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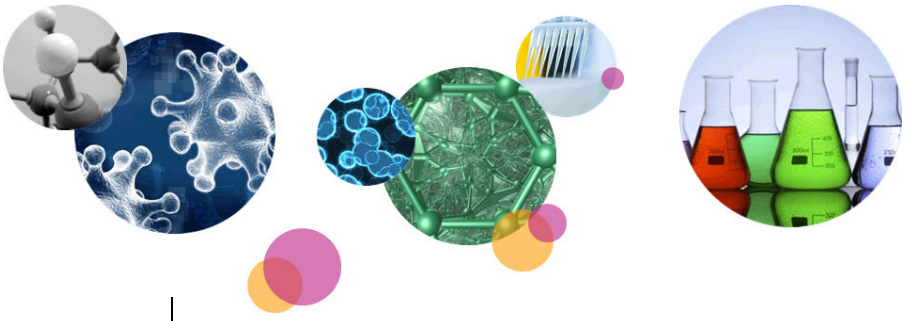

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On behalf of the Organizing Committee, we take great pleasure in welcoming you to Barcelona (Spain) for the NanoBio&Med2019 International Conference.

This event, after successful editions organized within ImagineNano in Bilbao 2011 & 2013, and in Barcelona in 2014, 2015, 2016, 2017 & 2018, is going to present 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&Med2019 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.

As in previous editions, an industrial forum will be organized to promote constructive dialogue between business and public leaders and put specific emphasis on the technologies and applications in the nanoBioMed sector.

We are indebted to the following Companies and Scientific Institutions for their support: Institute for Bioengineering of Catalonia (IBEC), NANOMED Spain and American Elements.

We would also like to thank the following entities for their participation as exhibitors: Mecwins and the Distributed Biomedical Imaging Network (ReDIB).

In addition, thanks must be given to the staff of all the organising institutions whose hard work has helped planning this conference.

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Vesicle chemotaxis at the nanoscale: principles and applications.

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Abstract
Directional locomotion or taxis is possibly one of the most important evolutionary milestones, as it has enabled many living organisms to outperform their non-motile competitors [1]. In particular, chemotaxis (i.e. the movement of organisms either toward or away from specific chemicals) is the most common strategy adopted by many unicellular organisms to gather nutrients, escape toxins and help coordinate formation of colonies. Recently, we demonstrated that chemotaxis can be achieved by creating a nanoscopic vesicle loaded with enzymes, whose membrane have an asymmetric distribution of permeable domains [2]. When placed in the presence of a chemical gradient that act as substrate for the enzyme, the asymmetric distribution of flow across the vesicle membrane create a biased slip velocity around the vesicles and a consequent propulsion.
This, in turn, allows the vesicles to move chemotactically toward higher concentration of the substrate allowing long-ranged targeted delivery. Here, I will expand this concept using uniquely biological molecules with the final aim to demonstrate that a minimal organization of biomolecules can create the conditions for complex behavior such as chemotaxis. I will briefly present the physics associated with chemical gradient and how these create convective drifts and how these depend on both density gradient as well as diffusophoresis process associated with chemical interaction with wall surfaces.

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PHYSICAL PROPERTIES OF PARTICLES CAN CONTROL CELL SPECIFICITY

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Abstract

Growing evidence shows correlation between cancer aggressiveness and the mechanical properties of tumor cells. For many cancer diseases it was found that the deformability of the cells is higher in cancer compared to normal cells, and further raises with the degree of cell malignancy.

The goal of our multidisciplinary research is to investigate how physical properties of particles affect their internalization into non-cancer and cancer cells of varying malignancy potential, from the same origin. Such insight can promote specificity in uptake of drug delivery systems and can potentially be applied in diagnostic schemes. For that aim we used inert rigid colloids of different sizes, as well as nano particles of varying rigidity. Uptake of these particles was tested in non-cancer and cancer cells, including cells of increasing malignant potential. A wide range of biological and physical schemes was used and mechanistic insights were provided by a physical model.

A microfluidic based methodology to produce highly controlled nanoparticles was developed (1) followed by cell assays. We found that flexible particles are less internalized into the cells due to increased contact area that requires massive cell deformation in their engulfment (2).

A non-monotonic dependence on colloid size was found, explained by the energetic interplay between adhesion and cancer cell deformation. Importantly, uptake capacity of colloids was significantly higher in cancer compared to non-cancer originated cell lines, and also higher the more invasive the cancer cells were. The phagocytic capability of cells was found to correlate with the malignancy potential as well as with the mechanical deformability of the cells. Our study provides new mechanistic insights related to cancer progression that can potentially be applied in the rational design of drug delivery systems.

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Figures

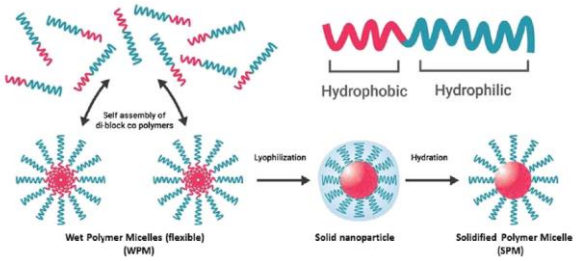

Fig. 1. Example of mechanical tuned particulate system, Illustration of soft (“wet”) polymeric micelles compared to semi-solid stiffer ones.

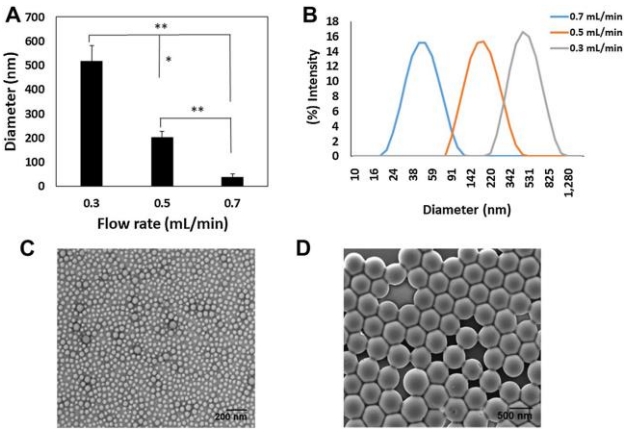

Fig. 2. An example of highly tunbnable polymer particles produced by a microfluidic device. A single chip can produce nano to micro range particles.

