthermore, the incorporation of metallic atomic clusters (ACs) within the bioREs enhances the sensor platform's fluorescence properties and signal amplification capabilities, setting it apart from conventional NP-based systems.[5]
As such, the DeDNAed project represents a pioneering endeavor poised to catalyze significant advancements in biosensing technology. By pushing the boundaries of innovation and harnessing the synergistic potential of nanotechnologies, it heralds a new era of bioanalytical sensing, offering transformative solutions to address pressing challenges in healthcare, environmental monitoring, and beyond.

Results and discussion

To integrate gold nanoparticles (AuNPs) with the DNA origami into a hybrid structure, the DNA origami was designed to have protruding single-stranded DNA (ssDNA) as attachment points (see Figure 1). The AuNPs were functionalized with complementary ssDNA oligonucleotides. This was accomplished by synthesizing oligonucleotides with a thiol group at the 5'-end. For assessing the efficiency of functionalization, the oligonucleotides were also tagged with a fluorescein dye at the 3'-end. These oligonucleotides were synthesized in-house using the Applied Biosystems 3400 DNA synthesizer to facilitate this attachment process. The three components of the bioRE (attachment to DNA origami, target-specific binding, and AC coordination) were individually analyzed and optimized.
For the synthesis of the AuNPs, previously reported CTAC/$NaBH_4$ seeded growth approach was employed. [6] To obtain the gold seeds 50 µL of a 0.05 M $HAuCl_4$ solution was added to 5 mL of a 0.1 M CTAC solution. 200 µL of a freshly prepared 0.02 M NaBH4 solution was then injected under vigorous stirring and after 3 min the mixture was diluted 10 times in CTAC 100 mM. With the aim of obtaining 50 nm particles, two growing steps were then carried out. In the first growth step, 10 nm nanoparticles (see Figure 2) were obtained by mixing 900 µL of the seed solution and 40 µL of 0.1 M ascorbic acid in 10 mL of 25 mM CTAC solution. Then, 50 µL of a 0.05 M HAuCl4 solution was injected under vigorous stirring 10 min. After that, in other to obtain 50 nm gold nanoparticles, the last growth step was then repeated using 30 µL of 10 nm gold nanoparticles as nucleating particles and adding 10 µL of a dilute sodium hypochlorite solution after one hour under vigorous stirring.

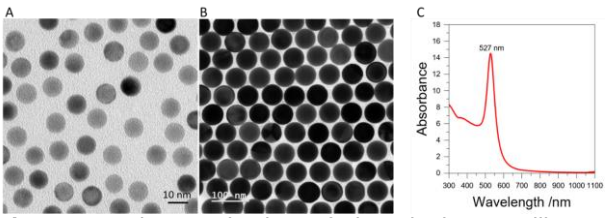
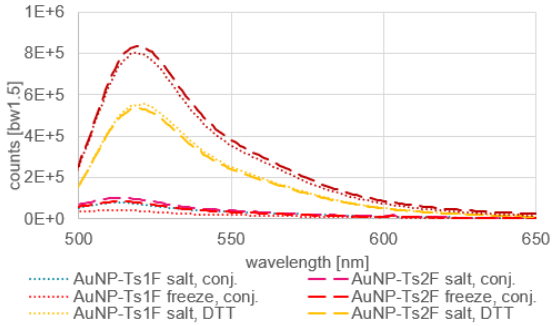


Figure 2. Characterization of the single-crystalline Au nanospheres. A: TEM image of intermediate growth step, showing NPs with an average diameter of 10 nm. B: TEM image of final growth step, showing 55 nm diameter AuNPs (after ligand exchange from CTAB). C: UV-Vis-NIR spectrum of the AuNPs in water displaying a sharp plasmonic absorption peak at 527 nm.

To attach the thiolated oligos to the AuNPs, two different approaches were employed, the salt-aging and the freeze-thaw method [7], [8]. The first step for both methods is the activation of the thiol by the addition of tris(2-carboxyethyl)phosphine (TCEP) to the oligo solution which was then mixed with the AuNPs in a concentration ratio depending on the size of the AuNPs (50-60 nm diameter NPs require a ration of 1:2000 [AuNP]:[oligo]).
To further distinguish between the loading ratios (oligos per NP) achieved with the two different functionalization methods, we developed a method to measure the oligo concentration after functionalization by utilizing the attached fluorescein dye and fluorescence spectroscopy (Figure 3).

a)



b)

	dye conc. [nM]	NP conc. [nM]	loading ratio
AuNP-Ts1F, freeze	164.7	0.30	549
AuNP-Ts2F, freeze	169.4	0.29	584
AuNP-Ts1F, salt	96.6	0.30	322
AuNP-Ts2F, salt	101.4	0.26	390

c)

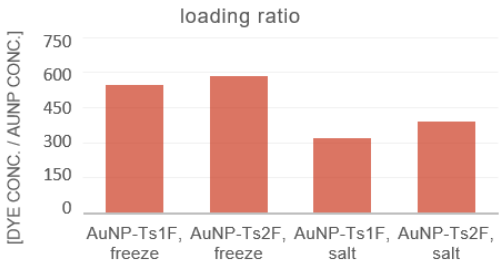


Figure 3: Quantitative loading analysis comparing the loading efficiencies of the salt-aging (*salt*) and freeze-thaw (*freeze*) functionalization methods. **A**: Fluorescence spectra of the fluorescein labelled oligos, before (*conj.*) and after (*DTT*) detaching them from the surface of the AuNPs. **B**: Table with the values for the dye and AuNPs concentration and the resulting loading ration of dye-labelled oligos per NP. **C**: Column chart visualising the resulting loading ratios for the two different oligo species and functionalization methods, taken from the table in **B**.

Figure 3 shows the results of this approach for the two functionalization methods. Using the previously calibrated peak values of the fluorescence spectra of the resuspended oligos together with UV-Vis-NIR spectra to determine the NP concentration, measured before dissociation, we determined the concentrations of the labeled-oligos and the AuNPs. Based on these values, we then calculated the loading ratio of oligos per NP. The measured values are given in Figure 3, B and the loading ratio is displayed as a column chart in Figure 83, C. The resulting ratios appeared to be slightly higher for the freeze-thaw method, making this the method of choice for the preparation of oligo-functionalized nanoparticles as amplifiers for the optical readout of a novel SERS-based sensor system

Conclusions

In summary, we successfully synthesized a DNA oligonucleotide sequence designed for a bioRE compatible with DNA origami bearing AC. This bioRE comprises three functional segments: a complementary DNA strand for attachment to the DNA origami, an aptamer for specific target binding, and a sequence for the coordinated synthesis of fluorescent AgACs. Additionally, we have successfully prepared gold nanoparticles functionalized with these sequences and quantitatively and qualitatively characterized the oligonucleotide-to-nanoparticle ratio.

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NANO-QSAR MODELS ON THE TOXICITY OF NANOMATERIALS

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Developing new materials often prioritizes enhancing specific properties, but assessing the potential risks these materials pose to human health and the environment is equally important. Computational methods offer a valuable solution, helping minimize this assessment's economic, ecological, and ethical impacts. Among these, QSAR models (Quantitative Structure-Activity Relationships) stand out for their widespread use and acceptance in regulatory evaluations, particularly for discrete organic molecules. Recently, researchers have expanded these models to cover nanomaterials (NMs), a field known as nano-QSAR, to better predict and understand these complex substances' behavior. [1]

Unlike traditional molecules, where describing substances solely by their chemical structure is enough (such as the SMILES code), nanomaterials have unique structural features, like size and complex composition (Figure 1), that significantly influence their physicochemical and biological behavior. In our recent review, [2] we recognized the need for a new classification of numerical descriptors to represent nanomaterials. This classification (Figure 2) distinguishes between direct descriptors, which offer a direct representation of the nanomaterial's structure, and indirect descriptors, which incorporate additional experimental parameters.

Direct descriptors provide information about the chemical composition of the core (a), surface substituents (b), or the physical structure of the particles (c). Indirect descriptors, on the other hand, capture experimental features that either depend on the structure (d) or cause changes to the structure (e). Additionally, we include descriptors that don't directly describe the nanomaterial but relate to the conditions under which endpoint measurements are taken (f). However, using experimental data brings its own challenges, as inconsistencies in methods and characterization across the literature complicate the creation of reliable modeling databases for nano-QSAR.

ProtoNANO, a module of the *in silico* prediction server ProtoPRED® [3], is at the forefront of advancing nano-QSAR models for various inorganic NMs. ProtoNANO is instrumental in assessing the risks associated with nanomaterials and improving their characterization by focusing on human toxicity, ecotoxicity, and physicochemical properties. In this presentation, we will explore the obstacles faced in developing nano-QSAR projects and highlight some of the models in ProtoNANO as case studies. These examples demonstrate how different features, including calculated descriptors and experimental data, can influence model outcomes and risk predictions.

This project has received funding from the European Union's Horizon 2020 research and innovation program under the Marie Skłodowska-Curie grant agreement No 896848 (NanoQSAR) and from the Horizon Europe Programme under the grant agreement No. 101137990 (CheMatSustain).

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Figures

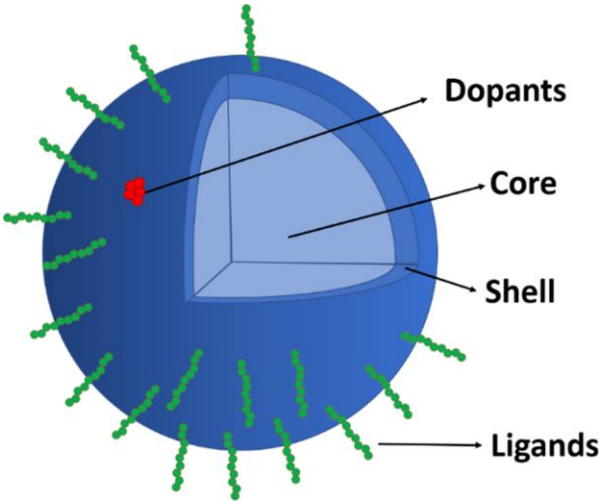

Figure 1. Schematic depiction of the parts of a complex nanoparticle.

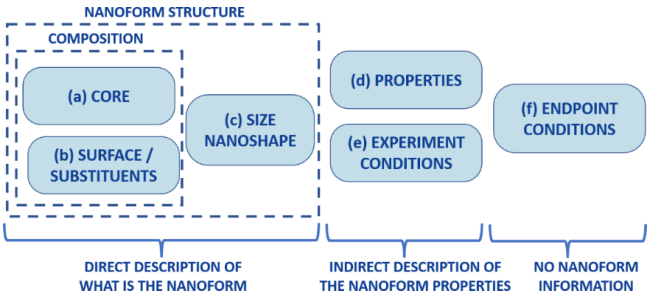

Figure 2. Classification of nanoQSAR descriptors

Highly effective and safe non-viral ionizable lipid nanoparticle as universal transfection vectors

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The use of oligonucleotide therapy offers significant potential for treating a wide range of diseases by delivering functional DNA or RNA into targeted cells or tissues and modulate gene expression. However, their clinical application has been hindered by the lack of safe and efficient oligonucleotide delivery systems. Normally, oligonucleotides have to be administered with a carrier; otherwise, they are rapidly degraded, can be immunogenic, and do not enter inside cells.

In the last decades, a high number of biocompatible materials has been engineered to form complexes, encapsulate, and deliver oligonucleotides with varying degrees of effectiveness [1]. Among the diverse formulations, lipid-based nanocarriers have been one of the most employed [2]. They present good biocompatibility and versatility, but their drug loading and cell penetration capacities are normally low, specifically for hydrophilic or polar molecules [3]. This led to the development of charged (cationic) nanocarriers to favor electrostatic oligonucleotide aggregation in the lipidic NP, dramatically increasing loading density, cell membrane interaction, and transfection efficacy. Unfortunately, the remaining positive charge in the nanocarrier also presented unacceptable levels of immunogenicity and toxicity at the necessary doses to produce therapeutically relevant effects [4]. In addition, they commonly presented hemodynamic toxicities, such as the activation of the complement system and an increase of blood coagulation time [5]. In this context, around the 2000s, Pieter Cullis at the University of British Columbia in Canada developed ionizable lipids as an answer to these problems, leading to the development of ionizable aminolipid NPs (iLNPs) [6], displaying cationic charge at acidic pH for high-density oligonucleotide encapsulation, small size and neutral charge at physiological pH for entering the cell by endocytosis (safer than by transcytosis and membrane fusion) [7], become protonated again during acidification of late endosomes recovering the cationic charge and disrupting the endosomal membrane, and delivering their cargo at the cytosol. Since then, effective ionizable cationic aminolipids have been identified in large-scale screening programs in several biotech companies [8]. The first approved for human use iLNP was Onpattro® (patisiran) containing the ionizable lipid MC3, $C_{43}H_{79}NO_2$, identified from a library of 56 ionizable lipids, with an apparent pKa of 6.44 [9]. Patisaran delivers small interfering RNA to treat polyneuropathies caused by transthyretin mediated amyloidosis in the peripheral nervous system. Followingly, BionTech and ModeRNA developed their own ionizable lipids for their vaccines, the ALC-0315 and SM-102, with pKa of 6.09 and 6.75 respectively. Initial studies showed a strong correlation between gene-silencing activity and the apparent pKa of iLNPs based on different ionizable lipid components [10]. The correlation followed a Gaussian distribution with an optimum pKa between 6.2 and 6.5. These type of lipidic NPs have been the preferred choice for COVID-19 vaccines, and are now the vehicle for next generation of mRNA vacines for cancer immunotherapy to reach the market. These lipids exhibit an inverted cone geometry which is said favor both, high curvature radii and for NP size, and high endosomal membrane disrupting capacities for similar geometrical reasons. It is interesting to note that these developments were industrialized very soon, therefore the description of formation degrees of liberty, intermediates, and characteristics of the structure activity relationship are not yet found in the scientific literature despite clinical penetration.

A high number of investigations conclude that the iLNPs formed by nanoprecipitation, displayed low cargo encapsulation efficiency, poor reproducibility, and heterogeneous therapeutic efficacy. Consequently, the technology has been developed by microfluidics where reagent solutions mix in very small volumes continuously. This is ideal for large scale production but is hampering the availability of simple and accessible methodologies for preclinical research, a prerequisite for therapeutic development.

This reported failure of iLNPs made without microfluidic devices led us to explore which boundary conditions lead to efficient iLNPs synthesis. The objective of this study was to investigate the formation of ionizable lipid nanoparticles (iLNPs) using a straightforward benchtop mixing technique that yields reproducible highly functional iLNPs characterized by monomodal size distributions and high encapsulation efficiency

for various oligonucleotide types, including antisense, mRNA, ssDNA, and plasmids with high loading, reproducibility and size dispersions smaller than some commercial products. Additionally, we assessed the transfection capabilities of the synthesized iLNPs across different cell lines, namely HEK-293, Jurkat, RAW 264.7, HepG2-NTCP, and ARPE-19, as well as in-vivo retinal cells in C57BL/6J mice. The obtained results confirm the capacity of the synthesized iLNPs to encapsulate diverse oligonucleotides, ensuring cargo protection and efficient delivery to all tested cells.

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Figures

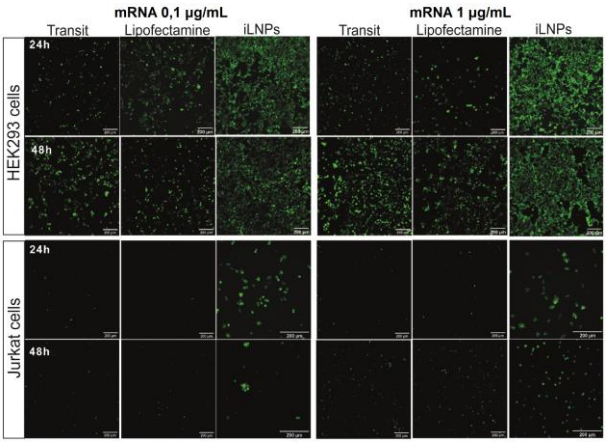

Figure 1. In vitro transfection experiments for EGFP mRNA encapsulated with in-house iLNP. Fluorescence microscopy images after 24 and 48 hours of transfection in both cell lines at different concentration of mRNA.

Design of polymeric nanoparticles selectively directed to the blood-brain barrier for depression treatment

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Major depressive disorder (MDD) has been considered by the World Health Organization as the third cause of the burden of disease worldwide in 2008, expected to become the first cause by 2030. This implies a worsening of life quality in a huge part of the population, since it produces a persistent low mood, lack or decreased interest in enjoyable activities, lack of energy, concentration, appetite, sleep…, turning into a big burden for society, being one of the leading causes of disability worldwide. It is believed that MDD is a multifactorial disease, recently associated with neuroregulatory systems dysregulations, causing disturbances in neurotransmitter systems, such as the ones involving serotonin, norepinephrine and dopamine. [1]

The primary problem of treating brain diseases is the lack of accessibility caused by the presence of the blood-brain barrier (BBB), a structure composed by endothelial cells that interact with pericytes, astrocytes, neurons and microglia. This barrier is highly regulated, with only some molecules being able to cross it, either due to their small size, or their interaction with a receptor.[2] For this reason, nanomaterials provide properties such as drug loading capacity, passive or active targeting, biodegradability and biocompatibility, which help the delivery of multiple substances into the brain. In this case, poly-(beta-aminoester) (pBAE) polymers are used. After a modification of their ends with oligopeptides (**Figure 1**), they acquire positive charge that allows them to electrostatically encapsulate genetic material, interact with cells and be able to escape the endosomes.[3]

In this project we work with pBAE nanoparticles, modified with four amino acid peptides consisting of one cysteine followed by three lysines, histidines or arginines (CKKK, CHHH, CRRR); which give the polymer a positive charge. A second modification being done is the addition of targeting peptides, that direct the system towards receptors overexpressed in the BBB. The peptides being used are SEQ12, a twenty amino acid long peptide, targeted towards the low-density lipoprotein receptor; and T7, a seven amino acid long peptide, targeted towards the transferrin receptor [4]. A second version of pBAE was synthesized by the introduction of a zwitterionic moiety on the side chain, firstly by a Steglich esterification with a chain transfer agent, followed by the addition of a sulfobetaine chain that is simultaneously positively and negatively charged (**Figure 2**). This zwitterionic modification is used to give the nanoparticle stealth characteristics, and thus after combining it with the polymers containing the targeting moieties, having a directed delivery system. [5]

After polymer modifications, different combinations of them are made either to improve their interactions with cells, or their endosomal escape, or their targeting abilities. The combinations are formed into nanoparticles by complexing with the genetic material of choice (DNA, mRNA, miRNA…). These formulations are characterized by Dynamic Light Scattering and proven to be correct when they have a size around 200 nm and a polydispersity index lower than 0.3. In this project we used a mixture of the polymer containing lysine (K), histidine (H), SEQ12, T7 and zwitterionic (Kz) in different proportions, generating the following formulations: KH, KHSEQ12, KHT7, KzH, KzHSEQ12, KzHT7 (60/40, 55/40/5, 55/40/5, 30/70, 25/70/5, 25/70/5 respectively).

To test the affinity of the different formulations for the BBB, we selected different cell lines, that in vitro, express the receptors we chose to target, such as BEAS-2B, CaCo-2, HeLa and BBMVECs. We confirmed the expression of the receptor on these cell lines by Western Blot, at different passages. Another experiment we carried out was studying the transfection efficiency and uptake of our desired formulations on these cell lines, also testing and proving their lack of cytotoxicity.

First results showed that after 4 h all the nanoparticles have been internalized by BBMVECs. On the comparative studies between cell lines, the transfection efficiency is higher for the nanoparticles that do not have the zwitterionic moiety, and in those that do contain it, when they also have the targeting peptides, their transfection increases. This is close to what we expected, since the addition of the zwitterionic moiety, generates stealth nanoparticles that should not interact with cells, but when we introduce targeting peptides to these formulations, they have a receptor-mediated interaction with cells, and not only by charge.

Therefore, we can say that out of the different formulations tested, the ones containing targeting peptides have a higher transfection efficiency and that those containing zwitterionic interact way less with the cells. By these results, we managed to formulate nanoparticles that are directed towards the brain and should reduce the secondary effects due to their stealth characteristics.

Next steps will study the most adequate genetic material-based loading to become therapeutic against MDD.

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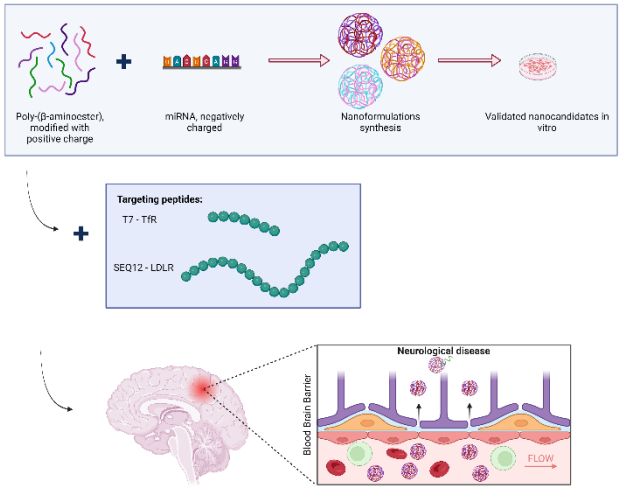

Figure 1. Polymer description of nanoparticle formulation, and peptide structures to direct the delivery system towards the brain.

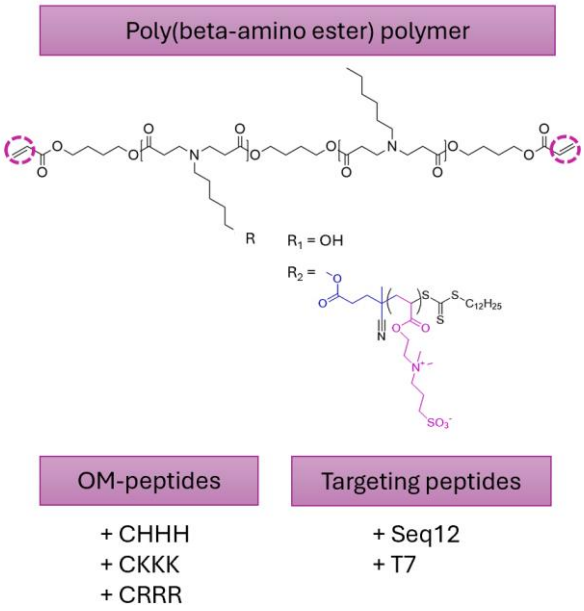

Figure 2. Poly-(beta-aminoester) polymeric main structure, with a 5-amino-1-pentanol chain and a hexylamine chain or a zwitterionic moiety, followed by the different possibilities for positively charged oligopeptides, and targeting peptides.

Advancing Nanomaterial Functionalisation: Stability and Dispersion of Metal Nanoclusters on PEGylated Graphene Oxide

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Graphene and graphene oxide (GO) are increasingly utilised in energy, materials, and healthcare sectors [1], [2]. Silver (Ag) and copper (Cu) nanomaterials, including their nanoclusters, are pivotal for advanced antimicrobial therapies and enhancing materials for energy and biomedical applications [3]. However, achieving uniform dispersion and stability in both hydrophobic and hydrophilic environments remains a challenge [4]. This study addresses these challenges by synthesising and characterising PEGylated GO_{30} (30% oxidised) functionalised with Ag and Cu nanoclusters through amide bond formation. As shown in Figure 1, PEGylated GO_{30} stabilises Cu and Ag nanoclusters by providing steric effects and limiting aggregation. Using molecular dynamics (MD) simulations, we identified optimal strategies for stable nanostructures, focusing on radial distribution function (RDF) and mean squared displacement (MSD) analyses. The oxidation and subsequent PEGylation of graphene significantly enhance the interaction energy of Ag nanoclusters by 239.47 kcal/mol and Cu nanoclusters by 259.98 kcal/mol. This functionalisation also substantially reduces nanocluster mobility, with MSD values of 20-30 $Å^2$ at 500 ps, compared to 150-175 $Å^2$ for non-functionalised clusters. RDF analysis reveals improved nanocluster dispersion on the PEGylated GO_{30} surface, supporting the formation of stable nanostructures. SEM and TEM analyses corroborate these findings, showing that PEGylation enhances nanoparticle dispersion and reduces aggregation on GO_{30} sheets, achieving a more consistent size distribution of 10-20 nm. UV-Vis spectroscopy indicates that PEGylated Ag nanoparticles exhibit a stable plasmonic response between 400-450 nm, which is crucial for their antimicrobial activity.

Overall, PEGylation significantly enhances the stability, dispersion, and antimicrobial functionality of metal nanoclusters on graphene-based materials, underscoring their potential for drug delivery, antimicrobial technologies, and sensing applications, while laying a strong foundation for future research in functional nanomaterials.

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Figure

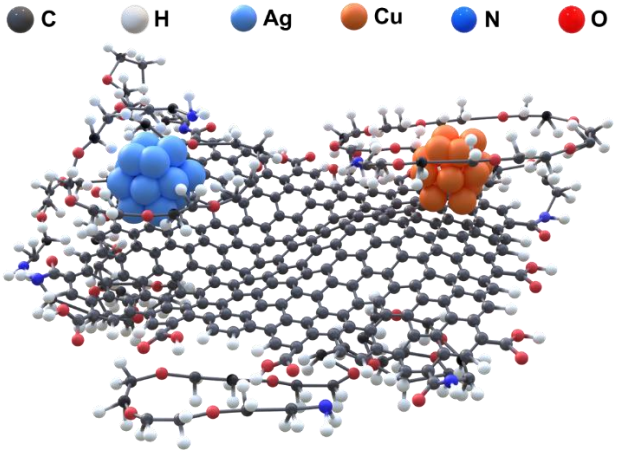

Figure 1. GO_{30} functionalised with PEG-NH_2 stabilises both Cu and Ag nanoclusters. The PEG chains provide steric stabilisation, preventing nanocluster movement along the GO_{30} sheets and limit aggregation by creating a physical barrier, reducing the likelihood of aggregation.

RANDOM LASER APPLICATIONS IN BIOMEDICINE

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Random laser (RL) is produced by the combination of an optical gain medium with a dispersive environment. In conventional lasers, optical feedback is provided by mirrors. However, in a RL the amplified light can be scattered multiple times, resulting in stimulated emission and laser action without the need of external mirrors. Furthermore, since RL phenomenon is based on scattering events, the emission is highly sensitive to the scattering characteristics of its own lasing medium.

In this communication, we present our results in RL obtained in tissues impregnated with different types of dyes. For instance, a chemically modified fluorescent anticancer drugs will be presented as an example of a dye molecule for RL [1]. Further investigations using commercial rhodamine dyes will also be shown. We have studied RL signal from mouse brain slices impregnated with a dye solution [2]. Moreover, a transgenic mouse model of Huntington's disease, which is a neurodegenerative disorder characterized by motor and psychiatric symptoms, has been studied. The RL emission data were explored using a multivariate statistical analysis based on principal component analysis and linear discriminant analysis. This statistical analysis allowed us to correctly classify the emission spectra from healthy and from transgenic mice [3]. Finally, our last results on RL from human blood samples will be presented too and the potential use of this optical tool for medical diagnosis assistance will be discussed. It includes an observational case study, in which a multivariate statistical analysis of the RL spectra allowed to differentiate the blood of Chronic Lymphocytic Leukaemia patients from that of healthy controls [4].

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Acknowledgements

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Figures

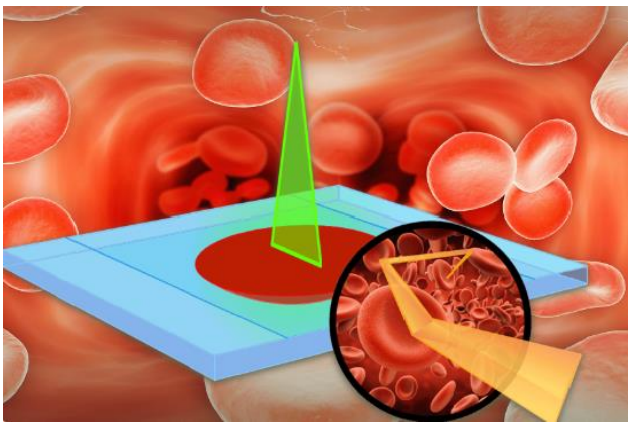

Figure 1. Scheme of the use of RL analysis using a human blood sample.

PMMA/Graphene drug delivery systems as promising candidates for intraocular devices to improve glaucoma surgery

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Abstract

Glaucoma is one of the major causes of blindness and in late stages it requires surgery. There are several glaucoma surgery techniques, for example, a common approach involves the application of an implant (valve) to drain the aqueous humor. However, postoperative wound healing produces an exacerbated response from the fibroblasts, clogging the introduced drainage canal [1]. Due to that, the development of new drug delivery systems have been receiving great attention in recent years due to its application to avoid the fibroblast proliferation without human intervention [2, 3]. The challenge is to develop biocompatible materials that do not affect the vision of the patients.
PMMA is a synthetic polymer that has arisen great interest in the biomedical field mainly due to its biocompatibility, transparency, high resistance to sunlight exposure and good optical properties [4, 5, 6].
The present study describes the development of a nanostructured smart drug delivery film adsorbed in thin substrates of PMMA that will be attached at the top of the glaucoma drainage device. This technique was applied by Mónica et al. in a recent study to develop a nanostructured and time-controlled film to release precise amounts of brimonidine (drug to control the Intra Ocular Pressure) at specific periods of time [7]. The presented multilayered films will be able to release precise amounts of the antimitotic drug 5-Fluorouracil (5-FU) used to control the fibroblast proliferation during the cicatrization time (one month) in postoperative glaucoma's surgery at specific periods of time.
The thin substrates of PMMA were made through drop cast method, dried and sterilized under UV light (figure 1).
Biocompatible films were composed of drug delivery layers and barrier layers. The drug delivery layers had 5-FU encapsulated in β-cyclodextrin (β-CD) to promote the solubility and reduce the toxicity of the drug. The barrier layers were made of a hydrossoluble polymer (poly (β-amino ester)) and graphene oxide [8, 9], to delay and control the drug's release. The drug delivery layers were alternated adsorbed with the barrier layers by the layer-by-layer assembly (LBL). LBL as a simple and versatile technique that allows to control the architecture of the film and can be repeated as many times as the desirable layers. The growth of the film was monitored by ultraviolet-visible spectroscopy. The acquired results demonstrated that the films are stable on the PMMA substrate with a perfectly linear increase of the absorbance intensity with the adsorbed layers (figure 2). These results are in concordance with the ones achieved in a previous study where it was used quartz substrates (figure 3.) In that study it was also showed that the presence of graphene oxide and a hydrossoluble polymer can control the drug release (figure 4). Specifically, it was shown that graphene oxide significantly slows the release of 5-FU, allowing for exact control of the dosage administered. This work helped to advance the creation of new drug delivery films in a biocompatible PMMA substrate, that can be applied to enhance the recovery of glaucoma surgery.

Acknowledgements:
All authors acknowledge the SmartGlauco project-PTDC/CTM-REF/2679/2020 and the PhD grant – 2022.11625.BD which are both financially supported by Fundação para a Ciência e a Tecnologia (FCT)

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Figures

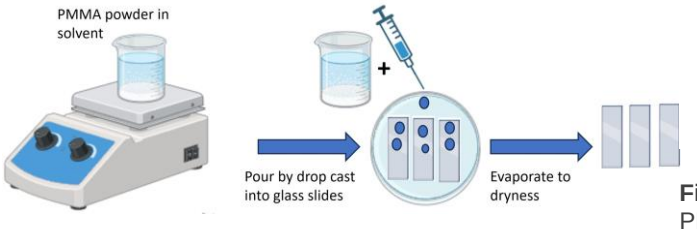

Figure 1. Fabrication of PMMA substrates by drop cast.

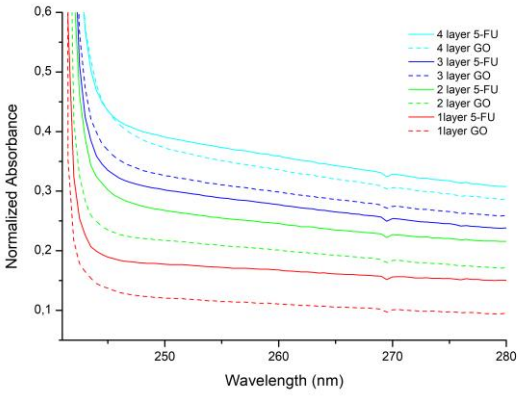

Figure 2. Normalized absorbance spectra of drug delivery film growth in PMMA substrate, representing that film layers are stable and grow sequentially.

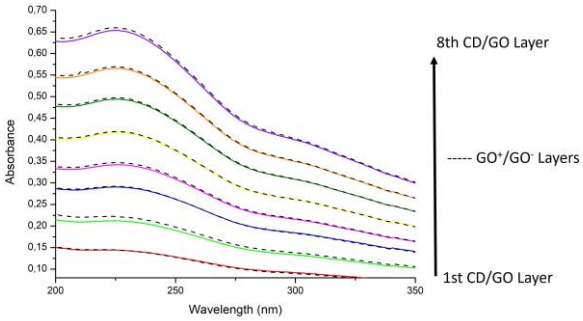

Figure 3. Absorbance spectra of drug delivery film growth representing that film layers are stable and grow sequentially.

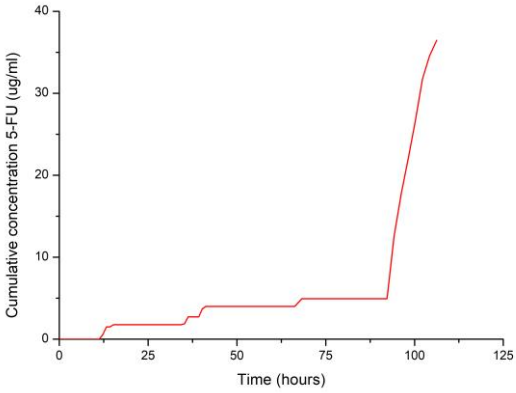

Figure 4. Cumulative concentration release of 5-FU in PBS solution over time.

Drug-loaded PLGA nanomotors as a new approach for bladder cancer therapy

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Bladder cancer is the 7th most common cancer type worldwide, with over 500,000 new cases and 200,000 deaths annually (1). Current bladder cancer treatments are hindered by drug sedimentation and poor retention in the bladder, leading to high recurrence rates and low long-term survival (2). In recent years, nanomotors (NMs) have been developed as drug delivery systems for therapeutic agents. Nanomotors are self-propelled nanoparticles capable of converting chemical energy from their surroundings into mechanical propulsion (3). This motion enhances their diffusion and mixing capabilities, as well as their internalization into tumors, compared to passive particles (4,5). Given these benefits, they are an excellent tool for improving bladder cancer treatment as has been demonstrated in in vivo experiments, where radiolabeled nanomotors reduced bladder tumor size in mice by 90%(6). However, the designs used so far have limitations for clinical applications due to their inorganic chassis, such as silica. Therefore, there is a need to develop new nanobots based on organic materials, which are more biocompatible, biodegradable, and FDA-approved.

In this study, we developed a new design of nanomotors based on poly (lactic-co-glycolic acid) (PLGA) to enhance the standard treatment for bladder cancer, Mitomycin C (MMC). MMC-loaded PLGA nanoparticles were synthesized using the double emulsion method. To achieve motion, the surface of the nanoparticles was modified for urease attachment by first adding polyethyleneimine (PEI) and then using glutaraldehyde as a linker for the enzyme. The polydispersity, size, and surface charge of the nanoparticles were analyzed by Dynamic Light Scattering (DLS) after synthesis and at each stage of functionalization. Additionally, the enzyme activity of the nanomotors was measured by the pH change promoted by urea catalysis using Phenol Red reagent. Moreover, motion studies were conducted by comparing nanomotors in the presence and absence of urea. After characterizing the drug-loaded nanomotors, their therapeutic efficacy was assessed in bladder cancer cells derived from mice (MB49 line) and compared with the standard treatment (free MMC), demonstrating that the motion and drug encapsulation enhanced MMC-induced cell death. Finally, the mechanisms of action of our formulation were studied by analyzing the nanomotors' cell internalization and their effect on bladder epithelial cells, showing that they can internalize into cancer cells within just one hour affecting only bladder cancer cells.

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Encapsulin Protein Nanocages for Targeted Drug Delivery

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Usually, drugs are distributed through the whole body and can cause side effects. Therefore, the drug efficiency is reduced at the site of interest. Targeting drugs to the pathologic site is then crucial and it requires a specific carrier, such as nanoparticles. Among the variety of nanoparticles developed for drug delivery, protein cages are good candidates due to their well-defined monodisperse shape, hollow structure and their biocompatibility.[1,2]

In our group we are working with homo-multimeric protein nanocages called encapsulin and that evolved in some bacteria and archaea by providing advantageous compartmentalization of certain processes. Currently, they are being studied for applications as drug delivery platform, nanoreactor and imaging agent for their convenient modification and production. We specifically study the encapsulin found in the bacteria *Thermotoga maritima* (Tm) and aims to modify it for targeted drug delivery. Tm encapsulin is composed of 60 monomers that self-assemble into a 24 nm particle according to the icosahedral T=1 symmetry (Figure 1). [3]

This encapsulin has the advantages to be easily engineered, easy to produce and it is not taken up un-specifically by cells. The surface has been successfully engineered genetically to insert at different positions, specific targeting peptides for key pathogenic cell types in liver diseases i.e. macrophages and hepatic stellate cells[4] or brain endothelial cells. [5,7] With the intention to deliver drugs to or across these cells, we are now working on actively openable nanocages by inserting light sensitive amino acids or cleavable peptide sequence in the encapsulin protein sequence to disrupt the cages in response to irradiation or specific enzymes respectively (Figure 2).

I will also present the progress on targeting a cytokine with anti-fibrotic effect to LX2 liver cells, the optimization for using the nanocages as transporter to cross the blood brain barrier and the investigation of the immunogenicity of the different cage variants.

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Figures

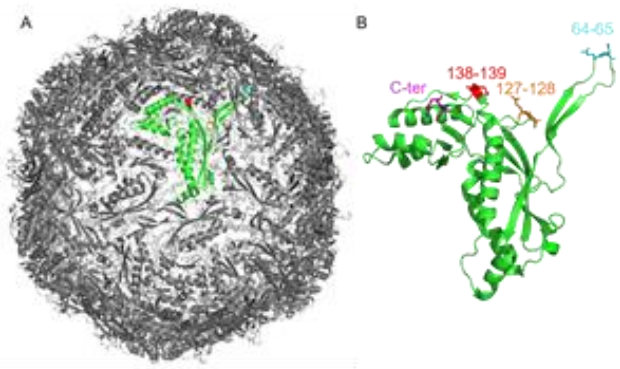

Figure 1. Model of Tm encapsulin with outside loops of interest highlighted. A is the cage with a single monomer in green. B is a monomer with the engineerable position in different colors.

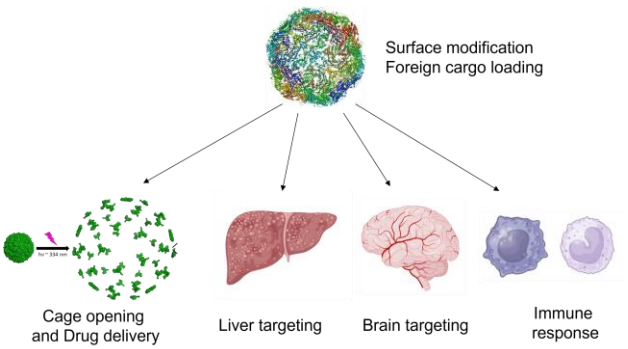

Figure 2. Different targeting drug delivery project using encapsulin nanocages.

CRISPR/Cas9-mediated genome editing in T-cells using non-viral nanovectors

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Abstract

Immunotherapy using Chimeric Antigen Receptor (CAR)-T cells is one of the most exciting recent developments in cancer treatment [1]. The therapy involves genetic modifications of a patient's T-cells to improve immune activation against cancer cells (Figure 1) [2]. The process requires the use of viral vectors for efficient and stable DNA editing of T cells with relevant side effects related to unsafety procedures, potential for integration into the host genome, long-term effects in terms of mutagenesis and carcinogenesis [3]. This is leading to the necessity of using alternative delivery vectors for genome engineering of T-cells.
Nanovectors (NVs) are promising delivery system having unique physical, chemical, and biological characteristics. Some of them, made of lipids, ceramic, metallic, polymeric materials, find use in the biological world [4]. A class of carbon-based nanomaterials, with sizes typically less than 10 nm, is represented by Carbon Dots (CDs), emerged as promising nanomaterials for gene delivery due to their properties including biocompatibility, since CDs are generally non-toxic and well-tolerated by biological systems, tunable surface functionality, and cellular uptake [5]. Giving that, we are developing biocompatible and biodegradable nanovectors, to prevent the side effects of viral carriers, coupled with the CRISPR/Cas9 technology for modifying precisely the genome of T-cells (Figure 2). In particular, we have chosen one of relevant gene involved in escape of immunosurveillance as *PDCD1* (encoding PD-1), an inhibitory receptor that, through the binding to its ligand, PD-L1, promotes self-tolerance (Figure 3) [6]. Turning off PD-1 genetically means inducing autoimmunity, so is more appropriate to endogenously tune the binding affinity of PD-1 with PD-L1. We provide evidences that new synthetized polymeric CRIPSR/Cas9-nanovectors (Cas9_NVs) are able to target *PDCD1* at genomic and protein level. We are aiming to introduce site-specific modifications of *PDCD1* which lead to tune the affinity for its ligands. This may pave the way for new therapeutic avenues offering highly innovative and promising technology for immunotherapy of cancer at genomic level.

Acknowledgements

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Figures

Figure 1. A schematic representation of CAR-T cell therapy. Peripheral blood is collected from a patient, T-cells are separated by apheresis from all other blood components and are genetically engineered to express a CAR receptor. Then, CAR-T cells are expanded and put back into the patient's bloodstream.

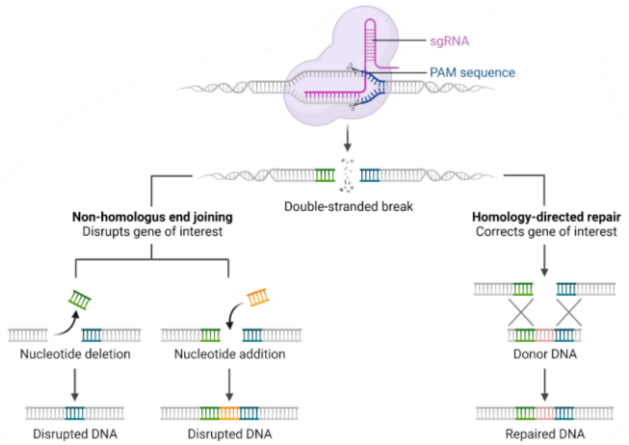

Figure 2. CRISPR-Cas9 genome editing system. This system targets the gene of interest by a single-guide RNA (sgRNA) that is designed to recognize the target DNA site via Watson–Crick base pairing. During the CRISPR editing process on successful recognition of the target, Cas9 undergoes a conformational change that involves its two nuclease domains. The nuclease domains cleave both strands of the target DNA about three nucleotides before the Protospacer Adjacent Motif (PAM) sequence, generating double-stranded DNA break (DSB). There are two mechanisms to repair the DSB: non-homologous end joining (NHEJ) and homology-directed repair (HDR).

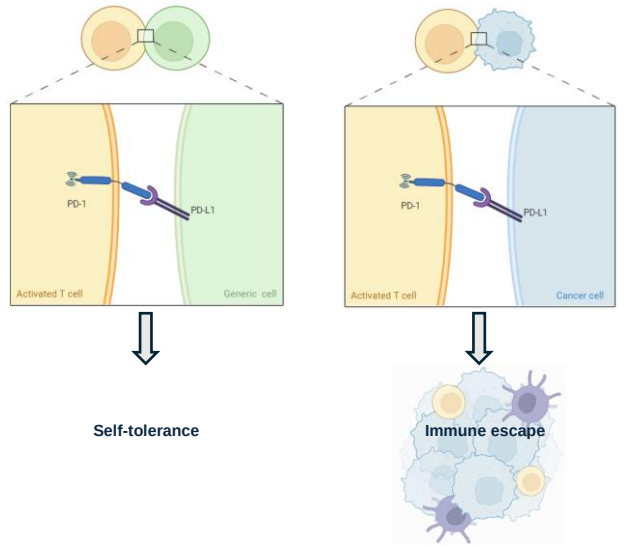

Figure 3. Biological role of PD-1 and its implication in cancer.

Biodegradable Light-Emitting Diodes (LED's) for biophotonic applications

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Abstract

Transient electronic devices, such as light-emitting diodes (LEDs), have garnered significant attention for optical-based biosensing, photodynamic therapy and optogenetics.[1-4] This technology focuses on using biocompatible and biodegradable materials that gradually degrade inside the human body without triggering a major inflammatory response, thereby avoiding potential harm to the patient.[5-6] Currently, the major challenge in designing a fully biodegradable LED for the biomedical industry is identifying the right combination of materials that have the proper electrical, optical, mechanical and biocompatible qualities, as well as, thermal stability and compatibility with the required fabrication processes.[7-9] In this work, we report a transparent and flexible LED device where all the components, including substrate, active layer and electrodes, are completely biocompatible and biodegradable. The substrate was made of chitosan, a polysaccharide present in the exoskeletons of insects and crustaceans widely used in drug delivery nanocarriers but poorly explored for developing transient electronic devices. The biodegradable electrodes were made of magnesium, a metal that easily dissolves in water and human body fluids. The bis[4-(9,9-dimethyl-9,10-dihydroacridine)phenyl]methanone (DMAC-BP), a biocompatible and hydrolysable thermally activated delayed fluorescence material, served as active layer of the device. The results showed that the LED device is biocompatible, biodegradable, flexible, operates at voltage (4-5V) and current values that are safe for the human body and exhibited a stable green emission at 510 nm, which can be used for activating photosensitizers (like doxorubicin) in cancer therapy.

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MULTILAYER NANOCARRIERS – A PROMISING TOOL FOR DELIVERY OF NEUROPROTECTIVE DRUGS THROUGH BLOOD-BRAIN BARRIER

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Despite an enormous progress in understanding molecular basis of age-related neurodegenerative diseases such as Alzheimer's disease and Parkinson's disease, no efficient neuroprotective strategy has been invented so far. One of the major limitations is an inefficient delivery of neuroprotective drugs through the blood-brain barrier (BBB). The very poor water solubility of most promising neuroprotectants limits their delivery to the affected part of the brain. Nowadays, nanoparticles (NPs) have attracted much attention as promising drug carriers that could deliver therapeutics to their specific molecular targets. Herein, we present a novel methodology for delivering hydrophobic neuroprotective substances through BBB using multifunctional polymeric-based nanoparticles (NPs).