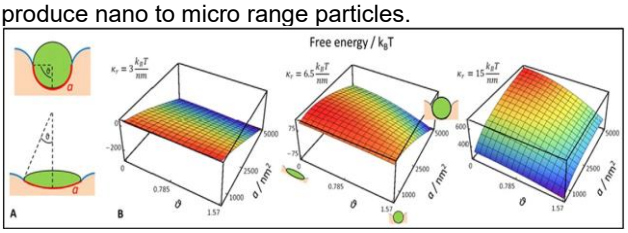

Fig. 3. Physicalmodel. **A**. A scheme of the model geometry, with definition of the flattening state,ϑ, and the contact area, a. **B.** Free energy plots as a function of ϑ and a for different values of the rim constants, κr, with ε ¼ 0:1 kBT. nm2 and c=20kBT.

Paper-based nanobiosensors: diagnostics going simple

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Abstract

Biosensors field is progressing rapidly and the demand for cost efficient platforms is the key factor for their success. Physical, chemical and mechanical properties of cellulose in both micro and nanofiber-based networks combined with their abundance in nature or easy to prepare and control procedures are making these materials of great interest while looking for cost-efficient and green alternatives for device production technologies. Both paper and nanopaper-based biosensors are emerging as a new class of devices with the objective to fulfil the “World Health Organization” requisites to be ASSURED: affordable, sensitive, specific, user-friendly, rapid and robust, equipment free and deliverable to end-users. How to design simple paper-based biosensor architectures? How to tune their analytical performance upon demand? How one can ‘marriage’ nanomaterials such as metallic nanoparticles, quantum dots and even graphene with paper and what is the benefit? How we can make these devices more robust, sensitive and with multiplexing capabilities? Can we bring these low cost and efficient devices to places with low resources, extreme conditions or even at our homes? Which are the perspectives to link these simple platforms and detection technologies with mobile phone communication? I will try to give responses to these questions through various interesting applications related to protein, DNA and even contaminants detection all of extreme importance for diagnostics, environment control, safety and security.

Creating a Bidirectional Bypass for Spinal Cord Injuries with Nanotechnology

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After a Spinal Cord Injury (SCI), the communications between the brain and the spinal circuits that dictate movement are interrupted. Innovative equipment has been developed to help patients regain control of limbs. The current strategies to remediate SCI based on computer-brain interfaces [1, 2], however, require the use of bulky devices, not implantable and without sensorial feedback.

We aim at developing a new generation of magnetic sensors and electrodes based on nanotechnology for neural interfacing with improved sensitivity and low tissue disturbance thanks to their nanostructure. The nanodevices are suitable for chronic implantation. It will be focused on restoring the transmission of electrical signals in the injured SC, acting as a bi-directional local bypass.

I will first describe the nanofabrication and performance of high-resolution magnetic field sensors working at room temperature, based on anisotropic magnetoresistive (AMR) LSMO films epitaxially grown on vicinal substrates. Then I will report on the production of nanowire-coated electrodes for neural excitation by template-assisted electrochemical growth (metallic) or nanoimprint technology (conductive polymer).

Finally I will show real-time measurements of both the pharmacologically induced activity of neural cord slices and the subsequent actuation on neural cord slices.

References

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Figures

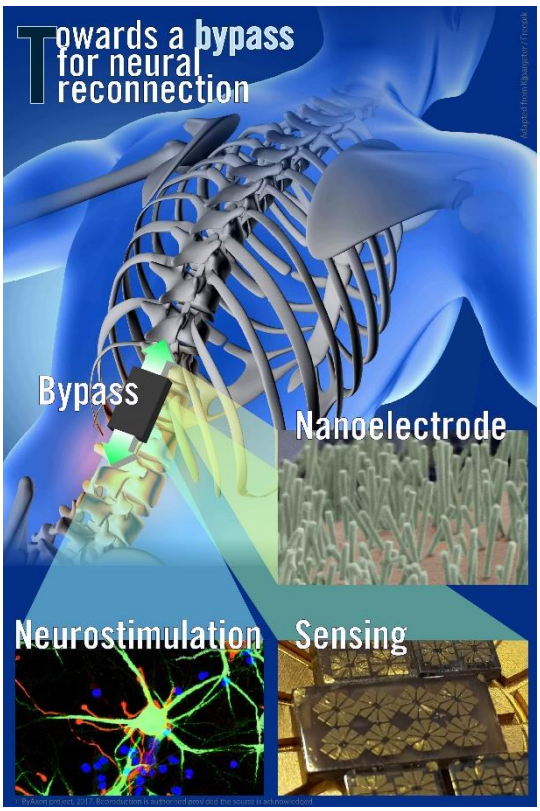

Figure 1. Scheme of the active By-pass for neural reconnection including magnetic sensing and nanoelectrodes for neurostimulation.

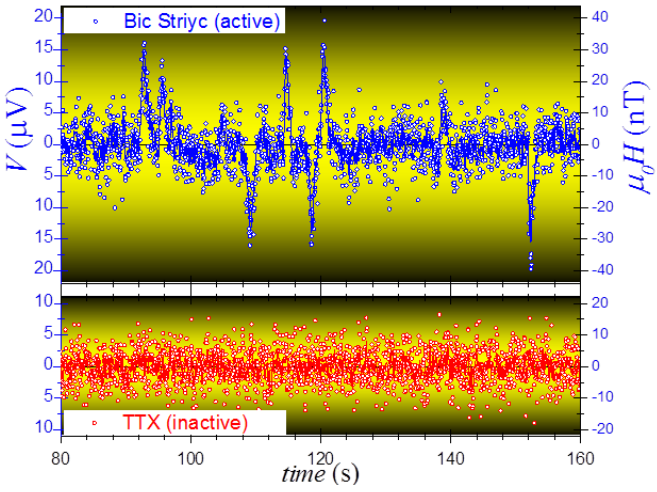

Figure 2. Real time measurements of the activity of a neural cord slice. The data have been taken at room temperature without any magnetic shielding.

Nanomagnets for biomedical applications: from spherical shapes to planar geometries

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Magnetic nanomaterials have risen the expectation to develop novel biomedical applications. Their unique properties and physical effects under external fields have attracted their interest in several areas; among them, cell manipulation through the remote action, diagnosis through high sensitive magnetic sensors or contrast enhancement in magnetic resonance imaging, and therapy through the heating effect under high-frequency fields.[1, 2] Magnetic nanoparticles (MNP) are chemically synthesized in compact forms like nanocubes, nanowires, and more conventionally spherical shapes.[3, 4] These nanomagnets are in a superparamagnetic state to be suitable for medical applications. Therefore, their size must be smaller than few tens of nanometers.

On the contrary, magnetic nanostructures (MNS) are fabricated using lithography techniques.[5] This approach produces planar geometries like disks or ellipses (Figure 1). This planar shape confers them new properties for magnetic actuation. However, MNS do not present superparamagnetic behavior due to their dimensions around hundreds of nanometers. Consequently, they must be designed with special magnetic configurations to achieve the zero remanence required in biomedical applications.[6]

We will discuss these special configurations and cost-effective routes to fabricate suitable planar nanomagnets. Magnetic properties and toxicity studies *in-vitro* models of tumor cells will be presented. We will also state the expected advantages of MNS in novel diagnostics and therapeutic applications.

This work was supported by AEI-MINECO FIS2016-76058, UE FEDER "Una manera de hacer Europa", the European Union´s Horizon 2020 research and innovation programme under the Marie Sklodowsks-Curie grant agreement No 734801, and the Basque government grant IT970-16, IT1162-19, and PIBA 2018-11.

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Figures

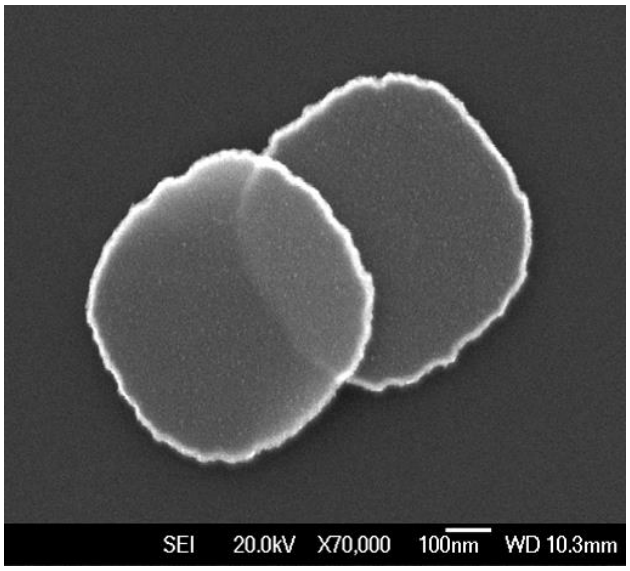

Figure 1. Magnetic nanostructures of NiFe fabricated by interference lithography

nanoBiomaterials for Neurology: one (powerful) tool, many applications

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Nervous system problems are common and encompass a large spectrum of traumatic injuries, diseases or iatrogenic lesions. The poor regenerative capacity, particularly in the case of the central nervous system (CNS), cannot be attributed to an intrinsic inability of neurons to sprout and re-grow after injury, as axons are able to regenerate in the presence of a permissive growth environment. One of the challenges facing the neuroscience field is the development of effective therapies that can enhance the regenerative capacity of the nervous system based on the advances achieved in basic research.

We have been dedicated to using nano-enabled solutions to the design of new therapeutic approaches based on biomaterials to promote neuroprotection and neuroregeneration. In this talk, two main strategies will be presented that represent two of the main lines of research of my group:

i) the design of biomaterial-based nanoparticles for targeted nucleic acid delivery to neurons [1];

ii) the design of bioactive hydrogels for neural stem cell delivery in the spinal cord[2].

Emphasis will also be given to the application of novel strategies proposed to assess the potential of the developed systems[3-5].

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Acknowledgements

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Novel DNA-Based Molecules and Their Charge Transport Properties

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Abstract

Charge transport through molecular structures is interesting both scientifically and technologically. To date, DNA is the only type of polymer that transports significant currents over distances of more than a few nanometers in individual molecules. Nevertheless and in spite of large efforts to elucidate the charge transport mechanism through DNA a satisfying characterization and mechanistic description has not been provided yet. For molecular electronics, DNA derivatives are by far more promising than native DNA due to their improved charge-transport properties.
In recent years we have invested great efforts to address the above issues. Measuring the charge transport in DNA was elusive due to great technical difficulties leading to various results. We recently devised an experiment in which double-stranded DNA is well positioned between metal electrodes. Electrical measurements give surprisingly high currents over 100 base-pairs (~30 nm) elevated from the surface. The temperature dependence indicates backbone-related band-like transport.
In collaboration with the Kotlyar group, We were also able to synthesize and measure long (hundreds of nanometers) DNA–based derivatives that transport significant currents when deposited on hard substrates. Among the molecules, metal containing DNA, which is true metal-organic hybrid, a smooth and thin metal coated DNA and G-quadruplex DNA. Step by step we improve the synthesized constructs and the measurement methods of single DNA-based molecules. I will present new and surprising results on dsDNA molecules. I will present new DNA-based molecules and report on our measurements of their properties.