The drug-loaded NPs were prepared from nanoemulsion template methods, i.e., the spontaneous emulsification solvent evaporation method [1]. Subsequently, NPs were modified using the layer-by-layer approach by creating multifunctional polyelectrolytes shells. Developed nanocarriers were characterized by determination of their size (below 250 nm), zeta potential and encapsulation efficiency (~ 100 %). For initial tests we have chosen two types of empty nanocarriers abbreviated as AOT/$(PLL/PGA)_2$-g-PEG and PCL/$(PLL/PGA)_2$-g-PEG without or with rhodamine B as fluorescent marker. As neuroprotectants we selected cyclosporine A (CsA) and tacrolimus (FK506) due to their anti-apoptotic, immunosuppressive and anti-inflammatory properties. Human neuroblastoma SH-SY5Y cell line was used to estimate the biocompatibility of nanocarriers loaded with appointed drug in the cellular viability quantification and cell death assessment using WST-1 and LDH tests, respectively. In parallel, we examined the neuroprotective potential of encapsulated drugs against oxidative stress-induced cytotoxicity. We also evaluated the capability of designed nanocarriers labeled with rhodamine B to pass through hCMEC/D3 cell monolayer to test if they can be considered as promising platforms for drug delivery. The immortalized human brain microvascular endothelial cell line (hCMEC/D3) was selected as well-characterized *in vitro* model of BBB and suitable for studying barrier permeability for neuroprotectants and drug carriers.

Results showed that the both kinds of new designed polymeric nanocarriers affected viability of SH-SY5Y cells only when used in the highest concentrations - in higher dilutions are devoid of cytotoxicity. We demonstrated that encapsulated CsA and FK506 moderately reduced H_2O_2-evoked cell damage in SH-SY5Y cells. In addition, both types of nanoparticles crossed BBB in its *in vitro* model in time-dependent manner. The maximum fluorescence intensity, which corresponds to the maximum quantity of NPs that passed BBB in chosen model, was obtained after 48 hour of treatment. Overall, these data point to biocompatibility and potential utility of proposed polymeric-based nanoparticles for transporting neuroprotective substances to central nervous system.

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ANTIBACTERIAL ACTIVITY STUDY OF COPPER OXIDE NANOFLUIDS FOR INFECTION CONTROL IN HOSPITAL ENVIRONMENT

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Abstract

Due to its antibacterial potential, copper and its alloys have attracted the attention of the scientific community. In the current pandemic context, new solutions and materials have been investigated for applications in methods of containing hospital infections. The use of nanoscale materials or coatings with antimicrobial properties is another approach that has been considered.
With the aim of evaluating the antibacterial potential of copper oxide nanoparticles, as well as evaluating their applicability in a new hospital disinfectant, nanofluids were prepared with different concentrations of CuO 40 nm nanoparticles in distilled water. Subsequently, they were applied to two strains of bacterial cultures.

Introduction

Technological development has boosted scientific research to achieve better results in the field of science and technology, particularly in the study of nanofluids and their applications with enormous potential in industry and biomedicine [1-3].

In this study, nanofluids of CuO 40 nm in distilled water were prepared by the 2-step method. The nanoparticles of the CuO were supplied by MK-Nano, with 99% purity and 6.32 g/cm^3 density. The nanoparticles were weighed on a KERN ALJ analytical balance, model 220-4 NM, were gradually added to the base fluid placed in a flask and homogenized in an Eco Stir magnetic stirrer for 10 minutes, model MS7-S. Subsequently, the sample was taken to a BANDELIN SONOPULS ultrasonic homogenizer, model HD2200, with a TT 13 titanium tip, for 60 minutes.
To evaluate the antibacterial power of nanofluids, samples of 5% and 7% concentration of CuO nanoparticles were performed and tested against two bacterial strains: one of Staphylococcus Aureus and one of Escherichia Coli. To verify the effect of CuO nanoparticles, an antibacterial study was carried out on a sample of 5% concentration of nanoparticles after it was centrifuged for 20 minutes at 4000 rpm, thus reducing the concentration of nanoparticles in the nanofluid. The procedure used in the preparation of the culture medium was based on the research procedure carried out by Yong-Wook Baek [4] using Mueller Hinton II agar, recommended for antimicrobial tests of common fast-growing bacteria.

Discussion and Results

The bacteriological study carried out on samples of nanofluids allowed us to conclude that the bacteria proliferated normally in the control petri dishes as well as in the petri dishes that contained the centrifuged sample of CuO nanofluid, as can be seen in Figure 1. This result can be explained by a considerable decrease in the concentration of nanoparticles of the centrifuged solution. However, the petri dishes relating to experiments 2 and 3, which contained CuO nanofluids with a volume percentage of 5% and 7%, do not show any bacteria. In view of the results obtained, it is possible to prove the antimicrobial activity reported in the literature that copper oxide, in high concentrations, can generate a wide range of substances harmful to the bacterial cell, leading to a compromise of the integrity of the bacterial cell membrane, production of reactive oxygen species (ROS) and, consequently, cell death [5,6].

Conclusions

The stability of nanoparticles is a crucial factor in the behavior of nanofluids, affecting the properties that make them so unique and highly sought after for future applications in the most diverse areas. In our study of stability of CuO 40 nm water nanofluids [7] it was possible to prove that the most stable nanofluid was the one sonicated for 1 hour. In this study was possible to verify the antibacterial potential of CuO nanoparticles. Bacteria normally proliferate in the control sample and the centrifuged sample. For the concentrated samples of CuO 40 nm, 5% and 7%, there was no bacteria, reaffirming that, in fact, copper oxide, in high concentrations, is capable of generating a wide range of substances harmful to the bacterial cell.

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Figures

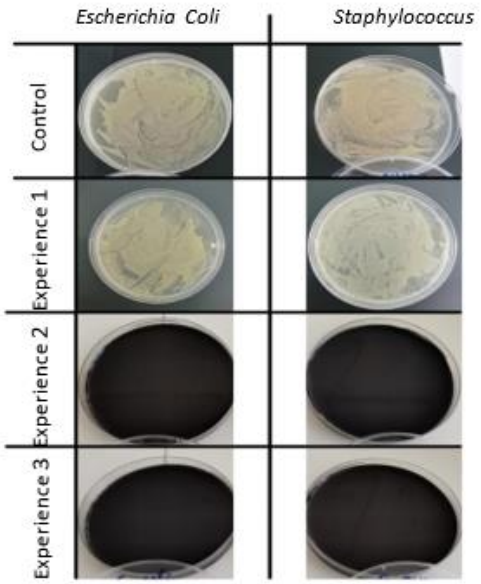

Figure 1. Evaluation of the antibacterial potential of CuO nanofluids

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EFFECT OF NANOPARTICLE-DRIVEN HYPERTHERMIA IN BIOMIMETIC NEUROBLASTOMA MODELS

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Introduction

Neuroblastoma (NB) is a rare developmental cancer. It is usually located in the abdominal region around adrenal glands, since it is a result of a defective evolution of the neural crest cells. Unfortunately, high-risk neuroblastoma causes relapse in approximately the 50% of affected children, eventually leading to child death [1-2].

Current translational gap created by conventional models hinders the development of efficient treatments. Therefore, there is a crucial necessity for innovative neuroblastoma models able to recapitulate complexity of human NB tumors. One of the main research fields on NB involves the production of biomimetic models resembling human tumor's core aspects. These cutting-edge models introduce complex features such as tumor composition in terms of cells and matrix, or three-dimensional structure. Additionally, they offer a more realistic representation of tumor aggressiveness, which is not achieved with traditional 2D models [3-4].

In parallel, bioengineering is also focused on the development of more efficient therapies, most of them based on nanomedicine principles. Emerging nanotherapies take advantage of the enhanced effect and selectivity provided by the addition of nanoparticles (NPs). Particularly, superparamagnetic iron-oxide nanoparticles (SPIONS) stand out as suitable candidates due to their double function of (1) absorbing near-infrared light, and (2) magnetism. SPIONS are being recently tested for hyperthermia techniques, consisting in exposing the tumor to temperatures above the physiological limits, causing cell death or cell sensitization. Therefore, we should highlight two hyperthermia techniques which will be highly favored from SPIONS's double functionality: photothermal therapy (PTT), and magnetic hyperthermia (MH). SPIONS in PTT are activated by local irradiation of a specific region of the tumor with a near-infrared light laser, and transform optical energy into heat. In MH, the whole tumor is homogeneously exposed to external alternating magnetic fields, so SPIONS convert the magnetic stimuli into heat [5-6].

Within this research, two novel nanoparticle-driven hyperthermia treatments (PTT and MH) were tested in biomimetic neuroblastoma models.

Methodology

In first place, we functionalized magnetite-based SPIONS with glucose, boosting tumor internalization of the nanoparticles. Then, three-dimensional models of human NB tumors were fabricated using tissue-engineering (TE) techniques. The TE-NB models included both the main components of human NB, collagen I and hyaluronic acid, and cells from SK-N-BE(2) NB cell line. Cells were seeded and cultured in the models for seven days. Another scientific publication by the same authors detailed these two fabrication steps [7].

Finally, glucose-modified nanoparticles were added to the TE-NB models. Samples were treated with the hyperthermia techniques (PTT or MH) 24h after the addition. Impact of the treatment was studied overtime (days 1, 2 and 5).

We evaluated the impact on cell apoptosis by immunofluorescence. Activation of caspase 3-7 apoptotic cascade was checked by staining histological slides with CellEvent™ Caspase-3/7 Green ReadyProbes™ Reagent (#R37111, ThermoFisher).

We quantified cell proliferation using the Quant-iT Picogreen dsDNA Assay Kit (#P7589, Invitrogen).

Results and discussion

Activation of caspase 3-7 apoptotic pathway

Regarding TE-NB exposed to PTT, we noticed activation of the apoptotic cascade in both scenarios with and without the nanoparticles (Figure 1). These results indicate that PTT in these experimental settings is influencing the studied apoptotic route of neuroblastoma cells independent of the addition of nanoparticles. Besides, activation in the edges of the models suggests unexpected propagation of the effect, since the models were irradiated locally only in the inner core. In contrast, TE-NB models treated with MH showed activation of the apoptotic cascade only in the condition with nanoparticles (Figure 2). These data indicate that an additional level of selectivity is being achieved with these experimental conditions for MH.

Picogreen quantification of cell proliferation

We studied cell proliferation at 1, 2, and 5 days. Regarding PTT, no significant differences were found between conditions (Figure 3A). Nevertheless, MH-treated samples showed a significant decrease of tumor cell proliferative activity with respect to the control condition at day 2 (Figure 3B).

Conclusion

Considering the experimental conditions for this project, PTT is not recommended as optimal for suppressing tumor growth. However, results from MH propose this technique as a promising nanoparticle-based hyperthermia approach.

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Figures

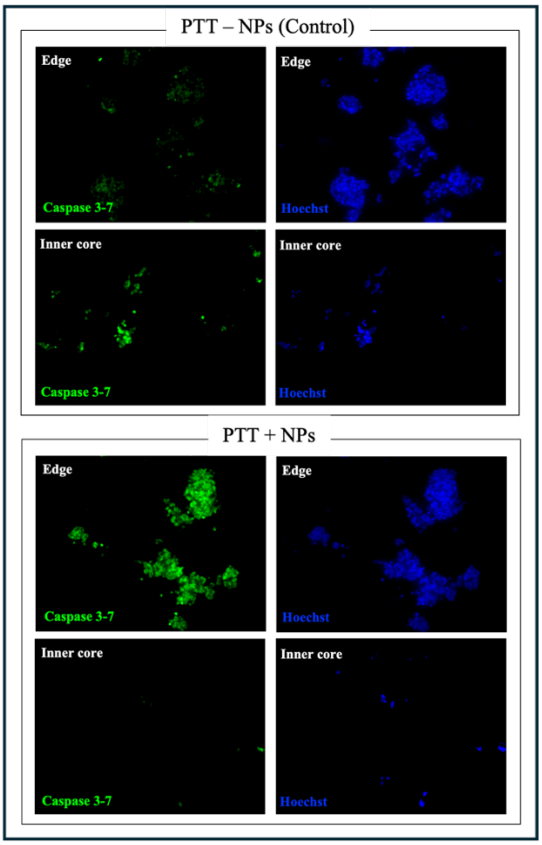

Figure 1. Activation of apoptotic caspase 3-7 cascade in TE-NB models subjected to PTT.

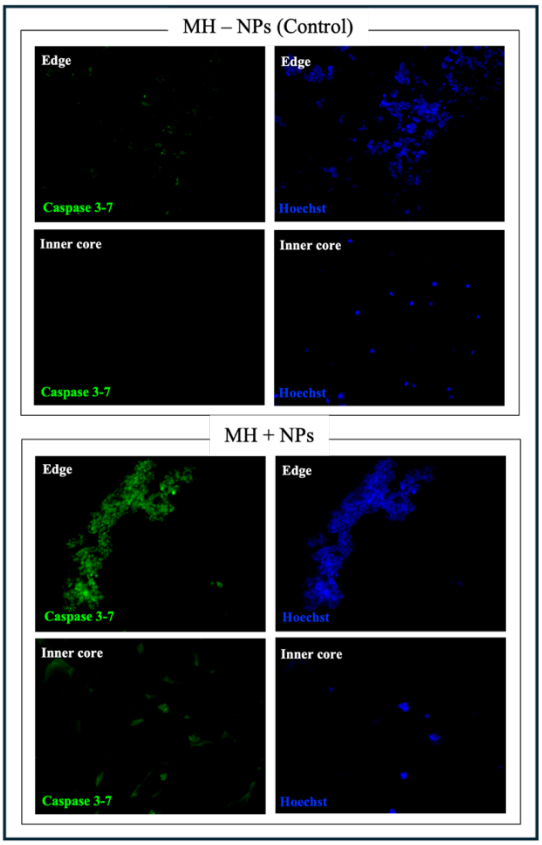

Figure 2. Activation of apoptotic caspase 3-7 cascade in TE-NB models subjected to MH.

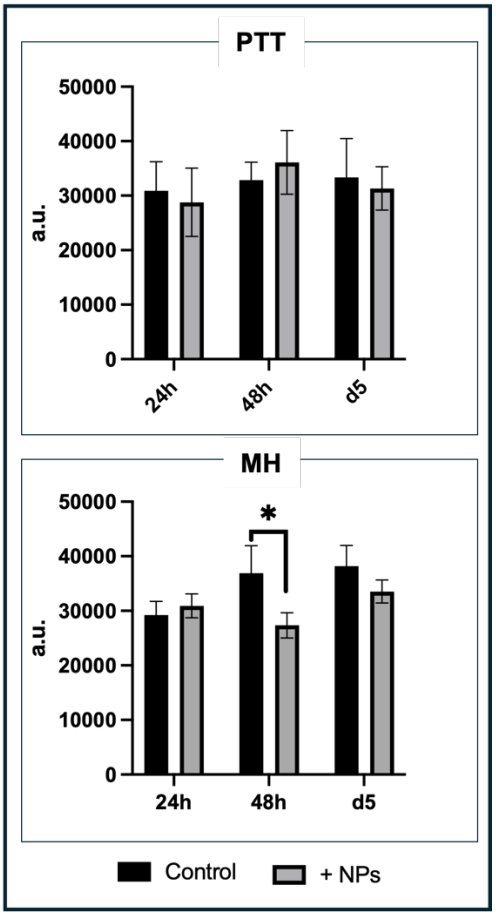

Figure 3. Picogreen quantification of cell proliferation in TE-NB models subjected to (A) PTT, and (B) MH. P-values < 0.05 are marked with "*"

Bioinorganic functionalization to enhance the uptake of nanoparticles in Gram-negative bacterial cells.

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Antimicrobial resistance is a growing health concern associated with high mortality rates. Alongside the increased number of resistant bacterial strains identified and the reduced number of new antibiotics in the pipeline, the development of new antimicrobial therapies is crucial.[1] Silica nanoparticles present unique properties for the design of novel drug delivery systems for antibiotics that are unable to be internalized in Gram-negative bacterial cells. The chemical versatility of their surface and framework proposes an advantage for the design of innovative antibiotic delivery systems.[2,3]

Iron is one of the most important nutrients for bacterial-cells, as it is involved in a large variety of metabolic processes. Bacteria cells can obtain extracellular iron in different ways, by siderophore-based iron uptake is the most efficient strategy. Siderophores are natural Fe(III) chelators produced by bacterial cells with high Fe(III) affinity that after scavenging Fe(III) from the growing media are internalized through highly specific siderophore surface receptors.[4] Due to this unique properties, Siderophore-Antibiotic conjugates (SACs) have been widely explored to enhance the antibacterial activity of several antibiotics following the “Troyan horse” strategy.[5] Nevertheless, this approach has never been applied to nanoparticles.

Hence, to enhance the nanoparticle cell interactions and subsequent particle internalization, aminocarbocylate-based Fe(III) complexes that mimic natural siderophores were developed. Previously developed luminescent vancomycin-loaded silica nanoparticles[2] were *in situ* functionalized with the Fe(III) complexes (Figure 1). The particles displayed optimal luminescent properties for imaging and sustained antibiotic release. The particles displayed high antibacterial activity. Even lowering the minimal inhibitory concentration of vancomycin, a non-active antibiotic against Gram-negative bacterial cells, against a wide range of tested Gram-negative bacterial cells. Electron and structured illumination microscopy revealed high nanoparticle internalization in bacterial cells, compared with uncoated and Fe(III)-free coated particles. Upon overexpression of siderophore receptor, the activity and internalization of nanopartciles was increased, suggesting that these receptors are involved in the mechanism of action of the nanoparticles. Similarly, *S. aureus*, *E. coli* and *P. aeruginosa* strains with inactivated surface siderophore receptors were employed to identifies specially the involved receptors. The results depicted that the nanoparticles interact with specific carboxylate- and aminocarboxylate-based siderophore receptors, which facilitates the nanoparticle internalization.

These results represent the first example in the literature of a nanoparticle-based drug delivery system interacting with siderophore surface receptors, expanding the application of siderophore-conjugated from SACs. Furthermore, the enhanced nanoparticle internalization could be used to repurpose antibiotics that, currently, are not being employed due to their lack of penetration abilities in Gram-negative bacterial cells.

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Figures

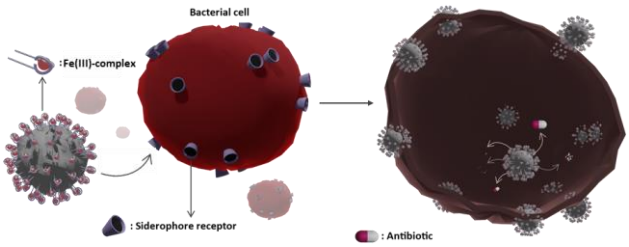

Figure 1. Scheme of the developed Fe(III)-aminocaboxylate coated silica nanoparticles.

Enhanced quantification and cell tracking using dual-fluorescent labeled extracellular vesicles

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Extracellular vesicles (EVs) are membrane-limited lipid bilayer vesicles released by many cell types, including both prokaryotic and eukaryotic organisms. One of the main functions of EVs that makes them so interesting, is their key role in cell-to-cell communication. [1], [2]. According to the Minimal Information for Studies of Extracellular Vesicles (MISEV) 2023 guidelines, EVs subtypes are classified as a function of different parameters, i.e., size, biogenesis, and composition [3], [4]. Accordingly, EVs have been divided into three classes: small-sized extracellular vesicles (also known as exosomes), microvesicles (MVs) and apoptotic cell-derived vesicles [5]. Nevertheless, EVs subtypes show overlapping dimensions, share some biomarkers and are similar in shape and density. Consequently, it remains difficult to cleanly isolate and distinguish between them. For this reason, sometimes it is difficult to refer to one specific type and, thus, they are referred to as small EVs (sEVs), including the group whose size comprises 30-150 nm [6].

Due to their natural tropism, there is an increasing interest in using sEVs, for diagnostic and therapeutical applications [7]. Regarding this last application, sEVs have certain advantages that make them a preferred choice for new therapeutic developments. sEVs display a more defined composition and tend to be more homogenous in terms of size compared to other types. and sEVs show an increased specificity and efficiency in targeting or delivering cargo to recipient cells [8]. In addition, since they exhibit unique biocompatibility, low immunogenicity, and reduced toxicity, so they can travel safely throughout extracellular fluids, sEVs have been explored as natural drug nanodelivery systems [9].

Despite the well-defined properties of EVs, their clinical studies and applications present technical and biological limitations that make it still marginal [10]. Distinguishing specific subpopulations of EVs from heterogeneous EVs remains a challenging task due to their heterogeneity and the limitations of current detection methods and the development of new strategies is needed since it allows a proper control of targeted EVs. These limitations, together with the lack of exclusive biomarkers, cost-effective quantification methods, and high-resolution visualization techniques, complicate the development of reliable and efficient techniques for purification, characterization, quantification and tracking studies of sEVs [11]. For this reason, is essential the development of advanced techniques that allow the distinction and quantification of sEVs within any type of sample.

An interesting approach to better distinguish sEVs from the background, consists in their selective labelling. The development of fluorescence-based monitoring tools has become of great interest for developing new techniques that ease the research in sEVs and their applications, including, cargo loading, recipient cells uptake and biodistribution [12], [13]. One promising method is the production of fluorescently labelled EVs by generating genetically engineered donor cells lines expressing fluorescent proteins fused to other proteins that are enriched in EVs, with minimal disturbance of their physicochemical and biological properties.

A single labelling of a tetraspanin (EV transmembrane protein) is not enough to identify a specific type of EVs population. In order to overcome this drawback, in this study, we aimed to develop a dual fluorescence reporter system using a tricistronic vector, which enables stable co-expression of two transgenes along with antibiotic resistance for mammal cell cultures selection [14], [15]. For this, eGFP and mCherry were fused to the N-terminal domain of CD63 and CD9, respectively, which are tetraspanins specific or enriched in sEVs.

Double fluorescently labelled sEVs were produced in a stable transfected HEK293SF-3F6 cell line. Fluorescently labelled sEVs were characterized using a variety of techniques (**Figure 1**), including, Flow cytometry (FC), Nanoparticle Tracking Analysis (NTA), Transmission Electron Microscopy (TEM), Western Blot (WB) and Confocal Spectral Fluorescent Microscopy (CSFM). The results about protein expression showed that the fused proteins were efficiently incorporated into sEVs. The size distribution and concentration of modified sEVs were compared with controls, indicating that the genetic engineering did not affect the structure and morphology of sEVs. The functionality of fluorescently labelled sEVs was assessed by recipient cells uptake assay. Results showed that sEVs functionality persists post-purification (**Figure 2A and B**). Moreover, fluorescently labelled sEVs were quantified by nanoflow cytometry, allowing to distinguish sEVs from other EVs or particles, and these values were compared to direct fluorescence measurements, obtaining a linear correlation, statistically validated, enabling a sEVs quantification method. In conclusion, this study describes a feasible, cheap, and fast methodology for EVs characterization, trafficking, and intercellular communication, as well as for sEVs quantification.

In conclusion, the production of dual-fluorescent labelled extracellular vesicles, specifically for small-sized EVs subpopulation, enhances the precision of sEVs tracking and enables a robust, cost-effective, and rapid quantification and characterization for sEVs of interest. This method also improves control over dosage-dependent studies involving sEVs. Likewise, modified sEVs preserved their attributes and their functionality, as demonstrated by executing the current MISEV guidelines. This strategy is a valuable tool for therapeutic sEVs development and drug delivery.

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Figures

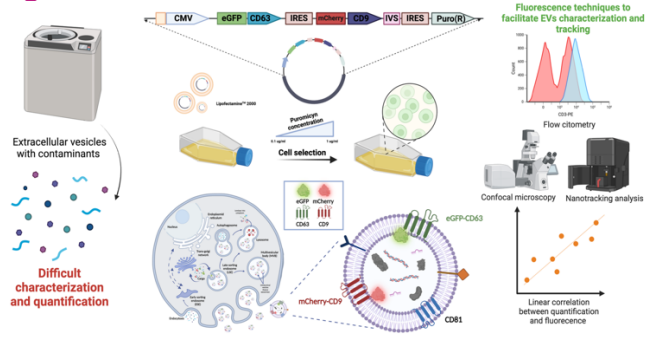

Figure 1. Existing problematic and followed methodology for obtaining double labeled stable sEVs and subsequent characterization.

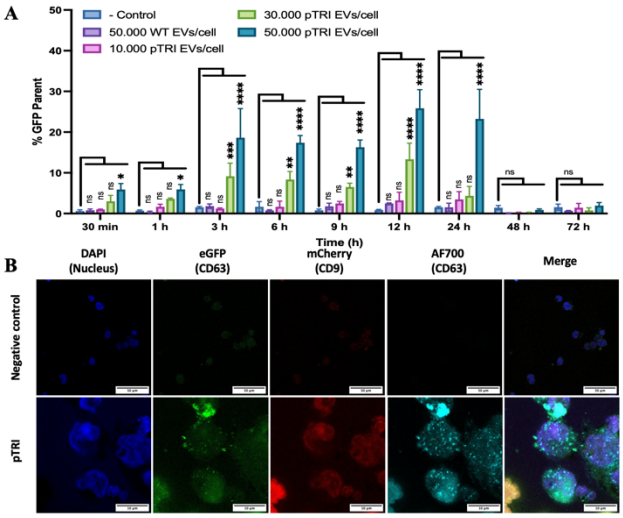

Figure 2. HEK293SF-3F6 double labelled EVs uptake study. **A.** Fluorescent eGFP positive percentage quantification assay with three different EVs concentrations. **B.** Qualitative evaluation of the uptake levels of 50.000 part/cell after 4 h and localization, upper negative control with non-modified EVs

The Antimicrobial, Anti-Inflammatory and Wound healing Effects of Silver Nanoparticles Synthesized from Cotyledon Orbiculata

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Cotyledon orbiculata, commonly known as pig's ear, is an important medicinal plant of South Africa [1]. It is used in traditional medicine to treat many ailments, including skin eruptions, abscesses, boils and acne [2]. Medicinal plants have also been used in the synthesis of metallic nanoparticles [3,4]. Nanomaterials produced in this way are more biocompatible and thus more suitable for biomedical applications [5]. This study aimed to synthesize silver nanoparticles using *C. orbiculata* aqueous extract and to investigate the antimicrobial, anti-inflammatory and wound healing properties of the synthesized nanoparticles and the plant extract. The *C. orbiculata* aqueous extract succesfully synthesized silver nanoparticles which were then characterized (Figures 1 and 2). The antimicrobial activity of the extract and nanoparticles was evaluated against common skin pathogens (S. Aureus, MRSA, S. epidermidis, P. aeruginosa, C. albicans). The immunomodulatory activity of the extract and nanoparticles was evaluated by determining their effects on cytokine production. The cytokine levels (TNF-alpha, IL-1 beta, and IL-6) were measured using the enzyme linked immunoassay. Their wound healing activity was assessed using the scratch assay and gene expression studies. The *C. orbiculata* aqueous extract was able to synthesize silver nanoparticles, which are 20-40nm in size. These nanoparticles exhibited good antimicrobial activity, with the highest activity observed against P. aeruginosa (5 µg/mL). The nanoparticles also showed anti-inflammatory activity by inhibiting pro-inflammatory cytokine secretion in macrophages. Both the nanoparticles and the extract showed good wound healing activities. It can thus be concluded that *C. orbiculata* synthesized silver nanoparticles have antimicrobial, anti-inflammatory, and wound healing properties.

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Figures

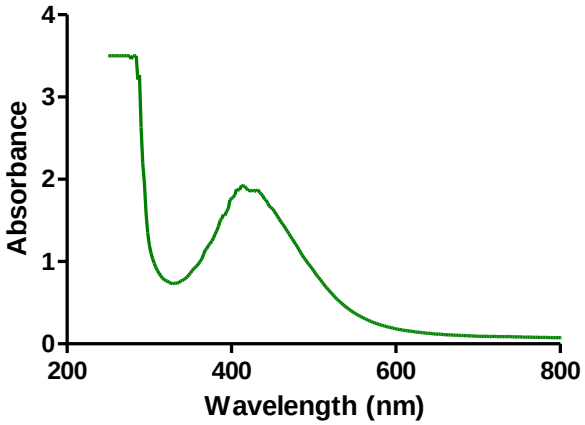

Figure 1. Characterization of *C. orbiculata* synthesized silver nanoparticles using UV-vis spectroscopy

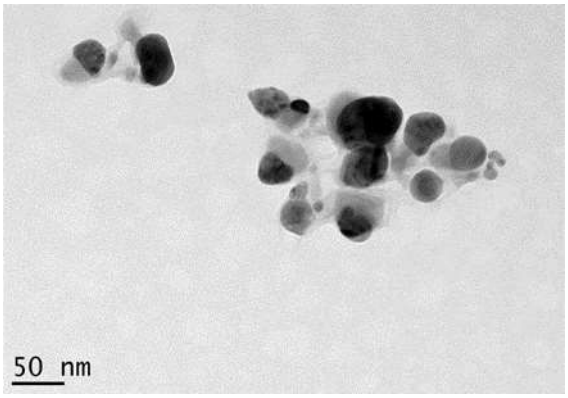

Figure 2. Characterization of *C. orbiculata* synthesized silver nanoparticles using HR-TEM

Immobilization of MUC-1 peptides on Supported Lipid Bilayers for Early Pancreatic Cancer Detection

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Pancreatic cancer is highly lethal due to the detection of the tumor at an advanced stage, in most cases after it has already metastasized. This is because it presents few symptoms in its early stages [1]. Currently, the main biomarker used for diagnosis in symptomatic patients is carbohydrate antigen CA19-9, with a sensitivity of 72%.[2] However, it has a much worse predictive value [3]. Hence, there is a need to develop new biomarkers for earlier detection and integrate them in diagnostic platforms that can be used to improve patient prognosis .

MUC-1 is a transmembrane protein that is overexpressed and aberrantly glycosylated in tumoral cells [4]. Circulating antibodies against tumoral MUC-1 could potentially be used for early cancer detection [5]. In this project, we focused on immobilizing different MUC-1 variants that resemble tumoral MUC-1 on Supported Lipid Bilayers (SLBs). SLBs have highly superb anti-fouling properties, and their components can be tuned, including for example the addition of lipids that carry functional groups for click chemistry [6]. Specifically, we incorporated a DBCO-phospholipid that is suitable for Strain-Promoted Azide-Alkyne Click Chemistry (SPAAC) with azide-functionalized MUC-1 variants. Once MUC-1 was immobilized, human serum samples from healthy and sick patients could be introduced to verify the specific and selective recognition of healthy and sick individuals.

Different MUC-1 variants were synthesized to study their binding to the SLB. Initially, with an azide group on the peptide and a DBCO-modified phospholipid in the SLB, the SPAAC reaction was monitored using Quartz Cristal Microbalance with Dissipation (QCM-D). The results showed that the reactivity of the click chemistry varied depending on the amino acid closest to the azide group, with the presence of cysteine leading to higher efficiency (Figure 1B) . However, when the functional groups were reversed—DBCO on the peptide and azide on the SLB—the amino acid had no influence on binding (Figure 1C). Once MUC-1 was immobilized on the SLB, its recognition by the anti-MUC-1 antibody SM3 was studied. In both cases, MUC-1 was specifically recognized by the antibody (Figure 2). These results demonstrate that MUC-1 can be immobilized on an SLB without losing its recognition properties, making it a promising system for a diagnostic tool.

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Figures

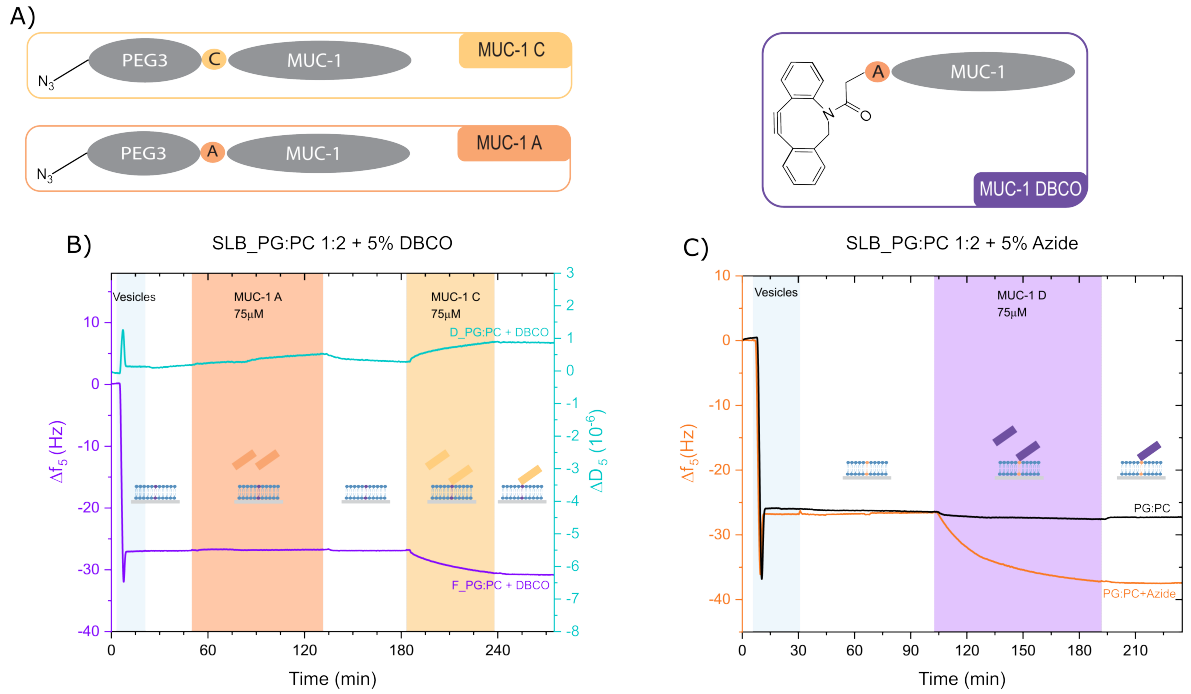

Figure 1. **A)** Scheme of the different MUC-1 peptides. **B)** MUC-1 A does not bind to the DBCO-SLB (orange area) since there is no frequency change instead, MUC-1 C binds successfully (yellow area). **C)** MUC-1 DBCO specific coupling to the Azide functionalised SLB. QCM of Azide functionalised SLB (orange) versus control SLB (black) analysing the binding of MUC-1 D (purple area), where there is a change in frequency only in the functionalised SLB.

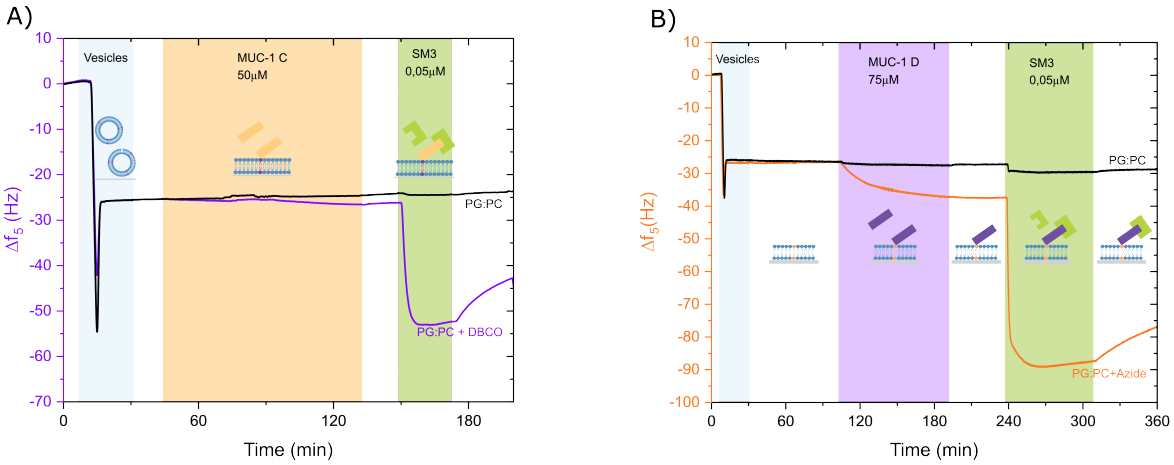

Figure 2. **A)** Antibody recognition of bound MUC-1 C to the DBCO-SLB. QCM of functionalised (purple) and control (black) SLB, in the yellow area the MUC-1 C was flushed and it just bound to the DBCO-SLB. After, in the green area the antibody binds specifically to the MUC-1 C. **B)** QCM of the azide functionalised SLB (orange) and control SLB (black) with MUC-D (purple area). Specific binding of the SM3 antibody (green area) to the MUC-D + Azide SLB (orange).

Label-free High Amplification SERS Detection with Nanostructured Substrates

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Surface enhanced Raman scattering (SERS) is a sophisticated analytical sensing method for biological and chemical sensing applications capable of detecting extremely low concentrations of a target analyte, even one molecule [1,2]. Its powerful detection capabilities are emphasized by the analyte identification without the use of artificial probes or labels (label-free detection). The detection of a fast ultra-low concentrations of analytes is of great significance for medical diagnostics, biomedical monitoring, food safety, and therapy [3,4].

In this work, simple, repeatable and cost effective methods [5-7] for obtaining ordered distribution of gold nanoparticle platforms with very high enhancement Raman signal are presented and successfully used for SERS detection of several analytes. Self-ordered distributions of nanoconcavities (Figure 1) and nanomounds substrates are obtained with several formation methods [8-12]. They all are decorated with metallic self-ordered nanoparticles formed by sputtering deposition and thermal annealing (Figure 2). The dependence of the size and shape of the resultant nanoparticles on the sputtering and thermal parameters is studied in depth, as well as the influence of the distribution of the patterned substrates (separation and diameter of the nanoconcavities and nanomounds).

The fabricated platforms are demonstrated to be excellent sensing SERS substrates for the detection of a broad range of molecules and medicines [13,14]. Also a complete evaluation of the different parameters of the fabrication steps parameters is presented.

Acknowledgements

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Figures

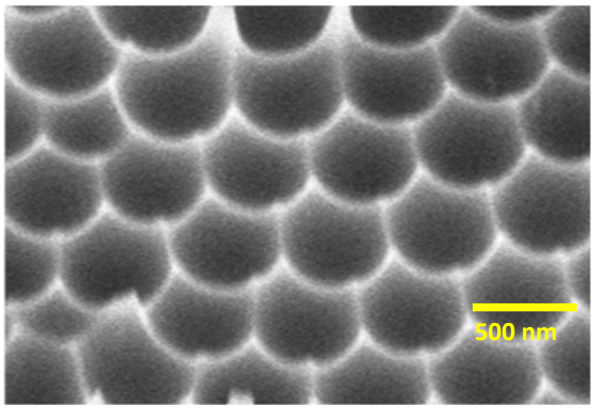

Figure 1. FESEM image of Al nanobowls of diameter ~500 nm.

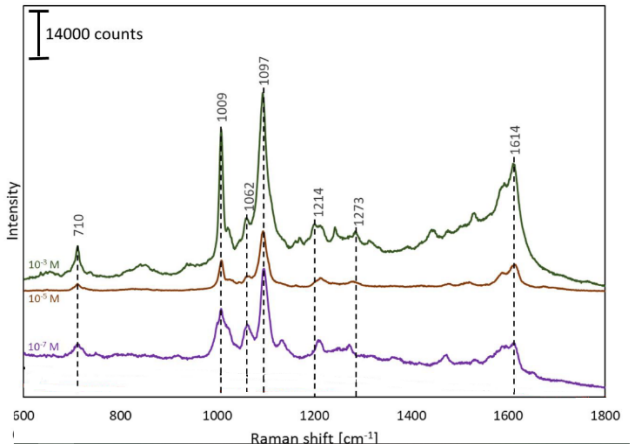

Figure 2. SERS spectra obtained with one of the developed substrates detecting several concentrations of 4-Mpy.

ZIF-8 and maghemite based nanoparticles as radionuclide carriers and hyperthermia agents in bi-modal cancer therapy

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This study focuses on developing novel nanoplatforms for therapy in cancer. We investigated the radiolabeling of ZIF-8 (a metal-organic framework) and Zn/Mn-substituted γ-Fe_2O_3 nanoparticles. Using polyol synthesis, we produced flower-like γ-Fe_2O_3 nanoparticles with multicore structures (23–67 nm), Figure 1.

The $Zn_{0.098}Mn_{0.447}Fe_{2.455}O_4$ nanoparticles exhibited moderate antiproliferative activity against tumor cell lines HeLa, LS174 and A375, while showing no activity against A549 and normal MRC-5 cells (IC_{50} > 200 μg/mL). After coating the nanoparticles with citric acid (CA), they were inactive against all cell lines. he cytotoxicity was linked to the high concentration of Fe ions on the surface área [1].

Investigating the changes in heating efficiency due to the partial substitution of iron ions with Zn and Mn in the parent compound while preserving the flower-like morphology and maghemite structure revealed significant variations in the ILP (Intrinsic Loss Power) values. ILP varied from 0.34 to 5.77 nH·m²/kg. The high ILP value for the $Zn_{0.098}Mn_{0.447}Fe_{2.455}O_4$ sample suggests its potential as an agent for applications in magnetic hyperthermia (both *in vitro* and *in vivo*). Our ongoing work focuses on *in vitro* magnetic hyperthermia studies.

The coated sample, $Zn_{0.098}Mn_{0.447}Fe_{2.455}O_4$@CA, was labeled with the therapeutic radionuclide ^{177}Lu. The maximum labeling yield of ^{177}Lu-$Zn_{0.098}Mn_{0.447}Fe_{2.455}O_4$@CA achieved was 89%, as determined by ITLC-SG. After 72 hours of incubation in saline and human serum, the labeled nanoparticles demonstrated very high stability.

The results show that ZIF-8 metal-organic frameworks are an effective potential carrier of the therapeutic radionuclide ^{177}Lu for nuclear medicine (cancer therapy). The maximum labeling yield was 92%. Stability testing over 72 hours showed 100% stability of the labeled ZIF-8. Zeta potential measurements show that the surface of ZIF-8 is positively charged at pH 7.

In order to identify the relevant binding sites within the porous framework of ZIF-8, the electrostatic potential distribution was calculated on the basis of periodic DFT methods implemented in CRYSTAL code. The analysis of the electrostatic potential mapped on an electron density isosurface (0.001 au) reveals well defined regions of negative potential located within the four- and six-membered apertures of ZIF-8 (red regions in Figure 2). These nucleophilic zones originate from the electron cloud density of the 2-methylimidazole linkers and can serve as main adsorption sites for Lu^{3+} ions, primarily by strong electrostatic interactions.

The results indicate that magnetic nanoparticles can be used in bi-modal therapy (magnetic hyperthermia and radionuclide therapy), while ZIF-8 has potential for use in radionuclide therapy.

Funding

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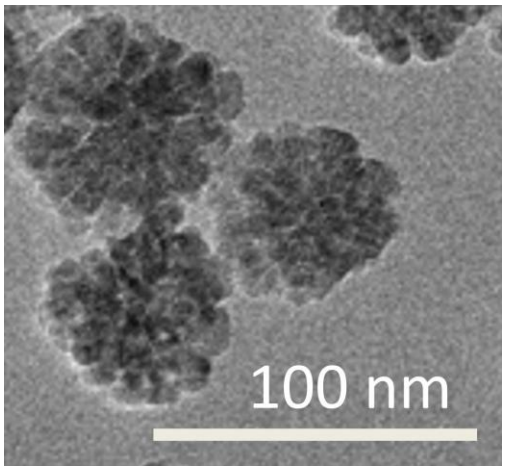

Figure 1. TEM micrograph of particles with flower-like morphology.

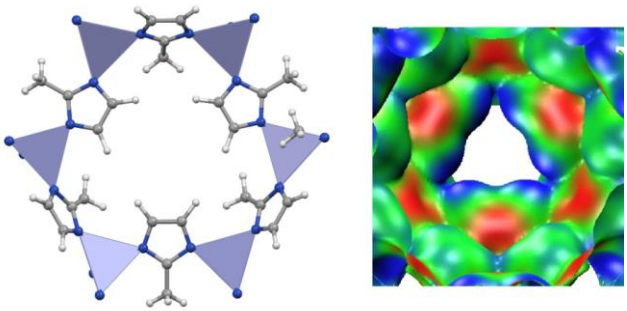

Figure 2. Six-membered aperture of ZIF-8 (left); Electrostatic potential mapped on the charge density isosurface (right). Red, green, and blue regions indicate negative, zero, and positive values of electrostatic potential.

Iron-Reduced Graphene Oxide Core-Shell Micromotors for Photothermal Therapy under Second Near-Infrared Light

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Core-shell nano-micromotors have garnered significant interest in biomedicine owing to their versatile task-performing capabilities [1,2].

We present a novel core-shell micromotor that combines magnetic and photothermal properties. It is synthesized via the template-assisted electrodeposition of iron (Fe) and reduced graphene oxide (rGO) on a microtubular pore-shaped membrane. The resulting Fe-rGO micromotor consists of a core of oval-shaped zerovalent iron nanoparticles with large magnetization. At the same time, the outer layer has a uniform reduced graphene oxide (rGO) topography. Combined, these Fe-rGO core-shell micromotors respond to magnetic forces and near-infrared (NIR) light (1064 nm), achieving a remarkable photothermal conversion efficiency of 78% at a concentration of 434 μg mL^{-1}. They can also carry doxorubicin (DOX) and rapidly release it upon NIR irradiation. Additionally, preliminary results regarding the biocompatibility of these micromotors through *in vitro* tests on a 3D breast cancer model demonstrate low cytotoxicity and strong accumulation. These results suggest that such Fe-rGO core-shell micromotors could hold great potential for combined photothermal therapy.

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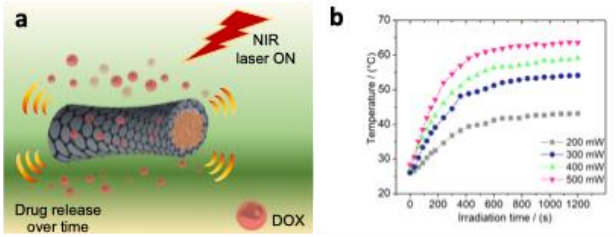

Figure 1. a) Scheme of the core-shell iron graphene oxide micromotors under NIR irradiation. b) Temperature change vs. irradiation time at various power intensities.

Mechanical phenotyping of breast cancer cells based on stochastic intracellular fluctuations.

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Recently, it has been observed that cancer cells undergo changes in their mechanical properties during carcinogenesis [1]. Cellular stiffness has been the most studied mechanical property due to the existence of Atomic Force Microscopy [2], a well-established and universal technique.

A correlation exists between the amplitude of the fluctuations of intracellular particles at short time scales, in the range from tens of milliseconds to tens of seconds and cell malignancy [3]. Even though this is viable approach to study cellular mechanical properties, it has a limited throughput and it only provides very local information.

In order to improve the viability of intracellular stochastic fluctuations in these studies, we propose the use of Digital Holographic Microscopy (DHM) and three breast cancer cell lines with different degrees of malignancy, MCF-10A (Healthy), MCF-7 (Non-metastatic) and MDA-MB-231 (Metastatic). DHM has proven method to achieve label-free quantitative imaging of the whole intracellular dry mass fluctuations with unmatched sensitivity. DHM images of the cell lines are present in fig. 1a).

For these means we record 100 s videos of our cells, fig 1b), and posteriorly determine our regions of interest fig. 1c). In order to quantify the amplitude of this fluctuations we calculate their root mean square (RMS), fig 1d), which scale with the malignancy [4].