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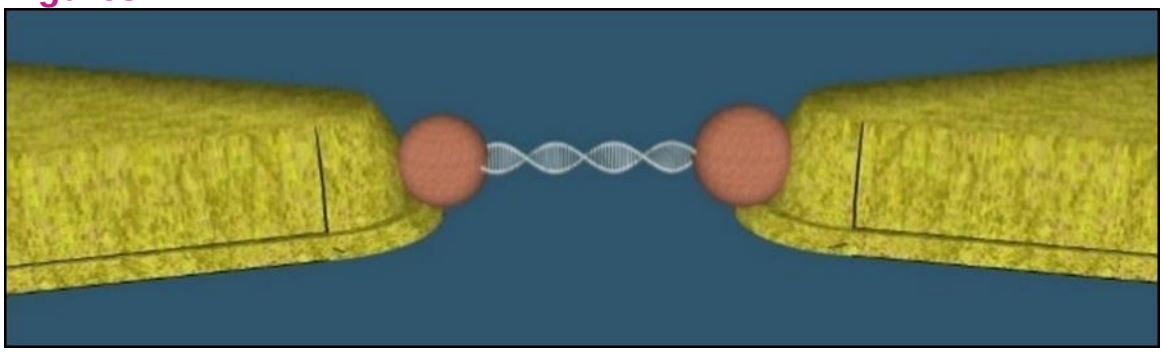
Figures



Detection of particles, micro-organisms and biomarkers using CMOS image sensors

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We will present recent developments in using images sensor arrays to detect minute quantities of transparent materials, small particles, micro-organisms and biomarkers. The designs of the large-field of view imagers are novel and exploit scattering and phase (interferometric) effects in the classical and quantum regime.

Medical Nanoparticles: Principles of Design

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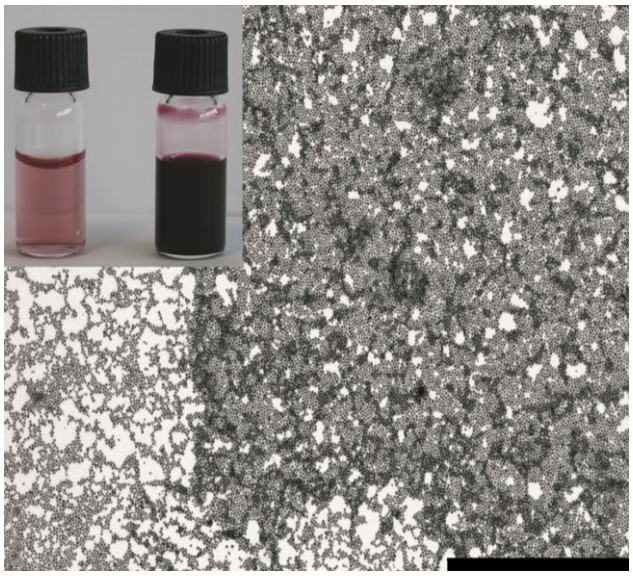
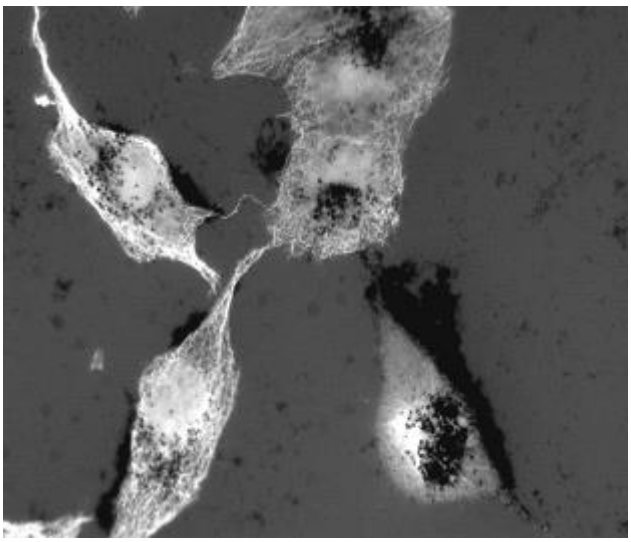
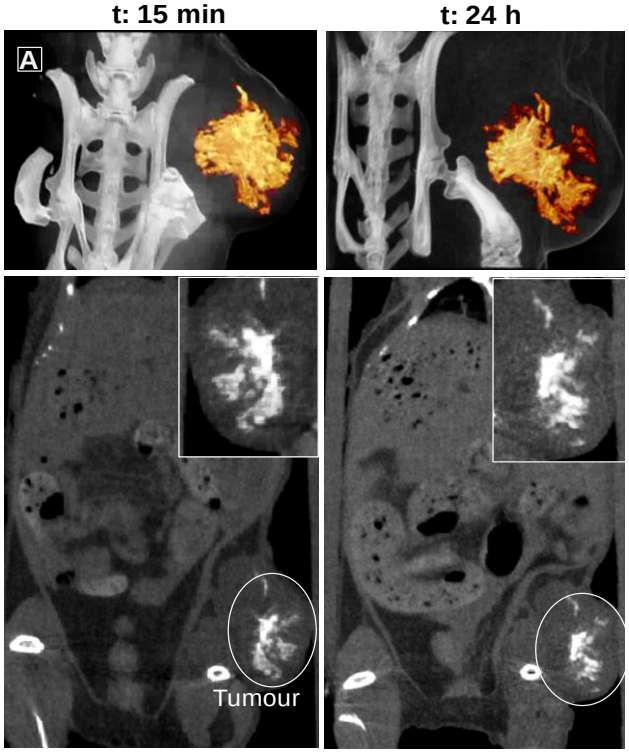
From last two decades of intense research at the nanobiointerface a series of conclusions can be drawn with respect a general set of rules for inorganic nanoparticle design for medical applications. This includes, not only purity, sterility and monodispersity but also feasibility and traceability, metabolization and excretion. In this context, safety is paramount and efficacy has to be developed in a context of a real pressing and precise medical need. Safety is mandatory not only in the development of medical tecyhno9logies but indeed any new technology has to be introduced safely, nanoparticles also. Finally, therapeutic doses has to be achieved, that may be high for colloidal NP stability (always at ease at low concentrations). Similarly, when thinking on dosing, persistency of NPs and potential accumulation has to be carefully controlled to avoid long term damage. This implies to develop a full nanoADME (administration, distribution, metabolization and excretion) model to enable NPs to be used in medicine.