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Figures

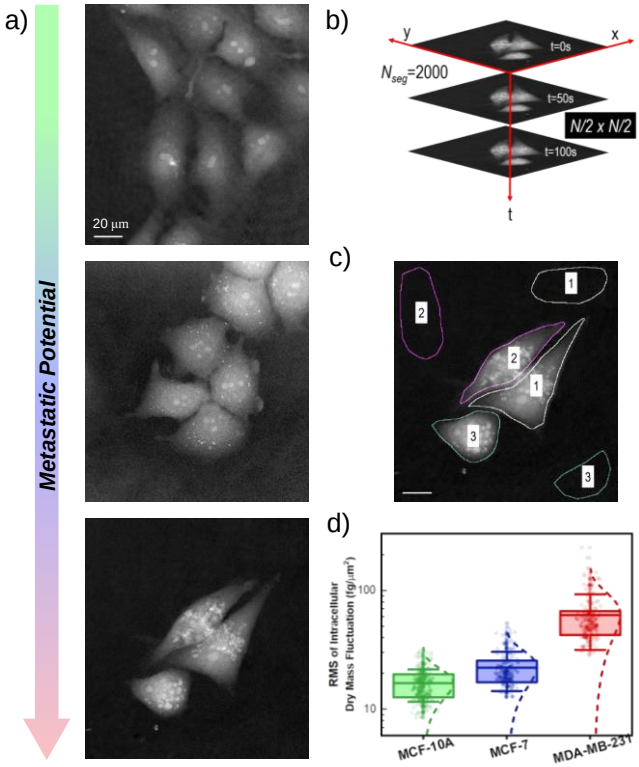

Figure 1. DHM fluctuation measurements. a) Morphology of the three cell lines. b) Schematics of image acquisition. c) Region of interest determination. d) Amplitude of stochastic intracellular fluctuations that scales with malignancy.

Stability of nanofluids CuO (40nm) in ethylene glycol or water base fluids with CTAB surfactant for biomedical applications

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Abstract

In this work, some properties of CuO nanofluids in distilled water and ethylene glycol base fluid were studied, comparing the stability of the nanofluid in samples with and without surfactant and measuring the electrical conductivity as a function of temperature and concentration.

Introduction

The dielectric properties of nanofluids based on water and ethylene glycol, of great relevance for several applications, have been studied by some researchers [1-5]. Some models used in estimating electrical properties describe equations that consider various factors such as volume concentration, nanoparticles shape, among others [1,5,6].
In this study, nanofluids were prepared by the two-step method, in 50 ml samples, with 40 nm CuO nanoparticles, supplied by MK-Nano, with 99% purity and 6.32 g/cm^3 density and distilled water and ethylene glycol base fluid, provided by Fisher Scientific, with 99% purity and 1.13 g/cm^3 density. Five volume concentrations from 0.1% to 0.5% were considered. The nanoparticles were weighed on a KERN ALJ analytical balance, model 220-4 NM, were gradually added to the base fluid placed in a flask and homogenized in an Eco Stir magnetic stirrer, model MS7-S. Subsequently, the sample was taken to a BANDELIN SONOPULS ultrasonic homogenizer, model HD2200, with a TT 13 titanium tip.
The electrical conductivity of the nanofluids was obtained from measurements performed with a HANNA Instruments conductivity meter, model HI 2550.

Studies have shown that the charge of the nanoparticles, the interaction between them and the dispersant directly affect the stability of the suspension [7,8]. To verify the improved stability of the nanofluid and the change in electrical conductivity behavior, samples of nanofluids were also prepared with distilled water and CTAB cationic surfactant, supplied by the company PanReac AppliChem.

Discussion and Results

The electrical conductivity was evaluated as a function of the concentration of nanoparticles, expressed as a percentage of sample volume and as a function of the temperature increase for the interval between 298,15 to 328,15 K, with increments of 5 K.
The results obtained for CuO and distilled water based nanofluids show that there is an almost linear increase in electrical conductivity with increasing temperature and that for the same temperature, electrical conductivity increases with increasing concentration. This evidence can be explained by several mechanisms proposed by different authors, nonetheless the most explain this phenomenon mainly by the formation of the electrical double layer [2, 6, 9, 10]. An analogous electrical conductivity study was carried out for the CuO and ethylene glycol based nanofluids, considering the same ranges of concentration and temperature. In this case, the results show that there is an increase in electrical conductivity with increasing temperature of the nanofluid with ethylene glycol base fluid, as happened with distilled water, however, this variation is not so linear. In the same way, it is verified that for the same temperature, the electrical conductivity increases with the increase of the concentration of nanoparticles.
When comparing the results obtained between the different nanofluids, it was verified that there is a more accentuated growth of the electrical conductivity of nanofluids with fluid based on demineralized water. Thus, it is possible to reach the conclusion that the electrical conductivity is strongly influenced by the base fluid, especially regarding to its polarity [1,6]. Regarding the stability of the nanofluids, it was found that the samples prepared with ethylene glycol base fluid were more stable over time, showing less deposition of nanoparticles. For this reason, only surfactant was used in nanofluids based on distilled water. The results obtained allow us to understand that the use of surfactant increases the stability and useful life of the samples but that it changes their properties, namely the electrical conductivity of the nanofluids, where there was a huge increase in the conductivity values.

Conclusions

The stability of nanoparticles is a crucial factor in the behavior of nanofluids, affecting the properties that make them so unique and much sought after for biomedical applications in order to harness the antibacterial power of CuO. [11]. In this study it was possible to prove that the electrical conductivity of nanofluids increases with the concentration of nanoparticles and with temperature and that nanofluids based on distilled water have higher electrical conductivity than nanofluids with ethylene glycol base fluid. It was found that the use of the CTAB surfactant leads to improvements in the stability of the nanofluid but that it significantly alters its electrical conductivity increasing significantly.

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Acknowledgements

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Nanobowled Aluminum Platforms Based on Nanoporous Anodic Alumina for SERS Applications

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In recent years, surface-enhanced Raman scattering (SERS) has garnered a lot of attention due to its high potential to be utilized in chemical and biological sensing as well as biomedical diagnostics.

In this work, the development of nanobowled aluminium (Al) platforms for SERS applications is presented, recreating the interesting honeycomb configuration on an Al substrate and its adaptable functionalization [1,2]. The initial formation of a nanoporous anodic alumina (NAA) sacrificial layer allows the tuning of the self-ordered nanobowls distribution on the Al surface. Using phosphoric acid and oxalic acid electrolytes, an anodization technique that consisted of two steps was utilized in order to create the NAA initial layer. Al nanobowls with diameter of ~500 nm were obtained with phosphoric acid electrolyte. In this case, a self-ordered pore structure of NAA was created by hard anodization with phosphoric acid of the Al templates at a voltage of 195 V [3]. The second step of the anodization process was carried out for a period of three hours while maintaining the same temperature and voltage parameters. Al nanobowls with diameter of ~100 nm were obtained with oxalic acid electrolyte, by applying 40 V for both anodization steps.

In order to obtain the nanobowls morphology at the surface of the Al template, the NAA substrates were submerged in a stirred aggressive combination of chromium trioxide (0.2 M) and phosphoric acid (0.42 M) for two hours while the temperature was maintained at 70 ºC. The resultant Al nanoconcavities are presented in Figure 1. For further functionalization, the resultant Al substrates were sputtered with gold for 120 s followed by thermal annealing at 400 °C for 1 h (Figure 2). The sputtering time and thickness of the gold film, followed by the shape of the aluminium template, are the factors that determine the size of the resultant nanoparticles, as the gold layer interacts with the topography of the Al nanoconcavities [4].

The findings of this study demonstrate an effective and robust process for assembling gold nanoparticles onto dense nanoarrays that were manufactured by electrochemical means. These nanoarrays have a shape similar to honeycombs and have a high spatial resolution. Experiments have been conducted to assess their plasmonic characteristics across a broad spectrum, ranging from the visible to the near-infrared area. These results have demonstrated that they are effective substrates for very sensitive applications, particularly for SERS [5,6]. Hypothetically, these interstitial spots were caused by concentrated electromagnetic fields that were coupled with intense localized surface plasmon resonance [7,8]. It is necessary for the nanoparticles to have a shorter separation distance in order for interparticle plasmon coupling to take place. This will result in a large rise in the near field intensity as well as SERS or an increase in the sensitivity of the LSPR refractive index [8,]. These fascinating substrates are highly helpful for SERS studies to detect various probe molecules, such as 4-Mpy, and antibiotics as well, owing to their high enhancement factor for low concentration. These prepared substrates have the potential to be functionalized and control morphological parameters that could be employed in various further applications in the sense of detection and sensing platforms.

Acknowledgements

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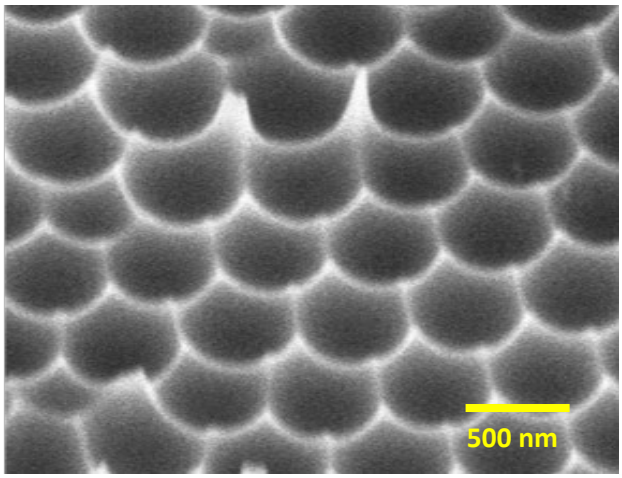

Figure 1. Top view FESEM image of Al nanobowls with diameter ~500 nm, obtained with phosphoric acid electrolyte.

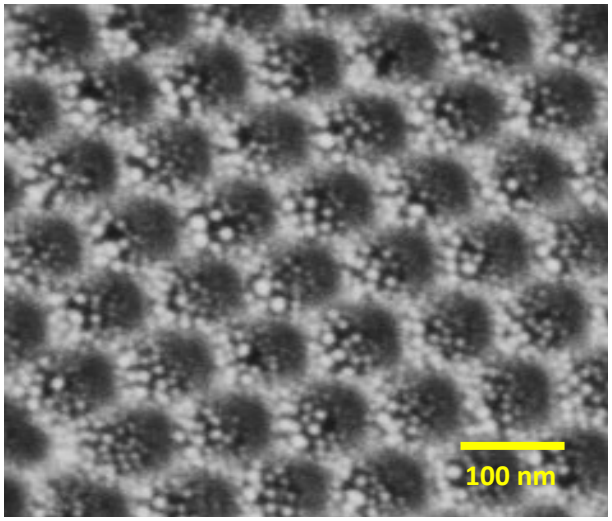

Figure 2. Top view FESEM image of Au nanoparticles formed in the Al nanobowls after thermal annealing. The diameter of the nanobowls is ~100 nm.

A MAGNETORESISTIVE BIOSENSOR FOR CIRCULATING EXOSOME DETECTION

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Abstract

Extracellular vesicles (EVs) are small, membrane-bound particles released by cells into the extracellular environment. These vesicles play a crucial role in cell-to-cell communication by transporting bioactive molecules, such as proteins, lipids, and nucleic acids (Figure 1) [1].
EVs are classified into different subtypes based on their biogenesis and size, with the main categories being exosomes, microvesicles, and apoptotic bodies. Exosomes, originating from endosomal compartments are released upon fusion of multivesicular bodies (MVBs) with the cell membrane and typically range from 30 to 150 nm in diameter. Microvesicles, formed by the outward budding or shedding from the plasma membrane, exhibit a broader size range (100 nm to 1 µm). Apoptotic bodies, released during programmed cell death, are larger vesicles (1 to 5 µm) containing cellular organelles and fragments from the dying cell [2].
EVs are a promising source of diagnostic biomarkers and have gained a wide interest in the biomedical and biosensing field [3]. Their presence in body fluids, such as blood, urine, saliva, and cerebrospinal fluid has opened new avenues for understanding physiological processes and exploring diagnostic and therapeutic applications [4]. Therefore, EVs have been proposed as novel diagnostic biomarkers for several pathologies such as cardiovascular diseases, autoimmune diseases, and cancer. Tumor-derived exosomes have been found to accumulate in human fluids and to be enriched in a set of membrane and soluble molecules reflecting the status of cells [5]. Thus, targeting exosomes could provide a promising tool for tumor biology and early disease detection, without invasive biopsy [6]. However, the implementation of exosomes as a diagnostic and prognostic tool has been hampered by the lack of highly reliable, sensitive isolation, characterization, and quantification techniques. Standard ultracentrifugation isolation is time-consuming and yields low recovery and low purity. In addition, in a clinical setting, fast results are also required [7]. Biosensing platforms, designed to detect and analyze EVs, offer several advantages, including non-invasiveness, the ability to access valuable biological information, and potential applications in diagnostics and therapeutic monitoring. The aim is to translate their importance in cell communication into measurable signals in point-of-care (POC) setups [8]. Biosensors offer significant advantages compared to traditional methods like ELISA, including the potential for miniaturization, automation capabilities, rapid and sensitive analysis, leading to cost-efficient designs [9].
This project aims to develop a portable magnetoresistive (MR) biochip platform to detect, characterize, and quantify exosomes in biological samples, order of magnitudes better than the state-of-the-art diagnostic devices. The core concept is to detect very low concentrations of biomarkers by coupling a magnetic sensor technology, easily integrable into microfluidic device, with iron oxide magnetic nanoparticles (MNPs) functionalized for the efficient capture of circulating exosomes. The detection scheme is based on an immune-sandwich assay, where exosomes from biological samples will be captured by MNPs, previously coated with antibodies against the tetraspanin protein CD63 [10], an enriched surface marker in exosomes (Figure 1). Streptavidin-coated MNPs are intended to be used, due to their consistent and reproducible properties. They will be incubated with biotinylated anti-CD63 antibodies, exploiting biotin-streptavidin strong interaction for MNPs functionalization. The biosensor will be functionalized with detection antibodies for the tetraspanin protein CD9, another common exosome marker, also reported as breast, ovarian, melanoma, and pancreatic cancer exosomal marker (Figure 2) [11]. Anti-CD9 antibodies will be immobilized on the surface of the sensor by amine-coupling according to standard protocols, in order to recognize and affinity-capture the MNPs labeled exosomes, by applying a magnetic field for separation of the MNPs from the solution.
The binding of the exosomes on the sensor brings the MNPs close to the surface of the sensor, leading to a measurable voltage change. The difference in voltage before and after the bio-recognition event indicates the amount of EVs in the sample. This detection strategy could allow the enrichment of the EVs from complex samples such as serum.
The biosensor will be feasible not only for the quantification of total exosomes but could be also implemented to detect multiple exosome markers simultaneously, providing a more comprehensive understanding of their profile in each sample. Importantly, the development of miniaturized and portable MR biosensors could

facilitate POC applications, allowing for exosome detection in diverse settings, including clinical environments and resource-limited areas.

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Figures

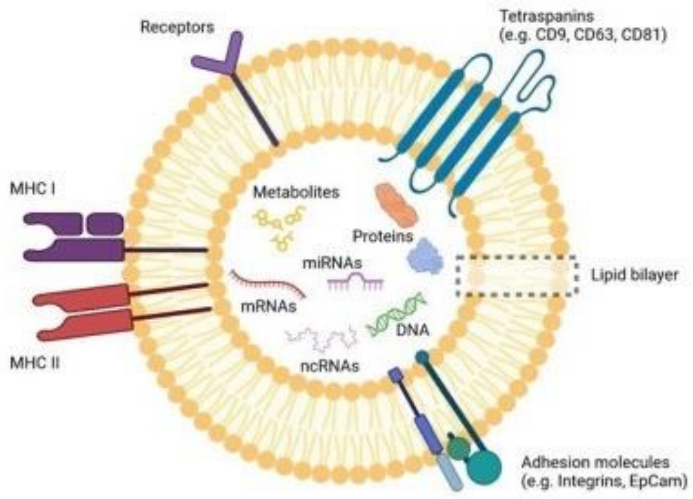

Figure 1. Schematic representation of the structure of EVs

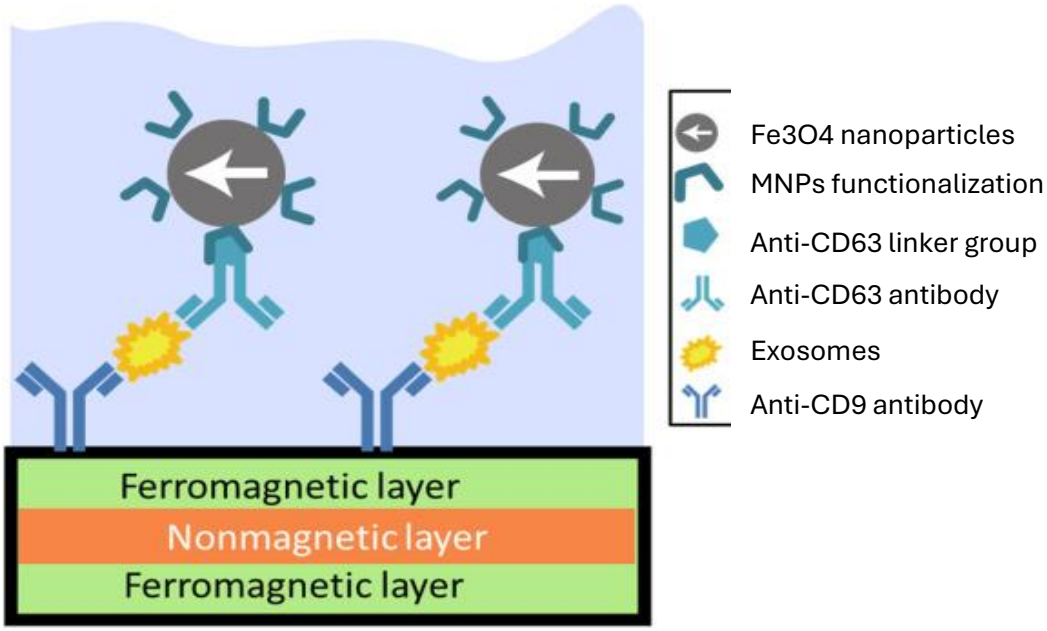

Figure 2. Schematic representation of the magnetic biosensor for exosome selective capture

SYNTHESIS, PHYSICOCHEMICAL PROPERTIES AND LIPOSOMAL FORMULATIONS OF NOVEL BODIPY DERIVATIVES AS EFFECTIVE ANTIBACTERIAL AND ANTICANCER AGENTS

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Boron dipyrromethene derivatives (BODIPY) constitute a new class of photosensitizers with interesting optical properties, including strong fluorescence. The optical properties make derivatives of BODIPY also promising candidates for practical applications, especially in the photodynamic therapy (PDT) of cancers and non-cancer lesions, as well as bacterial infections [1,2].

Photodynamic therapy (PDT) is a promising treatment successfully applied for localized cancers and other premalignant or non-malignant dermal lesions, and a perspective modality to treat microbial infections (photodynamic antimicrobial chemotherapy, PACT). PDT involves the use of a photosensitizer (PSs) and light of appropriate wavelength to induce oxidative stress leading to the eradication of targeted cells. PACT is a very promising therapeutic option to treat antibiotic-resistant microbes, but currently no photosensitizers are approved for antimicrobial photodynamic treatment. For that reason, the development of novel compounds for PACT represents an urgent need for research [3].

Novel BODIPY derivatives and its brominated and iodinated derivatives were synthesized and characterized using MS, UV-Vis spectrophotometry, and various NMR techniques including 2D methods. Photochemical studies allowed to evaluate absorption and emission properties as well as singlet oxygen generation ability of obtained compounds. Liposomal formulations were obtained by thin-film lipid hydration method. *In vitro* photodynamic activity studies were performed two bacterial strains gram-positive *Staphylococcus aureus* and gram-negative *Escherichia coli*, and two cell lines, ovarian cancer cell line (A2780) and triple-negative breast cancer (MDA-MB-231). It was found that the brominated and iodinated derivatives possess high singlet oxygen generation yields, which is considered a crucial cytotoxic agent in PDT. Liposomal formulations of BODIPY derivatives possessing bromine and iodine atoms revealed high activity towards both bacterial and cancer cells.

Acknowledgements: This work was supported by the National Science Centre, Poland, under grant no 2021/41/N/NZ7/00371. MF acknowledges Center of Excellence “Towards Personalized Medicine” operating under the Excellence Initiative—Research University at Nicolaus Copernicus University in Torun, Poland.

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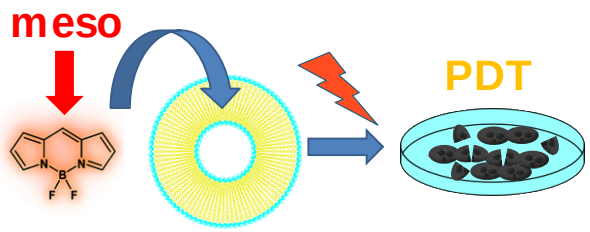

Figure 1. Schematic illustration of the procedures during studies.

Biodegradable Electrospun Nanostructured Polymer Dressings for Enhanced Burn Wound Healing.

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Abstract

The pursuit of the ideal wound dressing has remained a persistent challenge throughout human history. The demand for treatments that are rapid, effective, affordable, and painless has catalysed the development of skin substitutes and grafts for injuries that compromise natural protection, hinder healing, and prolong suffering, thus exacerbating tissue damage and pain. Recent technological advancements, particularly the advent of nanotechnology, have rekindled interest in electrospinning—an ancient material fabrication technique widely utilised in industry—which is now being harnessed with innovative substances for health research. The emergence of the nanometric scale has prompted the scientific community to reassess traditional manufacturing methods, including the time-honoured process of electrospinning, which has proven efficient in producing nanofibres from both synthetic and biological materials, woven at micro, sub-micro, and nanometric scales[1]. These nanofibres have demonstrated promising results when employed as skin substitutes[2].

This study seeks to evaluate the applicability of a biodegradable polymer dressing in the treatment of burn wounds. The randomised, controlled experimental trial was conducted with patients presenting with clean or potentially contaminated wounds, who were hospitalised in Manaus, Brazil. The average diameters of the poly(ε-caprolactone) (PCL) nanofibres and drug-modified PCL nanofibres were 1.16 ± 0.99 µm, 1.53 ± 0.92 µm, and 0.98 ± 0.66 µm, respectively. Figure 1 illustrates scanning electron microscopy (SEM) images and histograms depicting the fibre diameter distribution during the fabrication of PCL[3,4].

All dressings resulted in a significant reduction in pain among the treated patients, with no incidence of infection, thereby confirming the efficacy of the sterilisation method and its suitability for wounds extending to the deep dermis. The production of these dressings was rapid, uncomplicated, and cost-effective.

Keywords: Electrospinning; Biodegradable Polymers; Wound Healing; Burn Treatment; Nanofibres; Poly(ε-caprolactone) (PCL)

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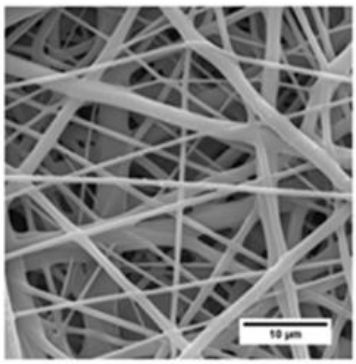
Figures

(a)



(b)

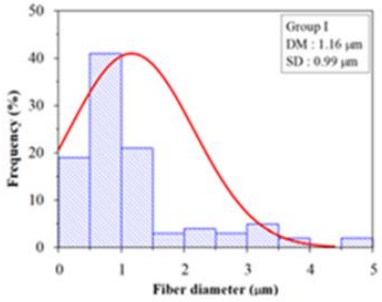


Figure 1. Figure 1. SEM images and histograms of fiber diameter distribution of the fabrication of PCL.