Figures

Figure 1. Concentration of AuNPs to reach theraputical dose of the conjugate.

Figure 2: Dark Field Confocal microscopu pm A549 exposed to AuNP-CisPt for 24 hours

Fig.3. CeO2NPs Tumor enhanced Computer Tomography X-ray images.

Bio-Inspired Peptide-Based Functional Coating

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The lecture will present bio-inspired functional coatings that are spontaneously formed by short peptides. These peptide-based coatings self-assemble on metals, oxides and polymers under mild conditions without any need for a curing step. The coating can serve in many functions. One application is preventing biofouling - the undesirable adhesion of biomolecules and organisms to surfaces. [1-2] This process leads to numerous adverse phenomena including hospital-acquired infections, blockage of water desalination facilities and food contamination. We showed that this coating prevents the first step of biofouling, which involves the adsorption of bioorganic molecules to the substrate. Moreover, the coating significantly reduces the attachment of various organisms such as bacteria and fungi to surfaces. Another function that these peptide-based coatings can mediate is the adhesion of mammalian cells to implants. [3] This function is important for the integration of implants into the human body. Finally, we showed that these peptides self-assemble in solution into particles that adsorb and release active compound that synergistically reduce the number of bacteria on the surface. [4]

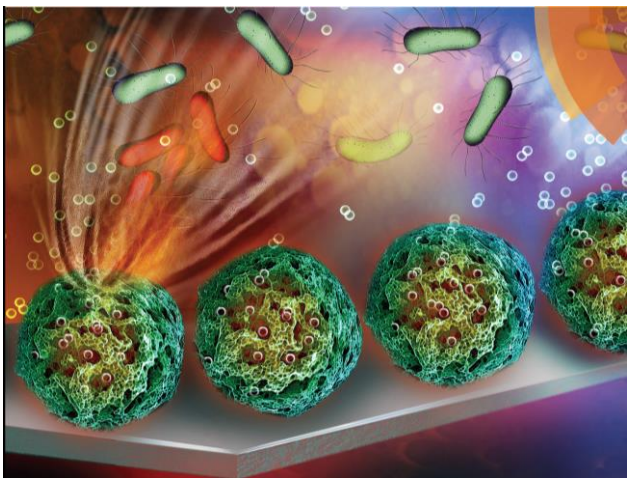

Figure 1. Peptide-based antifouling particles on surface release antimicrobial agents.

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Force pathway to synaptic clustering at the neuro-muscular junctions of embryonic flies

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Abstract (Arial 10)

Memory and learning in animals are mediated by neurotransmitters that are released from vesicles clustered at the synapse. Vesicle clustering has been believed to result primarily from biochemical signaling processes that require the connectivity of the presynaptic terminal with the cell body, the central nervous system, and the postsynaptic cell. We show, using embryonic *Drosophila* motor neurons, that vesicle clustering at the neuromuscular presynaptic terminal depends on mechanical tension within the axons [1]. Neurons generate this tension within the first two hours of synaptogenesis, and actively maintain the tension of about 1 nN by employing acto-myosin machinery. If the rest tension is perturbed mechanically, axons restore the rest tension either by relaxing or by contracting over a period of about 15 min. Vesicle clustering vanishes upon severing the axon from the cell body, but is restored when mechanical tension is applied to the severed end of the axon. Clustering increases when intact axons are stretched mechanically. We finally reveal the underlying mechanism by which tension and vesicle clustering are linked [2]. The role of mechanical force in vesicle clustering is a new paradigm in the understanding of synaptic functions. This force paradigm may lead to new treatments for neurological diseases.

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Figures

Figure 1. Nervous system of an embryonic Drosophila (left). The axons of motor neurons emanate from the Central Nervous System (CNS) to form Neuro Muscular Junction (NMJ) with muscle. One of the axons is resected by a laser beam. The neurotransmitter vesicles de-cluster after resection. But if the cut end is pulled by a pipette, vesicle clustering is restored.

Nanomotors: Artificial active matter for nanomedicine

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Engineering nanomotors which convert chemistry into motion is of fundamental relevance and interesting for specific applications. Self-propelled micro- and nano-motors are opening many avenues in fields such as nanomedicine, robotics, biosensing, microfluidics, and environmental field [for reviews see 1-3]. Due to the versatility of fabrication techniques available nowadays, we can synthesize nano- and micromotors of different shapes and materials almost on demand, from nanoparticles, microcapsules to nano- and microtubes.

The combination of biological components and artificial ones emerges into what we call hybrid machines/bots. Alike bacteria or small swimmers found in nature, artificial nanobots convert bio-available fuels to generate propulsion force to swim at the nanoscale. One of the dreams in nanotechnology is to engineer small vehicles which can eventually be applied in vivo for medical purposes. Major advances have been demonstrated towards that end, however, questions like -how to swim at the nanoscale, how to achieve motion control and how to image these nanobots- need to be properly addressed.

Here, I will present our recent developments in the field of nanomotors that can autonomously swim and perform complex tasks in vitro. Our hybrid “bots” combine the best from the two worlds: biology (enzymes) and (nano)technology (nano- micro-particles) providing swimming capabilities, remote control, multifunctionality and actuation.[4]
I will present some of the fundamental aspects that we have studied for enzyme nanomotors such as the role of asymmetry, catalysis and enzyme quantity [5]. Moreover, I will show some proof-of-concept applications such as the efficient transport and the enhanced release of drugs into cancer cells [6] and spheroids [7], sensing [8].
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Figures

Figure 1. Micro-and nanomotors, from fundamentals to applciations

From basic principles of protein self-assembly to rational design of multifunctional fibers

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Self-assembly is the autonomous organization of components into patterns or structures without human intervention. A number of different assemblies can be formed by proteins. A particularly interesting example of protein self-assembly is a formation of highly ordered, nearly one-dimensional fibrilar structures. This high-level, long-range ordering is relatively independent of the molecular identity of the protein monomers. Interestingly, in nature, such structures can perform either beneficial roles or appear as aberrant protein aggregation, which is in a latter case results in the development of neurological disorders. The main objective of our research is to understand the evolution of protein complexes in the context of both biological function and malfunction as well as to draw the links between structure and properties of self-assembling materials based on natural polypeptides.

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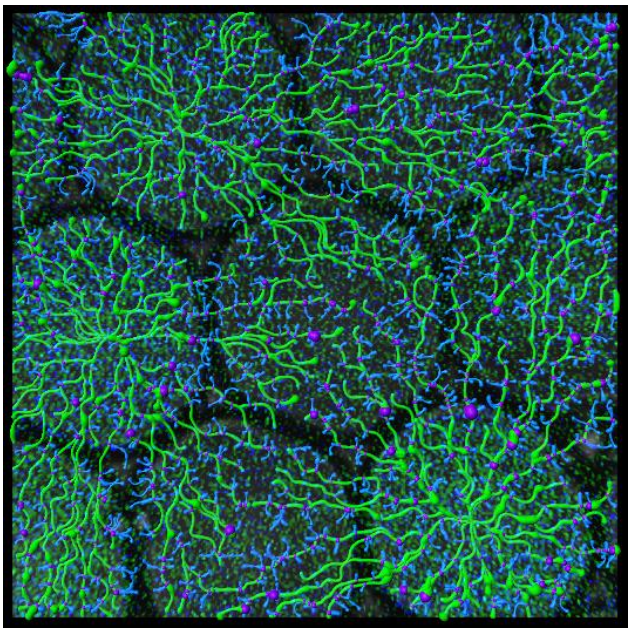

Figure 1. Microgels made of protein nanofibrils

Glycosylated amphiphilic polymeric nanoparticles in active drug targeting

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The overexpression of energy-dependent and independent glucose receptors and transporters has been identified in different cell populations (e.g., macrophages). For example, in pediatric sarcomas, overexpression of glucose transporter 1 (GLUT-1) is associated with the more efficient uptake of this vital energy source and faster proliferation and metastasis. Nanotechnology has made sound contributions to treat disease due to the ability to target drug-loaded nanomaterials by the enhanced permeation and retention effect (passive targeting). Furthermore, the design of nanocarriers surface-modified with ligands recognized by receptors overexpressed in specific cell types is an extensively investigated (though still unrealized in the clinical practice) strategy for active targeting and reduce off-target accumulation and toxicity. Among the platforms, polymeric micelles with core-corona nanostructure and amphiphilic polymeric nanocarriers displaying more complex self-assembly patterns have gained great attention owing to their ability to encapsulate and target hydrophobic cargos and the chemical versatility to modify their surface. Aiming to confer them with active targeting features, in recent years, we designed a plethora of glycosylated amphiphilic polymeric nanocarriers and demonstrated that by tuning their composition and size, they undergo selective accumulation in solid tumors [1-3]. In this presentation, I will overview the strategies developed in my laboratory to improve the efficacy of the pharmacotherapy in cancer and also tune the phenotype of immune cells by using this unique type of nanoparticle.

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Nanomechanics and Nanomechanical Systems for Biology and Medicine

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Millions of people dies of cancer or infection every year in the first world and developing countries, respectively. Most of these lives could be saved if patients had timely access to early detection. Mass spectrometry and multiplexed immunoassays have rapidly developed during last years with improved limits of detection. Still these technologies can hardly probe the deepest region of the plasma, at concentrations below the pg/mL level in the case of cancer protein biomarkers or below 1000 virions per mL in the case of viral infections. Overcoming these detection limits is required for early detection [1]. This clearly indicates the need of implementing novel ultrasensitive techniques can cover the inaccessible regions of the plasma. We here propose biological detectors based on nanomechanical systems for discovery and detection of cancer protein biomarkers and pathogens in plasma. We review the modes of operation of these devices [2], putting our focus on recent developments on nanomechanical sandwich immunoassays [3] and nanomechanical spectrometry [4]. The first technique enables reproducible immunodetection of proteins at concentrations well below the pg/mL level, with a limit of detection on the verge of 10 ag/mL as well as the detection of one pathogen in 10 mL of plasma. The second technique enables the identification of individual pathogens by two physical coordinates, the mass and stiffness, instead of the mass-to-charge ratio of the protein constituents. This technology can enormously simplify the identification of pathogens such as virus and bacteria that are not accessible to current mass spectrometry detectors (http://viruscanproject.eu/). Finally, I will show some of our most recent developments for deciphering the role of the cell stiffness in cancer.

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DNA-nanoparticle hybrids for single-molecule sensing

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Biofunctionalized nanoparticles are promising constructs for molecular sensing and drug delivery. Plasmonic nanoparticles are particularly attractive because the plasmon resonance enables single molecule detection by measuring shifts of the plasmon resonance [1,2], or by exploiting the fact that the fluorescence intensity of nearby fluorophores is strongly enhanced by the plasmon [3].

The functionality of such particle-based sensors is governed by their chemical interface, specifically by the number of functional groups per particle and particle-to-particle differences. The latter can only be resolved by single-particle and single-molecule approaches, so I will first show how we use correlative microscopy to characterize the number of functional groups on single particles.

We use a combination of super-resolution microscopy, single-particle spectroscopy, and atomic force microscopy to characterize and optimize functionalization protocols. I will then show the application of such biofunctionalized particles toward single-molecule sensors that can be applied for biomedical and environmental sensing applications.