Raman Spectroscopy applied to the characterization of rough plant surfaces of romaine lettuce

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Romaine lettuce is one of the most widely consumed vegetables worldwide, which is susceptible to pathogen contamination. In this study, we explore the application of advanced microscopy and spectroscopy techniques to characterize the surface of romaine lettuce, with the aim of identifying its structural and physico-chemical properties. Raman spectroscopy was used to identify specific functional groups and spectra, could potentially indicate the chemical compounds present, generally associated with plant protection against multiple biotic and abiotic stress factors [1,2]. Given that Raman spectroscopy is a non-destructive technique, it allows for the identification of chemical compounds in plants without altering their structure, making it an essential tool for studying the physico-chemical properties of plant surfaces. In this study, Raman mapping was also performed on the stomatal areas to investigate their heterogeneous composition. Their two surfaces, abaxial (lower) and adaxial (upper) sides, where compared. The results indicated similar chemical functional groups on both surfaces. However, the variation observed in Raman intensities was systematic, being generally smaller on the abaxial side. These areas were also investigated by advanced Atomic Force Microscopy techniques, providing information about the location of hydrophilic areas. FTIR studies provided the surface leave composition with a broader spatial scale. Understanding the surface properties of romaine lettuce is crucial, suggest that increased adhesion is associated with increased pathogen binding-on the edible parts of the leaves [3]. These areas are of special interest due to their role in gas exchange and transpiration. Our findings revealed the presence of carbohydrates, carotenoids, and hydrophilic nano-areas, which can influence microbial adhesion. This approach offers a comprehensive understanding of the physico-chemical and biological properties of the plant surface, highlighting their role in self-protection against biotic and abiotic factors.

By analyzing the surface properties of various species and organs, such as rose petals [4] and olive leaf [5], we can gain a deeper understanding of the interaction between plant surfaces and their environment. These characteristics not only affect pathogen adhesion but also reveal the heterogeneity of the chemical components of leaf surfaces, as defense mechanisms against pathogens and many other stress factors.

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Figures

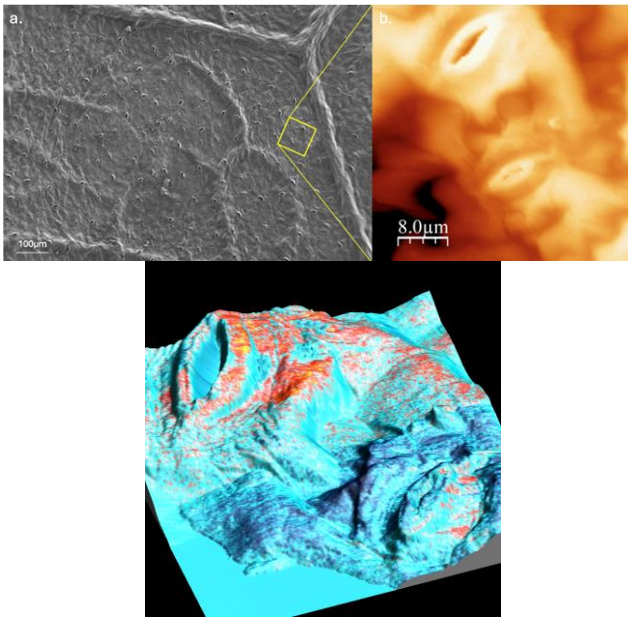

Figure 1. Scanning electron microscopy (SEM) and atomic force microscopy (AFM) images of the stomatal zones of romaine lettuce.

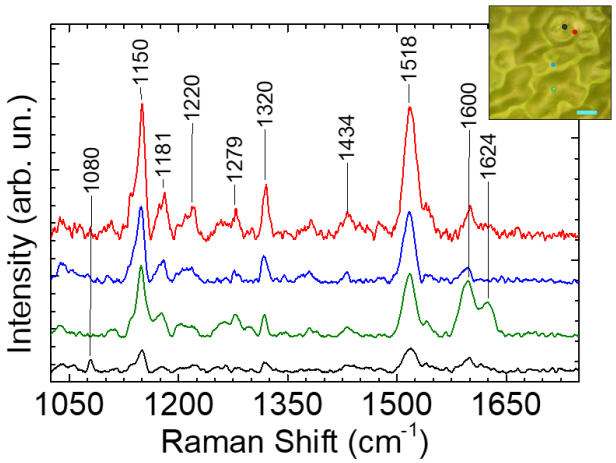

Figure 2. Raman spectroscopy spectra of four distinct regions within the stomatal zones of romaine lettuce, highlighting key chemical components. Notable differences between areas are observed in the peaks: 1080cm⁻¹ (carbohydrates, VM:$\upsilon(C{-}O){+}\upsilon(C{-}C){+}\delta(C{-}O{-}H)$); 1150cm⁻¹ (carotenoids with VM: $\upsilon(C{-}C)$, $\upsilon(C{-}O{-}C)$); 1320 cm⁻¹ (cellulose, aliphatics, phenylpropanoids, VM: $\delta(CH\_2)$); 1434cm⁻¹ (alkanes, waxes with $\upsilon(CH\_2)$); 1518 cm⁻¹ (carotenoids, VM: $\upsilon(C{=}C)$ in-plane), and 1600-1624 cm⁻¹ (phenylpropanoids, VM: $\upsilon(C{-}C)$ in the aromatic ring + $\sigma(CH)$). VM: Vibrational Modes).

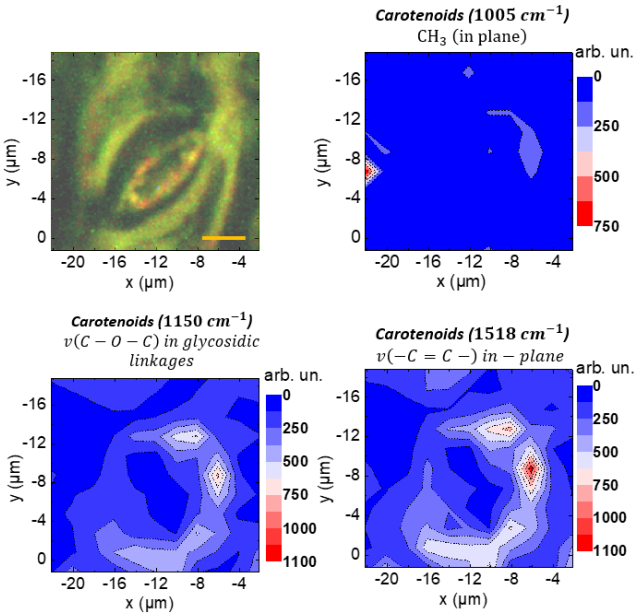

Figure 3. Raman spectroscopy maps showing the intensity of the modes corresponding to different functional groups in the stomatal regions of romaine lettuce. Each map represents different functional groups, with the color scales varying between maps. The yellow reference line represents a scale of 4 µm.

MODIFICATION AND DEPOSITION OF GRAPHENE OXIDE (GO) ON GOLD SURFACE AS A PLATFORM INCREASING SENSITIVITY AND STABILITY FOR A NEW TYPE OF IMMUNOSENSORS

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Protein – ligand interactions are fundamental to almost all processes occurring in living organisms. The ligands are most often ions, low-molecular weight compounds, peptides, nucleic acids and other proteins. This binding is most often reversible, highly specific and crucial in the regulation of the cell cycle. The interaction of proteins with ligands is also important in the development of new pharmaceuticals. The research for low molecular weight compounds (new drugs) that inhibiting enzymes and modulating the processes of protein complex formation is the foundation of modern medicine. Due to such extensive meaning, understanding of protein - ligand systems has become an important issue. Currently, tests based on the interaction of proteins with ligands are used not only for scientific purposes, but also have significance in diagnostics.

Biosensors are common devices for studies the interactions of protein - ligand. Nowadays, optical biosensors based on surface plasmon resonance (SPR) are becoming more and more important (Fig.1). They are widely used in scientific and pharmaceutical research, food research and in medical diagnostics. The main advantage of SPR technique indicates of biosensing without requiring any types of labeling (fluorescent, colorimetric, radioactive), which could interfere with the biosensing process, sensitivity, and real-time monitoring of biomolecule binding. In SPR techniques, the protein - ligand interaction is monitored directly in real time. Unfortunately, label-free SPR-based biosensing has low sensitivity for applications with small molecules and low concentrations of analyte. Nowadays, to improve the biosensing performance, researchers have proposed various types of material for the enhancement of optical properties of the transducer in SPR sensor.

The aim of the work was to deposit a layer or layers of GO and GO hybrids with polyelectrolytes/metallic nanoparticles of copper (Cu) or silver (Ag) on the gold surface in a controlled and repeatable manner. The physicochemical characterization of various GO suspensions and Ag/Cu nanopartilces suspensions obtained by chemical reduction methods were also carried out, including: size, zeta potential value, stability, composition and presence of different functional groups for GO using methods: DLS, UV-Vis, SEM and XPS. Additionally, using methods; AFM, SEM and ATR-FTIR determined the topology, coverage, surface roughness, structural and spectral properties of the tested systems. Quartz Crystal Microbalance with Dissipation monitoring (QCM-D), whose surface is covered with gold, and gold SPR sensors were used as model surfaces for deposition of graphene oxide/nanoparticles. The deposited GO layers should be characterized by high surface coverage, high stability and homogeneity. The layer-by-layer (LbL) technique was used to form the layers, which is based on the adsorption of oppositely charged nano-objects using methods: immersion, spraying (air brushing) and spin coating.

The planned research will contribute to the broadening of knowledge in the field of modern materials such as graphene oxide in physicochemistry and biochemistry with a special focus on SPR immunosensors.

Acknowledgements

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Figures

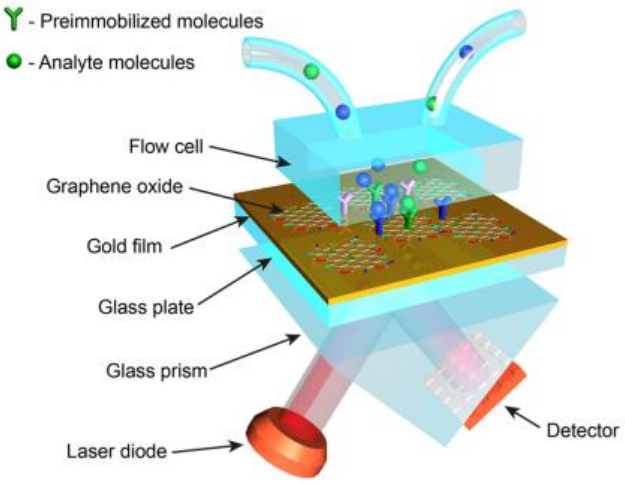

Figure 1. Schematic representation of the SPR biosensor comprising the SPR sensor chip with the graphene-oxide-linking layer, which forms in conjunction with the preimmobilized molecules that are highly selective to analyte [1].

OPTOPLASMONIC BIOSENSOR FOR ULTRASENSITIVE DETECTION OF INTERLEUKIN 11 (IL-11): TOWARDS SINGLE-CELL ANALYSIS IN LUNG CANCER

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Cancer remains one of the leading causes of premature mortality worldwide [1], with around 20 million new cases every year and over 10 million deaths reported in 2022. Lung cancer is the most frequently diagnosed cancer (12, 4%) followed by female breast cancer (11.6%). Projections indicate an increase of 77% in global cancer incidence by 2050 [2]. Notably, 50% of the cancers are detected at an advanced stage, when the prognosis is poor and treatment options are limited. Therefore, early detection is essential for improving survival rates and outcomes [3].

The development of advanced proteomic technologies for detecting small amounts of proteins at the single-cell level and low abundant proteins circulating in the bloodstream is crucial for deepening our understanding of cellular processes and disease mechanisms [4] and for the early detection of the disease. Traditional methods often lack the sensitivity needed to capture low-abundance proteins or the ability to resolve protein expression heterogeneity within individual cells – factors that are key to studying complex biological systems such as cancer progression [5].

Innovations in single-cell proteomics offer the potential to reveal critical insights into cell-specific protein dynamics, facilitating more accurate diagnostics, targeted therapies, and a greater understanding of molecular biology at an unprecedented resolution. Furthermore, early tumor proteins released into the bloodstream when the tumor is composed of only a few cells are found at very low concentrations (< 1pg/ml), making them undetectable for current proteomic technologies [6, 7] (Figure 1).

IL-11 is a member of the IL-6 cytokine family, which is important in regulating immune responses, inflammation, and hematopoiesis. It exerts its effects by signaling through the Interleukin11 (IL-11) receptor (IL-11Rα) and the gp130 protein, which in turn activates several key downstream pathways, including JAK/STAT, MAPK, and PI3K/AKT. These pathways are often dysregulated in cancer and contribute to tumor growth, metastasis, and resistance to therapies. Notably, IL-11 is overexpressed in bronchoalveolar lavage fluid of patients with lung adenocarcinoma compared to non-cancer controls and other histological subtypes of lung cancer, indicating its potential as a diagnostic biomarker for this disease [8].

In this work, we developed an optoplasmonic sandwich assay for detecting IL-11, using both purified proteins and non-small cell lung carcinoma (NSCLC) cell lines. Specifically, we used the H3122 cell line, which overexpresses IL-11, and the H1975 cell line, which does not express IL-11, serving as the negative control. This selection of cell lines allows for a clear comparison between a positive signal and a baseline or negative signal, helping validate the specificity and sensitivity of the assay in detecting IL-11 in NSCLC contexts.

Immunoreactions were carried out on biofunctionalized silicon wafers coated with capture antibodies, while detection antibodies were conjugated to gold nanoparticles (GNPs), which functioned as plasmonic labels (Figure 2). The optoplasmonic signal is measured using the automated dark field reading system, AVAC, from Mecwins, which allows the automatic measurement of 48 samples in just 40 minutes. For each sample, 119 images are captured to ensure robust statistical analysis of the gold nanoparticle (GNP) distribution on the surface. After the measurement process, the AVAC system automatically analyzes the acquired images. The system optically identifies individual nanoparticles, and their scattering properties are examined to characterize, classify, and count the nanoparticles present on the silicon surface due to IL-11 detection. This method offers high specificity in detecting IL-11, with a precise analysis of nanoparticle scattering contributing to the assay's sensitivity and accuracy.

The optoplasmonic assay was tested using varying concentrations of recombinant IL-11 protein, diluted in fetal bovine serum (FBS), to validate its specific recognition of IL-11. Remarkably, the assay achieved a limit of detection of just 1 x 10^{-18} g/mL (Figure 3).

After successful validation of the sensor, we tested the optoplasmonic biosensor with an IL-11 overexpressing NSCLC cell line (H3122) and an IL-11 knockout NSCLC cell line (H1975). The cells were initially cultured at a density of 10^5 cells/mL and subsequently lysed to prepare serial dilutions ranging from 10^5 cells/mL to 10^{-1} cells/mL. The optoplasmonic assay was then carried out using these dilutions. We successfully differentiated between the two cell lines, even in dilutions corresponding to a single cell or fewer (Figure 4). The observed decrease in the optoplasmonic signal at the highest concentrations is likely due to the high-dose hook effect. This phenomenon occurs when there is an excess of antigen relative to the capture antibodies, leading to falsely lower signal values.

In conclusion, we have successfully developed an optoplasmonic immunoassay for the ultrasensitive detection of IL-11. The assay achieved an

impressive detection limit of 1 attogram/mL using recombinant IL-11 diluted in fetal bovine serum (FBS). Additionally, the optoplasmonic immunoassay was successfully applied to cell lines with and without IL-11 expression, demonstrating its potential for detecting IL-11 with single-cell level sensitivity.

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Figures

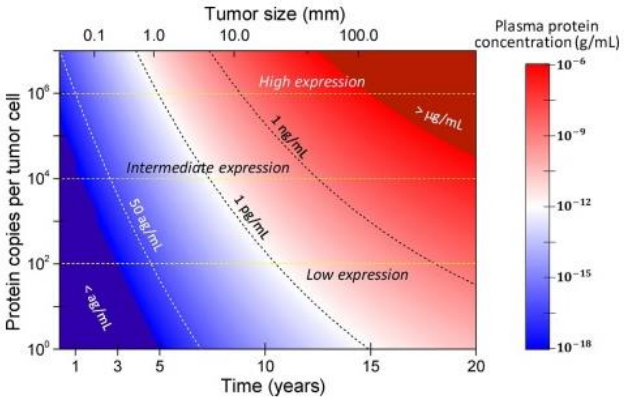

Fig 1. Mathematical prediction of the plasma concentration of proteins shed by the tumor as a function of the time and protein abundance in the tumor cells [7].

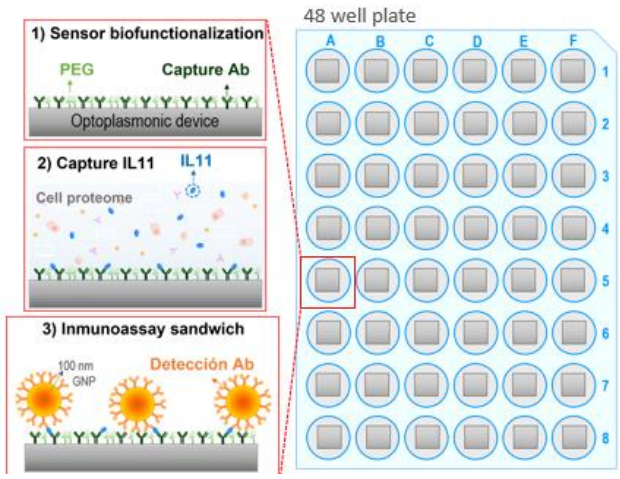

Fig 2. Scheme of optoplasmonic immunoassay procedure.

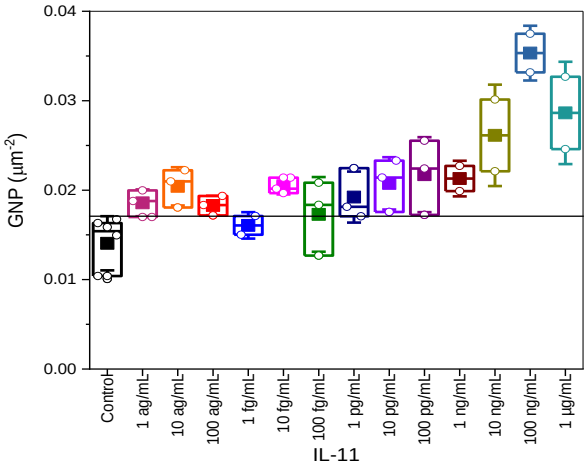

Fig 3. Successful detection of recombinant IL-11, with a limit of detection of 1 attogram/ml.

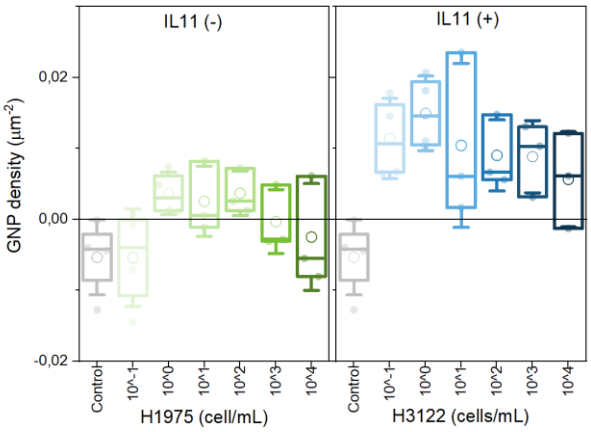

Fig 4. GNP density is significantly higher in cell line H3122 that overexpresses IL-11.

Drug-loaded PLGA nanomotors as a new approach for bladder cancer therapy

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Bladder cancer is the 7th most common cancer type worldwide, with over 500,000 new cases and 200,000 deaths annually (1). Current bladder cancer treatments are hindered by drug sedimentation and poor retention in the bladder, leading to high recurrence rates and low long-term survival (2). In recent years, nanomotors (NMs) have been developed as drug delivery systems for therapeutic agents. Nanomotors are self-propelled nanoparticles capable of converting chemical energy from their surroundings into mechanical propulsion (3). This motion enhances their diffusion and mixing capabilities, as well as their internalization into tumors, compared to passive particles (4,5). Given these benefits, they are an excellent tool for improving bladder cancer treatment as has been demonstrated in in vivo experiments, where radiolabeled nanomotors reduced bladder tumor size in mice by 90%(6). However, the designs used so far have limitations for clinical applications due to their inorganic chassis, such as silica. Therefore, there is a need to develop new nanobots based on organic materials, which are more biocompatible, biodegradable, and FDA-approved.

In this study, we developed a new design of nanomotors based on poly (lactic-co-glycolic acid) (PLGA) to enhance the standard treatment for bladder cancer, Mitomycin C (MMC). MMC-loaded PLGA nanoparticles were synthesized using the double emulsion method. To achieve motion, the surface of the nanoparticles was modified for urease attachment by first adding polyethyleneimine (PEI) and then using glutaraldehyde as a linker for the enzyme. The polydispersity, size, and surface charge of the nanoparticles were analyzed by Dynamic Light Scattering (DLS) after synthesis and at each stage of functionalization. Additionally, the enzyme activity of the nanomotors was measured by the pH change promoted by urea catalysis using Phenol Red reagent. Moreover, motion studies were conducted by comparing nanomotors in the presence and absence of urea. After characterizing the drug-loaded nanomotors, their therapeutic efficacy was assessed in bladder cancer cells derived from mice (MB49 line) and compared with the standard treatment (free MMC), demonstrating that the motion and drug encapsulation enhanced MMC-induced cell death. Finally, the mechanisms of action of our formulation were studied by analyzing the nanomotors' cell internalization and their effect on bladder epithelial cells, showing that they can internalize into cancer cells within just one hour affecting only bladder cancer cells.

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GRAPHENE NANOPARTICLES ON REFRACTIVE INDEX OF CANCEROUS BREAST TISSUES: SPECTRAL APPROACH

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The study of the refractive index (RI) in biological tissues is of great importance due to its numerous applications in medical optics. This physical quantity provides information on the interactions between light and tissue, and its quantification is essential and necessary for the identification and diagnosis of different pathologies and their respective therapies. Biological tissues show a structural complexity in response to the light beam, as their structures (cells, membranes and macromolecules) are integrated into a heterogeneous network of fibres. The scattering of the optical field that occurs in these structures is a complex phenomenon that is closely related to both the spatial variations of the RI and the morphological parameters of the scattering centres, such as the size, number density and shape of the particles. Therefore, any change in these parameters is reflected in the light scattering patterns [1].

This analysis is a very useful tool in the detection and characterization of tumours, as cancerous tissues have significant variations in their cell structures that lead to changes in light scattering. In addition to these variations, the absorption of light by endogenous tissue chromophores, such as melanin (RI =1.70) is also a feature that can be used to classify a tissue as pathological. These biomolecules, with their high light absorption efficiency, especially in the ultraviolet range (high RI values), are essential for the light protection of the body against ionising radiation [1].

It is also possible to relate the RI value to various parameters, such as dehydration, protein concentration, infections, elasticity, conductivity, cell division, oxygen saturation and metabolic rate, to name but a few. Another example is the optical mapping of RI in breast tissue, which makes it possible to recognise differences in cancerous tissue, as this has a higher RI value compared to healthy breast tissue.

Recent studies have shown important and significant differences in RI between healthy adipose tissue (which makes up the majority of the breast) and epithelial and tumour tissue [2].

This article presents the study of a nanofluid with graphene nanoparticles to be applied to breast tissue to improve its UV-Vis spectral analysis. Graphene, which consists of a single layer of carbon atoms arranged in a hexagonal structure, has unique and extremely important physical and chemical properties, it is an excellent electrical and thermal conductor, very resistant, impermeable to gases but permeable to water, has good elastic properties and exhibits excellent optical transparency [3].

This poster presents the preliminary study carried out to select the ideal concentration to achieve the proposed goal. For this purpose, we used glycerol and water in different concentrations as base liquid, x C3H8O3 + (1 - x) H2O and different concentrations of nanoparticles.