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Invited

Dynamic systems mimicking the systemic complexity in biology

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Abstract

One of the most inherent and interesting features of living systems is their adaptability to show complex behavior, in response to a variety of signals. Among these behaviors, autonomous motion has been an important source of inspiration for scientists who, over the years, have created a variety of synthetic motor systems, imitating biological motility[1]. For example, molecular motors[2], micro- and nanoscale sized Janus motors[3], self-assembled polymeric motors[4], movable tubules and rods[5] have been developed. Regardless of the excellent performance of these motor systems, there is a fundamental difference in the way movement is regulated in synthetic and natural systems. Cellular autonomous motion (e.g., vesicular transport and motility), displays adaptive features as a result of competing transient activation and deactivation processes, which are governed by enzyme-mediated energy input and consumption, and molecular interactions. Such dynamic processes are also referred to as out-of-equilibrium or dissipative; mimicking these behaviors in synthetic systems has recently drawn much attention from the scientific community. Introducing transient behavior into synthetic molecular or nanoscaled systems has been demonstrated for active materials with unique properties such as dissipative fibres[6], transient peptide hydrogels, vesicles or microcapsules[7], and non-equilibrium molecular recognition and colloidal systems[8]. In a stepwise fashion, we couple motility to a dynamic process, which is maintained by transient events[9]. This lecture will therefore focus on how we translate dynamic processes into motion – on both nano and micro scale.

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Figures

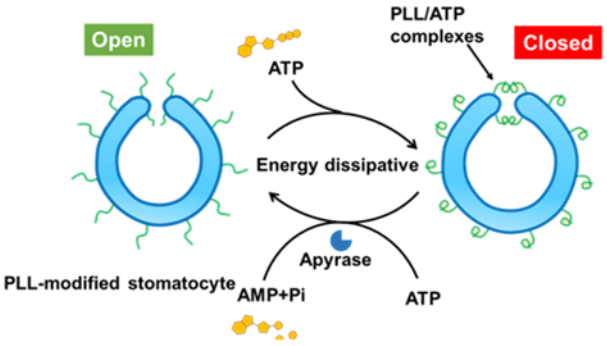

Figure 1. Schematic representation of the transient deactivation and activation of a stomatocyte nanosystem mediated by ATP.

Wound care and smart bandage technology based on Graphene monolayers

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After a decade and thousands of publications, graphene produced by CVD on copper foils remains at the sweet spot for quality and cost-effective production of large size, high-mobility graphene transferred on insulators. Use of these monolayers has been so far scarce in industrial applications. I will present the use of this technology for biological (1) and medical applications from both the academic and industrial point of view.

We have first explored the use of graphene-on -polymer for biosensing and tissue engineering. I will show results of in-vitro cellular growth (neurons and skin fibroblasts) on graphene-covered substrate which shows the stimulation of growth (2) and migration of cells promoted by the graphene substrate together with the possibility of probing their electrical activity down to the sub-cellular scale (3).

We are also developing a novel technology platform that exploits the features of monolayer graphene, for woundcare. The first-generation of our system is a graphene-based scaffold that looks like a very thin, transparent plaster. Based on that material, we are building an innovative bandage technology platform based on graphene-on -insulator film in order to better support chronic wounds.

In particular, I will insist on the possibility to combine therapeutics (bio electrostimulation & healing) with diagnostics (biosensing) features in the same device. Following these properties, we have elaborated a graphene-based scaffold that looks like a very thin, transparent plaster integrated in commercial bandage that is indented to be applied in direct contact with an open wound. We believe these films will have some impact in healthcare, as they target some important and poorly addressed diseases such as pressure ulcers and diabetic foot ulcers I will present the preclinical results on animal studies and the perspectives of their commercial (3) use for wound-care, in particular in the treatment and diagnostics of chronic wounds that affect the diabetics and elderly.

Our bandage platform (4) is based on the integration of a monolayer graphene polycrystalline layer back-bonded onto a biocompatible polymer layer (figure 1° . The resulting film can directly be applied onto the bed-wound and is inserted in a commercial bandage. Graphene surface combines healing (speed-up of wound closure) and antibacterial action, optical transparency and electrical conductivity. It is obtained by integrating a large uniform graphene monolayer into a bandage in order to provide a bio- stimulating and electrically-active platform directly applied in contact with the wound. It allows the development of a range of intelligent dressings that combine on the same product both therapeutic and diagnostic actions. -Therapeutic action: graphene functions as a growth matrix, promoting healing but at the same time acting as an electrode in close contact with the wound. This allows the application of electrical pulses whose actions promote faster healing and reduce pain.
-Diagnostic action: Graphene it plays at the same time the role of a biosensor to monitor the wound evolution and early stage detection of infection by pH and biomarker sensing.

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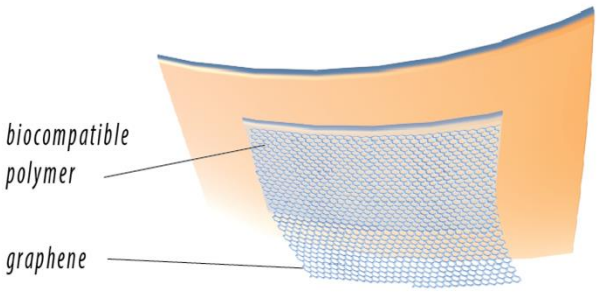

Figure 1. Principle of the graphene-coated bandage platform: a single monolayer of polycristalline, CVD Graphene is assembled onto a biocompatible polymer and integrated in a bandage.

Scanning probes for enzyme nanopatterning and for the spatial mapping of collagen micro-stiffness in tissue sections

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Scanning probe microscopy makes use of small probes, with size from tens of nanometers to few micrometers, scanned over the sample surface to obtain topographical and mechanical information with spatial resolution and force sensitivity. In a different application, scanning probes can be used as nanometric pens to locally modify the sample surface in contact with the probe. This second approach provides lithographic capabilities and it is called scanning probe lithography (SPL)[1]. In SPL, different inputs are used to generate the desired surface modification. In particular, when heatable cantilevers are employed to locally induce chemical reactions, the technique is called thermochemical nanolithography (tc-SPL)[2]. Both approaches can be of great interest for biological applications, to provide functional (nano and micro-mechanical) markers in biological surfaces (AFM) or for patterning purposes (tc-SPL). In my presentation, I will show the use of force volume AFM for the spatial mapping of collagen distribution in mammalian tissues with affordable timescales. This study points to the use of AFM as a routine tool in the biomedical research, to provide micromechanical data that correlate with outputs from other (optical, biochemical, histological) techniques[3]. I will also describe tc-SPL for the fabrication of nanoscale patterns in polymer films. These patterns can be used for enzymes anchoring with high throughput, high reproducibility and spatial control, till the single molecule level. Tc-SPL allows for the fabrication of 3D surfaces with independent control of topography and chemistry, and in my work it is employed to generate pockets (< 10 nm in size) that accomodate single enzymes[4,5].