Five samples were prepared with different volumetric concentrations of graphene nanoparticles, 0.01%, 0.025%, 0.05%, 0.075% and 0.1%, for the different glycerol-water based liquids, with a volumetric ratio of 100:0, 80:20, 60:40, 50: 50, 40:60 and 20:80. The nanofluids were prepared by the two-step method [4], using graphene nanoparticles with CAS 7782-42, size 11-15 nm, from *MK-nano*, and a glycerol solution from the commercial supplier *labKem*, with CAS 56- 81-5, together with distilled water obtained on the same day in the laboratory. A *Bandelin Sonopuls* HD 2200 ultrasonic homogenizer with a titanium tip (M72) was used to homogenise the solution. A *Kern* analytical balance with a resolution of 0.0001 g was used for mass measurements. The RI was measured with a *Kern* ORT-1 *Abbe* refractometer with a resolution of 0.0005. The FLEX STD UV-VIS spectrometer T24402 from Sarspec was used for spectra acquisition, together with the *Avantes* CUV-ALL-UV/VIS SF210125 sample holder and 10x10 mm quartz cuvettes from *PG Instruments*. A T17805 deuterium-tungsten lamp from *Sarspec* was used as the light source. The fibre optic cables used were UV/VIS with a diameter of 200 nm (T15717) and 600 nm (T05512), both 50 cm long. This configuration, as shown in Figure 1, was set up during spectra acquisition, with the dilution of the nanofluid used being 0.1 cc in 3 cc of base fluid.

The refractive index results for the different glycerol-water base liquids are shown in Table 1, where it can be seen that the index decreases with increasing water content.

Table 1 – Liquid Base refractive index

x $C_3H_8O_3$ + (1 - x) H_2O	RI
20:80	1,3675 ± 0.00003
40:60	1,3944 ± 0.00003
50:50	1,4085 ± 0.00003
60:40	1,4235 ± 0.00003
80:20	1,4475 ± 0.00003
100:0	1,4745 ± 0.00003

Table 2 – Refractive index (RI) of nanofluids with different concentration of graphene[1]

x % (V/V) graphene	RI
0.011%	1.4056 ± 0.00003
0.024%	1.4055 ± 0.00003
0.048%	1.4056 ± 0.00053
0.064%	1.4056 ± 0.00003
0.082%	1.4056 ± 0.00005

(1) Recalculation of the volume concentration after production of the nanofluids

It was found that the RI did not change significantly at low concentrations of graphene nanoparticles between the prepared samples. However, the RI decreased compared to the base fluid used in these samples (50:50).

Figure 2 shows the UV-Vis absorption spectra. It can be observed that at concentrations of 0.075% and 0.1% in the 190 to 200 nm range, the reader is saturated and reaches its maximum resolution. However, at lower concentrations, the peak around 200 nm can be seen, which is due to graphene.

The aim is to further investigate the study on nanofluids with graphene nanoparticles in both UV-Vis absorption and transmission and to apply a different method to measure the refractive index in order to quantify the RI of biological tissue immersed in the nanofluid. So, the next step is to apply these nanofluids to breast tissue samples to analyse the influence of graphene on the interaction of light with the tissue and evaluate possible differences in light scattering.

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Figures

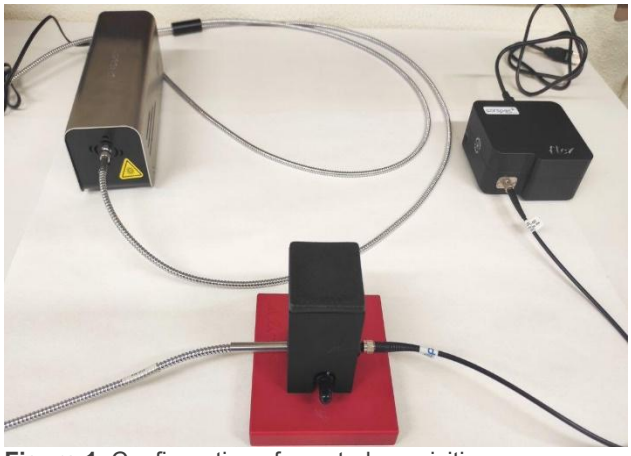

Figure 1. Configuration of spectral acquisition

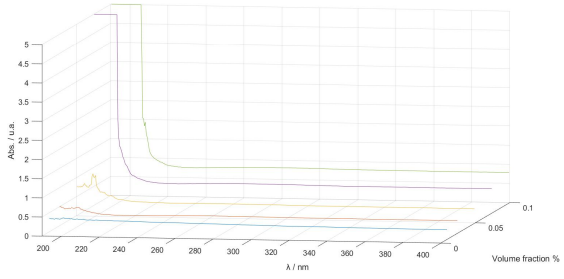

Figure 2. UV-Vis absorption spectra of nanofluids diluted in the base liquid with 0.01%, 0.025%, 0.05%, 0.075% and 0.1% graphene volume fraction

THE EFFECT OF TITANIUM DIOXIDE NANOPARTICLES SYNTHESISED USING GREEN TEA EXTRACT AND PURE EPIGALLOCATECHIN-3-GALLATE ON GLOBAL 5-METHYLCYTOSINE LEVEL IN PHYSARUM POLYCEPHALUM

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Titanium dioxide (TiO_2) is a crystalline material widely used in industrial applications due to its photocatalytic and superhydrophobic properties. Nano-sized TiO_2 is used in some consumer products, including paints, cosmetics, aerosols, or functionalized surfaces [1]. The release of TiO_2 NPs into soil, water or air during manufacturing or utilisation of the above mentioned products could be significant [2], leading to the possible bioaccumulation of this inorganic and stable nanomaterial into the environment. The toxic effects of TiO_2 NPs that were already demonstrated are multiple [3], including oxidative stress, DNA damage and inflammation (Figure 1). Green approaches are emerging as possible solutions for diminishing the toxic effects of conventional NPs as they use biomolecules in order to obtain more biocompatible NPs. Therefore, the aim of the present study was to obtain TiO_2 NPs by green routes, involving green tea (*Camellia sinensis*) extract and one of its major components in pure form, epigallocatechin-3-gallate (EGCG), and to study their effect on the growth rate and global 5-methylcytosine (5-mC) level by using microplasmodia of *Physarum polycephalum* as a model organism. *P. polycephalum*, a decomposer, eukaryotic organism naturally living under the forest leaf litter, tolerates the oxidative stress produced by relatively high doses of TiO_2 NPs as shown by recent studies [4,5]. However, epigenetic marks might respond differently to the presence of TiO_2 NPs, as the epigenome is generally more sensitive to the external factors. In this context, three types of TiO_2 NPs were tested (Figure 2): i. chemically obtained TiO_2 NPs derived from hydrolysis of titanium (IV) isopropoxide (TTIP) and condensation of the reaction products (chem-TiO_2 NPs); ii. green route obtained TiO_2 NPs from green tea extract assisted hydrolysis of TTIP (GT-TiO_2 NPs) and iii. green route obtained TiO_2 NPs from EGCG assisted hydrolysis of TTIP (EGCG-TiO_2 NPs). The method of synthesis of chem-TiO_2 NPs was adjusted from the previous work of Mahshid et al. [6]. The adjustments were described in detail in a previous publication of our research group [7]. Briefly, the synthesis of chem-TiO_2 NPs was performed by adding dropwise a solution of 8 mL TTIP:isopropanol (1:3) into 100 mL of distilled water. The mixture was stirred overnight on a heating plate at temperatures between 60-70°C until all the water was released by evaporation. A white precipitate was retrieved and dried for 2 h at 100°C. Green NPs were produced in a similar manner, excepting the water involved in hydrolysation of TTIP that was replaced by: i. a solution of 1 mmol EGCG in case of the EGCG-TiO_2 sample and ii. green tea extract, obtained from chopped aerial plant parts boiled in distilled water, for the GT-TiO_2 NPs sample, respectively. The physicochemical characterisation of NPs was performed using UV-VIS spectroscopy, scanning electron microscopy (SEM), Fourier-transform infrared spectroscopy (FTIR), X-ray diffraction (XRD) and dynamic light scattering (DLS). The identification and quantification of phenolic compounds in the green tea extract was analysed by UHPLC-MS/MS. Microplasmodial cultures of *P. polycephalum* maintained in a liquid semi-defined medium were exposed to 0.01, 0.1 and 1 mg/mL for 24 and 72 h for each of the three NPs samples. Culture solutions and procedures were detailed in a previous paper [8]. DNA from each treated culture was purified by performing the organic phenol-chloroform extraction and quantified using the commercially available Qubit™ dsDNA Quantification Assay Kit (Invitrogen, Life Technologies Corporation, Oregon, USA). Global 5-mC level in DNA samples was measured by Dot Blot technique performed on a nitrocellulose blotting membrane (pore size: 0.45 µm; Amersham™ Protran™, Germany) incubated with an anti-5mC antibody. UHPLC-MS/MS analysis of the green tea extract revealed 25 phenolic compounds, EGCG being the most abundant (0.527 mmol) followed by epicatechin (0.2 mmol). Formation of TiO_2 NPs was preliminarily confirmed by UV-VIS spectroscopy. Bulk TiO_2 absorbs radiation in the UV region of the spectrum, between 275 and 405 nm. The UV-VIS spectra of the three types of NPs synthesised by us had a similar pattern. Chem-TiO_2 sample had a maximum absorption (λ_{max}) at 226 nm, while for EGCG-TiO_2 NPs and GT-TiO_2 NPs it occurred at 232 nm and 212 nm, respectively. An additional peak at 273-278 nm was observed in the three UV-VIS spectra. FTIR spectra depicted a large absorption band (1000-400 cm^{-1}) with peaks around

550 cm^{-1} corresponding to Ti-O bonds stretching. Both NPs synthesised by green route presented a distinct region of absorption bands between 1040 and 1520 cm^{-1}. As these peaks had no correspondence in the spectrum of chem-TiO_2 NPs, they suggest the presence of moieties from EGCG solution and green tea. SEM images depicted spherical, uniformly dispersed and clustered particles with variable sizes within the nanometer range (<50 nm). XRD analysis proved that biomolecules might affect the crystalline structure of NPs. XRD spectra of both samples synthesised by the green route suggested their amorphous nature. In contrast, chem-TiO_2 NPs acquired a distinct crystalline structure comprising 73.3% anatase and 26.7% brookite. Zeta potential of the samples was variable, suggesting that EGCG-TiO_2 NPs were the most stable (-56.5 ± 6.43 mV). Electronegative NPs are considered stable under a zeta potential value of -25 mV. Chem-TiO_2 NPs and GT-TiO_2 NPs had a zeta potential of -20.3 ± 1.27 mV and -24.5 ± 0.50, respectively. The hydrodynamic diameter of TiO_2 almost reached 1500 nm. Both green synthesised TiO_2 NPs had a significantly lower average size when dispersed in water, 470 nm (GT-TiO_2 NPs) and 438.5 nm (EGCG-TiO_2 NPs). The amount of DNA in each tested culture was an indicative of the growth rate of microplasmodia. It should be noted that the *P. polycephalum* cultures exhibited similar growth in presence of the different NPs and in their absence. Dot blot revealed chem-TiO_2 NPs had a significant hypermethylation effect on the 5-position of cytosines in the DNA of *P. polycephalum* at 72 h, the effect being dose-dependent. Differences at 24 h were not statistically significant. EGCG-TiO_2 NPs acted reversely, causing a significant hypomethylation effect at 0.1 and 1 mg/mL after 72 h of exposure. The response of cytosine methylation state in *P. polycephalum* to exposure at GT-TiO_2 NPs was two-phased: i. hypomethylation was observed at 0.01 mg/mL in a time dependent manner and ii. at 0.1 and 1 mg/mL after 24 h of exposure the signal suggested global DNA methylation increased, while at 72 h decreased under the 5-mC level in the control culture. We concluded that a better control and standardisation of the NPs synthesis process were acquired by using pure EGCG instead of the entire green tea extract. The main advantage of using EGCG in the synthesis of TiO_2 NPs was the increased particle stability. Results suggested that EGCG inverted the effect of TiO_2 NPs on global 5-mC level in *P. polycephalum*. While EGCG and green tea acted barely similar on the particle properties, the epigenetic effect of GT-TiO_2 NPs did not follow an evident tendency. Response of *P. polycephalum* to GT-TiO_2 NPs could be described by a hormesis effect that might be caused by the variable biomolecules content of green tea.

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Figures

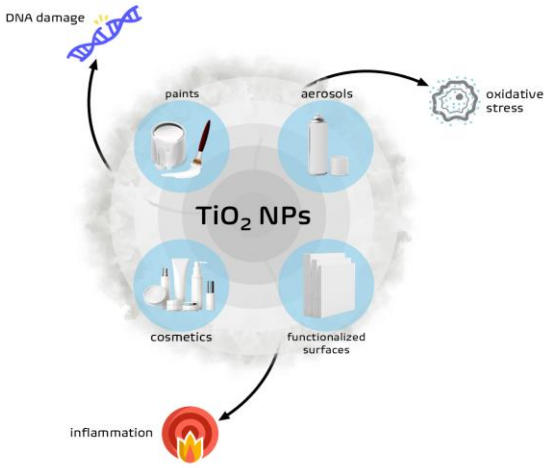

Figure 1. Main toxic effects of TiO_2 NPs and some of the consumer products prone to release NPs

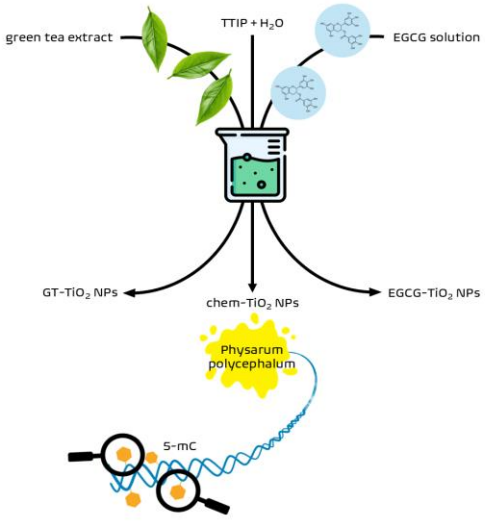

Figure 2. General flowchart of the experiment

AFM Spectroscopy for the Study of Lipid Bilayer Stability and Morphology

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Abstract

Atomic Force Microscopy (AFM) enables the visualization of nano-objects, biomolecules, and cells while simultaneously probing their mechanical properties. Force-distance curves derived from indentation experiments provide insights into the elastic and viscoelastic properties of macromolecules and living cells. For example, supported phospholipid bilayers can be used as mimics of cell membranes. And simulate cellular events *in vitro*. Moreover, puncturing the bilayer by AFM tip with a low bilayer radius creates a characteristic peak in the force-distance curve. Location of this break was called a “rupture event” and provides valuable information about the phospholipid membrane, such as its thickness, fluidity, or composition, etc. AFM can also capture protein-membrane interactions and the effects of specific agents on lipid bilayers or cell membranes, demonstrated here on pore-forming peptides with antimicrobial and anticancer potential.

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Synthesis, characterization, and applicability of aptamer-linked metalic nanoparticles against prostate cancer

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The anatomical location of the prostate poten-tiates several internal and external injuries, such as infectious agents, carcinogens, urinary reflux, hormonal changes, and physical trauma [1]. These factors can lead to chronic inflam-mation, resulting in the initiation and progres-sion of benign prostatic hyperplasia and pros-tate cancer [1–3]. This indolent carcinoma, with a non-invasive nature, presents one of the highest rates of neoplastic transformation in the human body, leading to death not only due to anatomical susceptibility to damage but also due to failures in controlling the metastatic process [1]. Prostate cancer affects more than 90% of men over 80 years old and is considered a serious threat to patients' lives [4]. It is among the five most prevalent cancers, repre-senting a significant global issue in terms of frequency and cancer-related mortality [5,6]. With the aging and rapid growth of the global population, it is expected that by 2030 more than 1.7 million men will be diagnosed with prostate cancer, with around half a million new deaths [1]. Currently, various treatments are available, including surgery, androgen depriva-tion therapy, chemotherapy, radiotherapy, and active surveillance by prostate-specific antigen. However, the choice of the most suitable treatment combination is still unclear, and the most common solution is total prostatectomy [7]. Given the limited potential of current ther-apies to prevent progression and treat prostate cancer, developing targeted, less toxic, and more efficient therapeutic strategies is crucial, especially for managing metastatic potential. Nanoparticles offer great potential for drug delivery and therapeutic applications due to their unique properties compared to macroscop-ic materials [8]. Various materials, such as iron, copper, cobalt, and some inorganic complexes, can be used to synthesize nanoparticles [9]. Thus, this work aimed to explore the synthesis of nanoparticles, functionalize them with fluorophores for tracking and aptamers for tar-geted therapy and test their effect on the pros-tate in vitro models.

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Figures

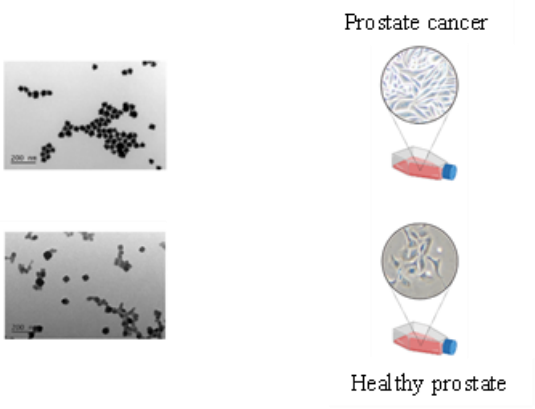

Figure 1. Figure ilustrating the fundamental question of this work: what is the impact of metallic nanoparticles against prostate cancer in in vitro models?

Unravelling the Interaction of Enzymatic PLGA-Nanobots with the Innate Immune System

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Our body is composed of highly complex and sophisticated environments, which are one of the key factors hindering the efficient delivery of drugs.[1] Nanobots, which are nanoparticles able to actively move at the individual and collective level, have shown enormous advantages in the presence of high viscous media,[2] mucus barriers[3] and tumors (*in vitro* and *in vivo*)[4]. Moreover, nanobots active motion have also shown enhanced nucleic acid delivery capabilities, allowing more efficient gene therapies.[5] With all, nanobots have brought wide attention as the next generation of drug delivery systems. Despite the clear relevance of this new approach, the unknown interaction with the immune system present *in vivo,* could provoke a decrease in the efficiency and severe side effects. There is a huge need for fundamental studies in this direction. Moreover, if we can understand the interaction between both systems, we will be able to develop new strategies for treating immune disorders or for modulating specific immune responses.[6]

Here, we present the design and characterization of a new kind of enzymatic nanobot based on (poly(lactic-co-glycolic acid) (PLGA), an FDA-approved material already used in clinics.[7] By conforming a positive layer of polyethylenimine (PEI), glutaraldehyde chemistry was used for functionalizing their surface with urease, resulting in PLGA-PEI-Urease nanobots. Our nanobots have been well characterized regarding size, surface charge, polydispersity, enzyme surface distribution (by STORM), degradation, enzymatic activity and motion (swarming behavior).

The immune system is responsible for our protection, defending our body from foreign threats such as pathogens and particles. The first line of defense is composed by the innate immune system, and it is characterized by being non-specific and quick. Then, a specific systemic immune response, known as adaptive, will take place against the recognized threat. In the first case, there are two main components: the physical barriers (skin and mucus) and the phagocytic cells (monocytes, macrophages and dendritic cells).[8]

Skin and mucus are considered biological barriers that limit the entrance of external agents, including drugs and nanoparticles, into the body. As already mentioned, enzymatic nanobots can be used to efficiently overcome mucosal barriers.[3] Here, we wanted to explore the capability of urease-nanobots to go through the skin, the first line of defense of our body. For this purpose, *ex vivo* pig skin was used as the most similar to human model. We have preliminary seen, how only in the presence of the fuel, nanobots are able to not get stacked in the epidermis and reach deep regions in the dermis.

A human monocyte cell line (THP-1) has been used for obtaining macrophages by culturing in phorbol myristate acetate (PMA). The differentiation was characterized by phenotypical changes (adherence and shape) and following the change in expression of markers CD14, CD68, CD80 and CD163 by flow cytometry. Then, we characterized the viability of human monocytes and macrophages with different concentrations of fuel (urea), nanobots, and a combination of both at different time points. With the optimal conditions, we checked the up-take by imaging and flow cytometry. Finally, we analyzed if the presence of our nanobots could activate the innate immune system cells by checking marker expression and cytokines secretion.

With all, we have established the first steps to unravel the interaction between our enzymatic-nanobots and the innate immune system, aiming to understand how to avoid immunogenicity or to modulate immune reactions to our desire.

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MULTIWALLED CARBON NANOTUBES, A PHYSICAL BARRIER TO CELL DIVISION

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Fibrous particles are extensively employed in a wide variety of industries, including construction, textile or medical industries due to their unique physical properties. However, at the nanoscale, these fibers have been associated with acute toxicological effects [1], raising significant health concerns. Asbestos is the best-known carcinogenic fiber, since it has been demonstrated that exposure to asbestos increases the risk of development of mesotheliomas and lung carcinomas [2]. The pathogenicity of fibers like asbestos has been attributed to specific physical characteristics, particularly their thinness, length and biopersistence, factors that enhance their ability to persist in biological tissues and cause harmful effects. The fiber pathogenicity paradigm recognizes fibers' geometry as their most important toxicological characteristic [3].

In the last decades, fiber toxicology research has increased, and several types of synthetic and natural fibers have been classified as carcinogenic or probably carcinogenic for humans according to IARC. Among these fibers, multiwalled carbon nanotubes (MWCNTs) have focused significant attention due to their widespread application in industry [4]. However, their fiber-like structure and their biopersistence in lung tissues raise concerns regarding their potential to induce adverse health effects, particularly in an occupational context. Several epidemiological and animal studies suggest that MWCNTs may induce mesothelioma in a manner similar to crocidolite asbestos [5]. However, the molecular mechanisms underlying this process remain a subject of debate.

In this study, we focused on the molecular mechanisms by which MWCNTs promote cell transformation *in vitro*. We employed a combination of cellular biology techniques, along with flow cytometry and microscopy to investigate the effects of MWCNTs exposure during cell division. Our results demonstrate that exposure to these fibers induces cellular phenotypes indicative of chromosome instability (CIN), including an increase in the frequency of binucleated cells and micronuclei after 48 and 72 hours of exposure. Additionally, we observed abnormal mitotic structures in MWCNTs-treated cells. Specifically, we observed lagging chromosomes upon cell division and a significant increase of aberrant mitotic spindle morphologies. Finally, flow cytometry analysis of DNA content, combined with Cdt1 protein detection, revealed an accumulation of tetraploid cells in MWCNTs-treated samples.

Taken together, our findings establish a model in which MWCNTs interfere with the cell division process by acting as physical barriers for the mitotic and cytokinesis machinery. This phenomenon leads to errors in chromosome segregation due to interference with mitotic spindle microtubules and to tetraploidization by disrupting the contractile ring resolution. These abnormal cell divisions trigger polyploid cell generation, which in turn exacerbates genomic instability in subsequent divisions, thereby promoting cancer progression.

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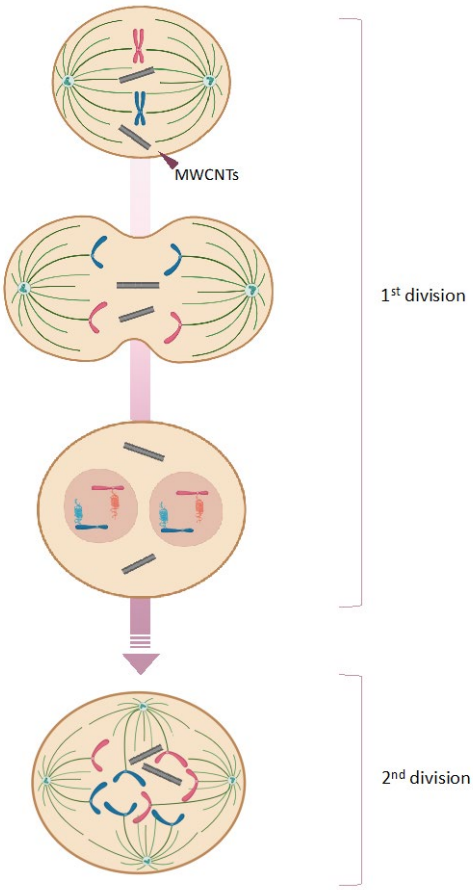

Figure 1. Proposed model explaining the mechanism by which MWCNTs interfere in cell division.

CeO2NPs modulation 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. It causes a sever hypoxia in the mother's , causing a systemic inflammation and oxidative stress with elevated levels of reactive oxygen species (ROS).

Trophoblasts, as placental precursors, play a pivotal role. Hence, we utilize this immortalized cell line HTR-8/SVneo cultured in a hypoxic chamber at 0.5% oxygen (hypoxia) to replicate PE conditions in vitro.

Furthermore, our research focus on cerium oxide nanoparticles (CeO2NPs), renowned for their anti-inflammatory and antioxidant properties, as a potential treatment for PE. This effect relies on the nanoparticles capacity to scavenge ROS. To ascertain ceria's ability to modulate the hypoxic impact on trophoblasts, we have analyzed the gene expression of several key genes. These genes include those involved in vascular remodeling (MMP9 and MMP2), hypoxia-inducible factors 1/2α, angiogenesis (VEGF and PlGF), pro-inflammatory cytokines (IL-1β and IL-6), and metabolic genes related to anaerobic glycolysis (GLUT1) and fatty acid oxidation (PPAR-ɣ). Typically upregulated under hypoxia, we've observed ceria's ability to mitigate this effect, in most cases in a dose-dependent manner. Additionally, migration assays indicate ceria' s modulation of trophoblast behavior. This correlate with the MMPs gene expression results involved in vascular remodeling and migration.

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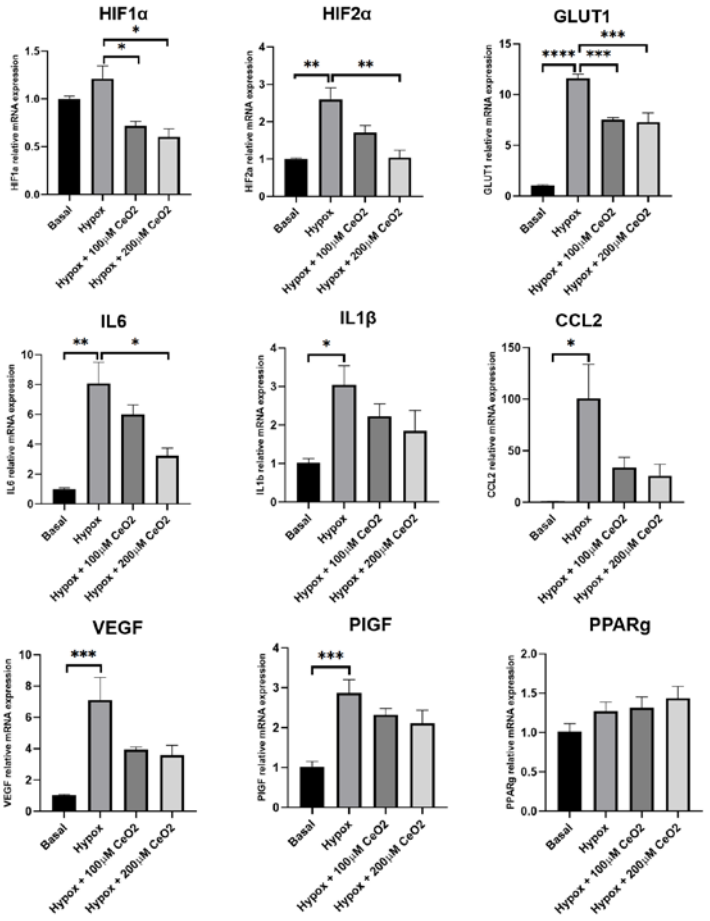

Figure1. Gene expression analysis of *HIF1α*, *HIF2α*, *GLUT1*, *IL6, IL1β*, *CCL2*, *VEGF*, *PlGF* and *PPARg* in HTR-8/Svneo cells. The expression of different genes were measured by real-time PCR. Data expressed as mean ± SEM and P-value ≤ 0.05.

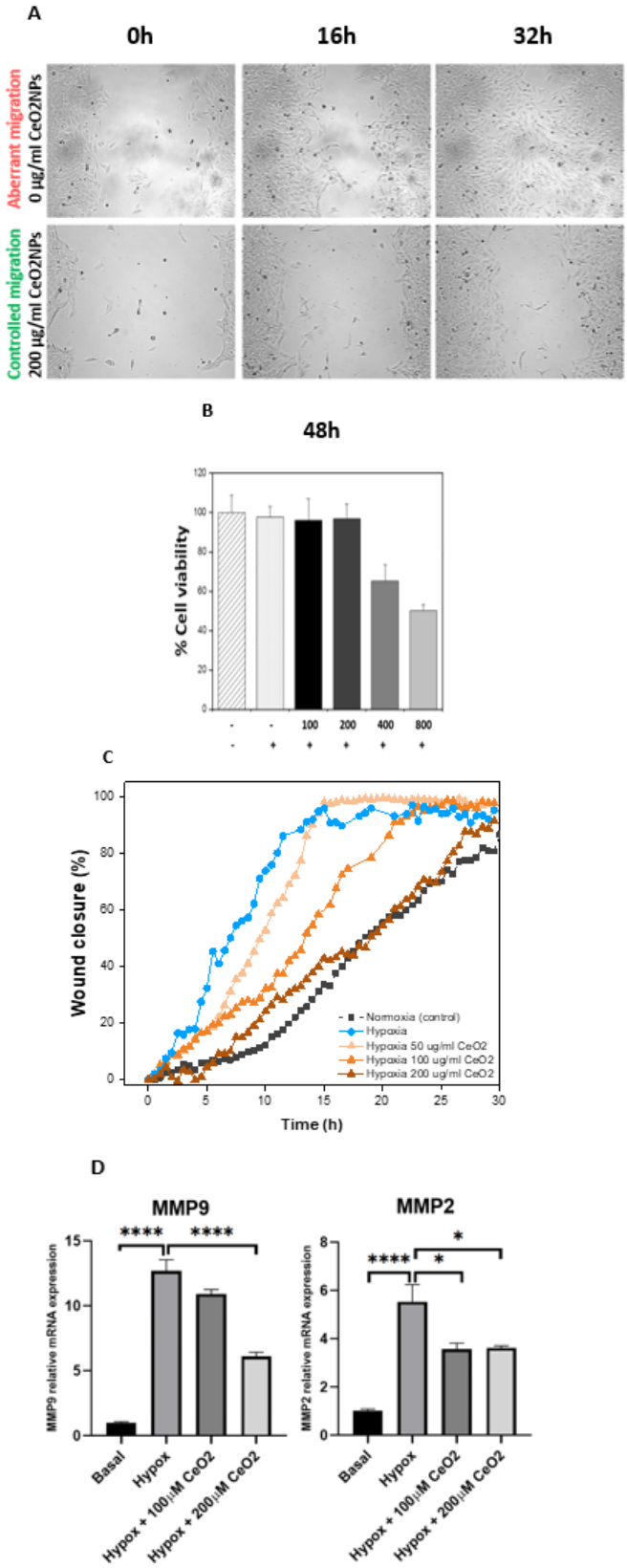

Figure2. Migration assay time-lapse confocal microscopy images. Untreated (above panels) and 200 µg/ml CeO2NPs treated (below panels) HTR-8/SVneo trophoblasts to 0.5% O2 at 0h, 16h and 32h (A). Cell viability in trophoblasts pre-treated with 100, 200, 400 and 800 µg/ml CeO2NPs and exposed to 0,5% O2 for 48h. Data expressed as mean ± SD (B). Evaluation of wound closure in % of untreated, 50, 100 and 200 µg/ml CeO2NPs treated HTR-8/Svneo trophoblasts (C). Gene expression analysis of *MMP9* and *MMP2* in HTR-8/Svneo cells. Measured by real-time PCR. Data expressed as mean ± SEM and P-value ≤ 0.05

Mass-Spectrometric Identification of Proteins and Pathways Responsible for Fouling on Poly(ethylene glycol) Methacrylate Polymer Brushes

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The prevention of protein fouling from blood plasma is a critical challenge in various biomedical applications, and significant research efforts have been dedicated to addressing this issue. The development of surface coatings that exhibit antifouling properties has seen substantial progress, with poly(ethylene glycol) (PEG) emerging as a widely recognized and effective solution. PEG and its derivatives, such as the dense PEG-like cylindrical brushes of poly[oligo(ethylene glycol) methacrylate] (poly(OEGMA)), have demonstrated remarkable potential in drastically reducing fouling from blood plasma proteins.

In this study, we present a comprehensive investigation into the variation of blood plasma fouling on poly(OEGMA) coated surfaces. Our research delves into the detailed analysis of protein deposition on these coatings after exposure to blood plasma from a diverse pool of donors. By examining the composition of the protein deposits, we aimed to identify the underlying mechanisms that influence the antifouling performance of poly(OEGMA) surfaces. Our findings reveal a significant correlation between the fouling behavior of blood plasma on poly(OEGMA) coatings and the composition of the deposited proteins. Notably, we observed that the activation of the complement system plays a pivotal role in the dramatically increased and accelerated deposition of blood plasma proteins on these antifouling surfaces. This activation occurs predominantly through the classical pathway of the complement system, which has been identified as the main contributor to the observed fouling phenomena.These results align with previous studies on PEGylated drug carriers, where similar issues of complement activation have been noted. The insights gained from this study underscore the importance of thoroughly understanding the interactions between antifouling coatings and their surrounding biological environment. Such understanding is crucial not only for optimizing the design and application of antifouling surfaces but also for anticipating potential challenges that may arise when these coatings are used in real-world biomedical contexts.

In conclusion, while poly(OEGMA) coatings offer promising antifouling properties, our research highlights the need for careful consideration of the complement system's role in protein fouling. The study provides a valuable contribution to the ongoing efforts in developing more effective and reliable antifouling strategies, paving the way for improved performance of biomedical devices and materials in contact with blood plasma.

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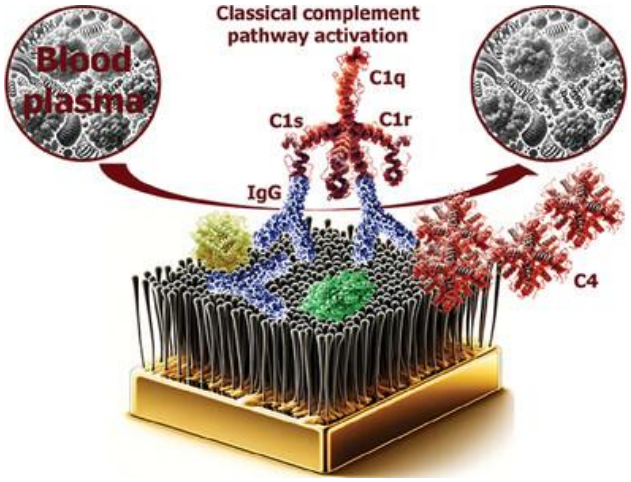

Figure 1. Scheme of polymer brush blood plasma interaction with focus on a classical complement pathway activation.

Acknowledgement

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Nanothin Fibrin-Heparin Coatings: A Dual-Function Strategy for Thrombosis Prevention and Vascular Implant Integration

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The prevention of thrombosis in artificial vascular implants remains a critical challenge in cardiovascular medicine. Traditional implant surfaces often trigger the coagulation cascade upon contact with blood, leading to thrombus formation and subsequent complications. In response to this issue, we have developed innovative nanothin fibrin-heparin coatings designed to both prevent thrombosis and support the endothelialization of vascular implants.[1] These coatings, with thicknesses in the nanometer range, are inspired by natural biological processes. They function not only as a barrier to platelet activation and inflammation but also as a platform for the controlled release of growth factors. Specifically, the coatings are engineered to bind and release fibroblast growth factor (FGF) and vascular endothelial growth factor (VEGF), which play crucial roles in endothelial cell proliferation and differentiation. In vitro studies using human umbilical vein endothelial cells (HUVECs) demonstrated that these coatings significantly enhance cell viability and promote the formation of mature endothelial structures. The synergistic effect of FGF and VEGF, delivered via the fibrin-heparin matrix, was particularly effective in accelerating endothelialization, a key factor in the long-term success of vascular implants.

Our research highlights the potential of nanothin fibrin-heparin coatings as a dual-function solution for both thrombosis prevention and the promotion of vascular healing. This approach represents a promising advancement in the development of next-generation cardiovascular implants, with the potential to significantly improve patient outcomes.

Figure

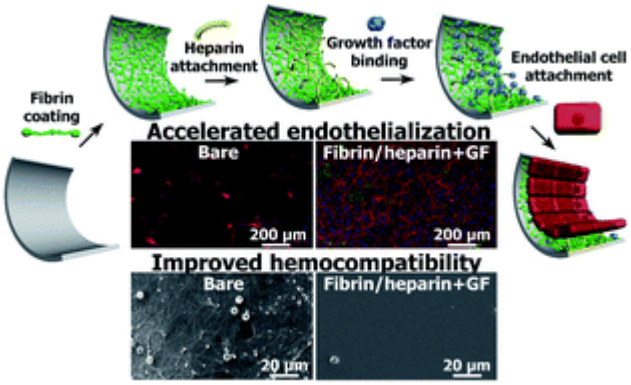

Figure 1. Scheme of a nanothin fibrin-heparin coating and its effect on hemocompatibility and endothelialization

Acknowledgement

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Sequential adsorption of charged nanoobjects as a method of formation of drug delivery systems

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The idea of drug targeting, proposed by Paul Ehrlich at the beginning of the twentieth century considered a hypothetical 'magic bullet' consisting of two principal components. The first component should recognize the target and bind to it, while the second component should perform therapeutic action. Polymeric nanocarriers are ideal candidates for use as a 'magic bullet' due to their potential to localize in a specific manner to the site of action and reduce or eliminate the possible side effects. To prepare targeted polymeric nanocarriers, the proper functionalization/surface modification should be performed. The sequential adsorption of oppositely charged nanoobjects (layer by layer (LbL) method) is a powerful technique for the fabrication of multifunctional coatings. The advantages of the LbL method are the ease of manipulation and the multifunctionality that comes from the possibility of modification of the multilayer shell by organic molecules, inorganic nanoparticles, carbon nanotubes, antibodies, lipids, or nanoparticles. That multifunctionality can be utilized for the preparation of targeted drug delivery systems. The polymeric nanocarriers were formulated using a method based on a nanoemulsion template. [1-2]. The polymeric nanocarriers were prepared by the self-emulsification and self-emulsification solvent evaporation methods respectively. Furthermore, polymeric nanocores were functionalized by layer-by-layer method to achieve targeted drug delivery systems. The polymeric nanocarriers with an average size of 80-100 nm were stabilized by an AOT/Polycation surface complex. Such nanocores were encapsulated with multilayer shells formed with biocompatible polyelectrolytes (poly-L-lysine hydrobromide PLL (MW 15000-30000), poly-L-glutamic acid sodium salt PGA (MW 15000-50000)). They were further modified for passive targeting by pegylation (adsorption of pegylated polyelectrolyte PGA-g-PEG as an external layer), for active targeting by immobilization of selected antibodies, whereas for magnetic targeting by iron oxide nanoparticles incorporation into a multilayered shell. The polymeric nanocarriers were also tested as a theranostic system i.e. the MRI-detectable drug delivery system. The developed systems may be considered as promising platforms for future nanomedicine.

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SELECTING THE BEST NANOPARTICLE-BASED DELIVERY SYSTEMS FOR TRANSPORT ACROSS INTESTINAL BARRIER

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The intestinal barrier represents a serious challenge for drug delivery. Its intricate architecture, mucus layers, and tight junctions act as gatekeepers, leading to degradation and poor absorption of conventional drug formulations [1]. In the quest for more effective drug delivery systems, nanoparticles (NPs) show a great promise. NPs-based delivery systems offer enhanced stability, targeted delivery, improved bioavailability, and controlled release, promising solutions to these issues [2].

In this context, we aimed to study the transport mechanism of NPs with different characteristics (chemical structures, sizes, surfaces) across intestinal barrier over time in order to provide a deeper understanding of NPs behavior in these biological systems and further innovative, mechanistic strategies for preventing toxicity and designing more effective drug delivery systems.

Gold, magnetic iron oxide and polymeric poly(lactic-co-glycolic acid)-PLGA NPs were selected for this study in order to cover the most common NPs that are used in biomedical applications.

Obtaining the intestinal barrier model first targeted the differentiation of HT-29-MTX cells towards goblet-like, mucus-producing cells by using a MTX solution (10^{-4} M). Afterwards, both cell types were seeded at the same time in a ratio of 7:3 (Caco-2 : HT-29-MTX) at a final density of 5×10^4 cells per 24-well insert on Transwell® plates with polyester membrane and 3 µm pore diameter, in DMEM medium with 10% fetal bovine serum. The medium was changed 2-3 days before the start of the transport study (day 21 after seeding).

The Caco2/HT-29-MTX co-culture was exposed apically to 25 µg/mL NPs and subsequently the level of NPs internalized and released into the basal medium was monitored over time (after 24, 48 and 96 h). The quantity of transported NPs, penetration times and permeability coefficients were calculated using a calibration curve for each type of nanoparticle.

To evaluate the proliferation of the obtained cell cultures, but also the permeability effect induced by NPs, the transepithelial electrical resistance (TEER) was monitored using the Millipore® Millicell Electrical Resistance system (ERS).

Our results showed that all three types of NPs tested showed increased internalization in the intestinal model and none of them were transported to the basal part in the first 24 hours. Instead, a transport rate of 14.6% was recorded in the case of gold NPs after 48 hours, and the permeability of the intestinal barrier to magnetic nanoparticles (6%) appeared only after 96 hours.

In conclusion, our findings revealed that gold NPs have the best transport rate across the gut barrier and they can be used in developing more effective medications in order to significantly impact the treatment of various gastrointestinal conditions.

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Figures

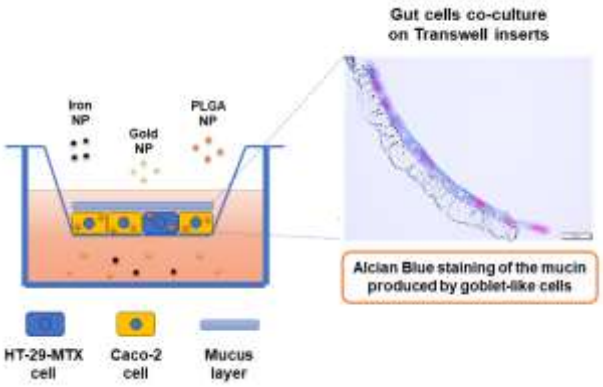

Figure 1. Dynamic transport of magnetic (iron), gold and polymeric (PLGA) NPs across intestinal barrier model.

Multimodal Characterization of liposomes and cells: Combining AFM with Confocal Microscopy

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Atomic Force Microscopy (AFM) traditionally offers high-resolution characterization of surfaces. Scanning the sample with a sharp tip in a piezo-electric controlled manner allows us to reconstruct the morphology of small macromolecules up to whole cells and tissues in a nano-meter scale. The force between the sample and the tip can be finely controlled, and resulting force-distance curves can be used to determine the elastic and viscoelastic properties of the sample. AFM used for studying biological samples are often coupled with optical or confocal microscopes, enhancing the range of imaging modalities. The presented poster will describe basic concepts in AFM technique and mechanical properties, together with real applications of this technique coupled with confocal microscopy. Viewers will see the results of dynamic changes of liposomes stiffness within a cytosol-imitating buffer, a morphological analysis of cells treated with a membrane-intercalating substance, and a mechanical mapping of cells undergoing mitochondrial clustering, confirmed by confocal imaging.

Acknowledgments

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Figure

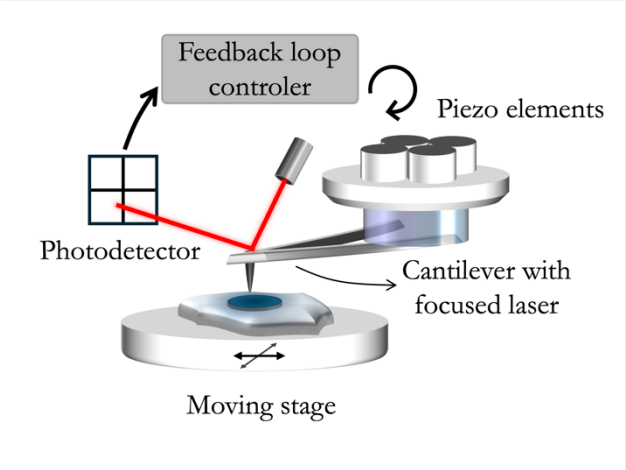

Figure 1. Schematic illustration of AFM. The cantilever bends due to interaction with the surface and this bending is detected by a laser, which is reflected from the back of the cantilever to a four-segment photodetector. The deflection is converted into an electrical signal by a controller. Constant force between the probe and the sample (setpoint) is maintained by a feedback system. The probe (or sample stage) is equipped with ceramic piezo elements, that can move the glass block with cantilever in the xyz axis with high sensitivity. Sample is placed on a stage that can be moved in the xy coordinates.

MWCNT–MANGANESE PORPHYRAZINE NANOHYBRID ELECTRODE MATERIAL AS A CATALYST FOR GLUCOSE AND H_2O_2 BIOSENSORS

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Porhyrinoids are very important macrocycle compunds which determine many biochemical processes. In porphyrazines (Pzs), aza-analogues of porphyrins, the conjugated molecular structure consists of four pyrrole rings linked by aza bridges. The presence of metal ion in the core and the plethora of peripheral substituents equips these macrocycles with many exceptional physicochemical properties, including optical and electrochemical. Carbon nanotubes (CNT) have gained considerable attention in research due to their unique electronic properties, chemical stability and affinity to biomolecules. It was found that the CNT can promote effective electron transfer reactions. Moreover, they can be used as a support for immobilization of different electron transfer mediators onto electrode surfaces able to improve their electrochemical properties. [1,2].

Enzymatic electrochemical glucose biosensors are very popular devices for glucose monitoring available on market. The research on the application of enzymes toward glucose sensing have been carried out widely over the past decades. Glucose biosensors monitor the redox current generated when electrons are transferred either indirectly or directly between an enzymatic receptor and a conducting electrode surface [3].

Based on the above considerations, synthethic route leading to the new macrocycle was performed following a two-step procedure. Obtained magnesium porphyrazine derivative was subjected to demetallation reaction with trifluoroacetic acid and subsequent remetallation using manganese (II) salt. The resulting products were carefully purified via flash column chromatography and characterized using various analytical techniques, especially NMR, MALDI and UV-Vis. The newly synthesized Pz was subjected to electrochemical studies and was deposited on MWCNTs. As a result, a promising electrode material revealing high electrocatalytic ability toward hydrogen peroxide oxidation was obtained. Moreover, the proposed hybrid nanomaterial was considered as a platform for immobilization of glucose oxidase. The resultant biosensor material was evaluated for glucose determination. According to the data, the novel PzMn(III) is a compound of choice for the development of electrochemical sensors of hydrogen peroxide or glucose [4].

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Figures

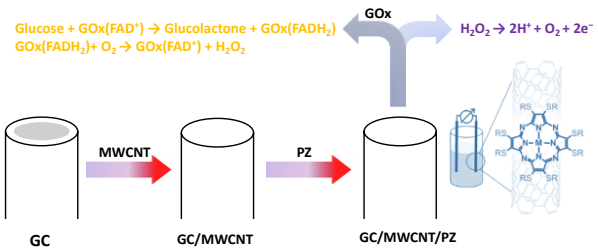

Figure 1. Chemical modification of glassy carbon electrode (GCE).

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