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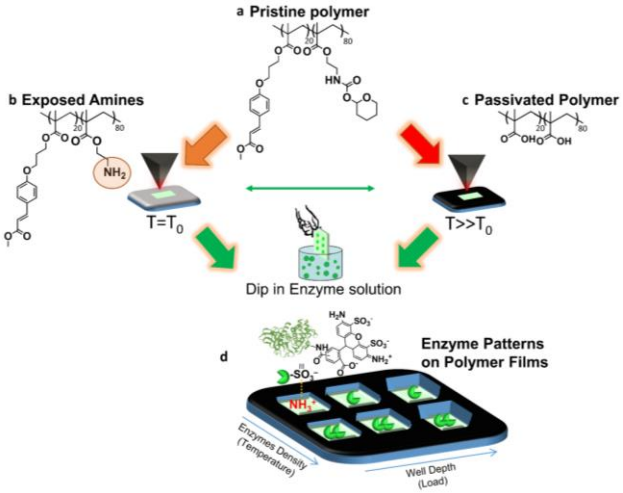

Figure 1. Enzyme patterning through tc-SPL.

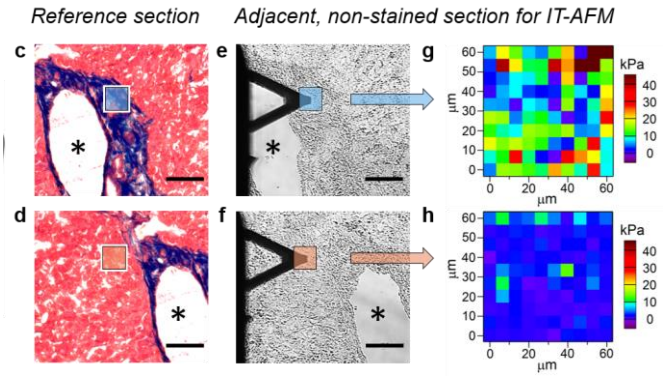

Figure 2. Force volume AFM in tissue sections from human liver.

Nanoneedle Arrays for Intracellular Sensing and Delivery

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Nanoneedles are rapidly emerging as a tool to interact with the intracellular environment of a large number of cells simultaneously, with limited perturbation of their physiological processes. This interaction provides characteristic advantages for minimally invasive cell and molecular biology investigations, as well as progression of biomedical translation of regenerative and precision medicine approaches.

A quick string of several successful proofs of principles have established nanoneedles' potential to efficiently deliver impermeant molecules and nanoparticles directly to the cell cytosol, and to sense the intracellular milieu across biological systems ranging from cells in culture to living organisms.

This talk presents an overview of our recent research advances in leveraging the nanoneedle biointerface to enable intracellular sensing and delivery in the context of diagnostics and tissue engineering.

High aspect ratio nanostructures provide a unique biointerface [1] that stimulates multiple mechanosensory elements within the cell, inducing non-canonical regulation of the YAP and Lamin A mechanosensors.[2] This interfacing also enhances payload delivery across the cell membrane and the harvesting of biomolecules from the intracellular space through a combination of enhanced endocytosis and increased membrane permeability [3].

Using nanoneedle arrays as a delivery vector for nucleic acids enables highly efficient transfection *in vitro* and localised delivery with minimal invasiveness in living organisms [4]. In particular the nanoneedle-mediated delivery of a plasmid coding for the vascular endothelial growth factor (VEGF) to the muscle of a mouse stimulates neovasculature formation driven by exogenous VEGF expression, in the first example of nanoneedle mediated gene therapy. This method of delivery does not impact on the structure of the tissue, induce cell death or elicit an immune response.

Nanoneedles can also sense the intracellular environment [5]. Nanoneedle sensors can map the activity of cancer biomarkers across cell cultures and tissues, with the potential to identify tumour margins and detect cancerous dissemination beyond the margin [6].

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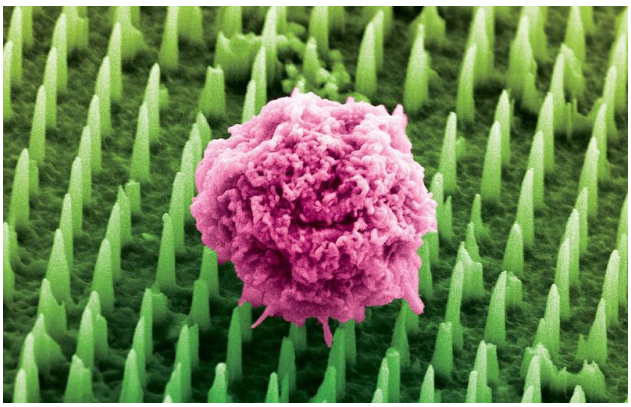

Figure 1. False-colored SEM micrograph of a human cell in the early stages of adhering to a nanoneedle array.

Membrane tension and domains: New method of measurement and visualization

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Organelles and cells are delimited by a lipid bilayer, which is highly deformable, a property essential to many cell processes such as motility, endocytosis, cell division. During these deformations, lipid membranes experience stretch causing membrane tension. Membrane tension is therefore a main regulator of the cell processes that remodel membranes, albeit, it is difficult to measure *in vivo*. **FliptR** (for Fluorescent LIPid Tension Reporter) can monitor changes of membrane tension by changing its fluorescence lifetime as a function of the twist between its fluorescent groups. We show that **fluorescence lifetime** depends linearly on **membrane tension**[1-3], allowing for an easy quantification of membrane tension by fluorescence lifetime imaging microscopy (**FLIM**) and **Fast-FLIM.**

Given that **FliptR** tremendously facilitates membrane tension measurements, we are currently studying membrane tension on the organelles[4], plasma membrane and tissues during different cell processes.

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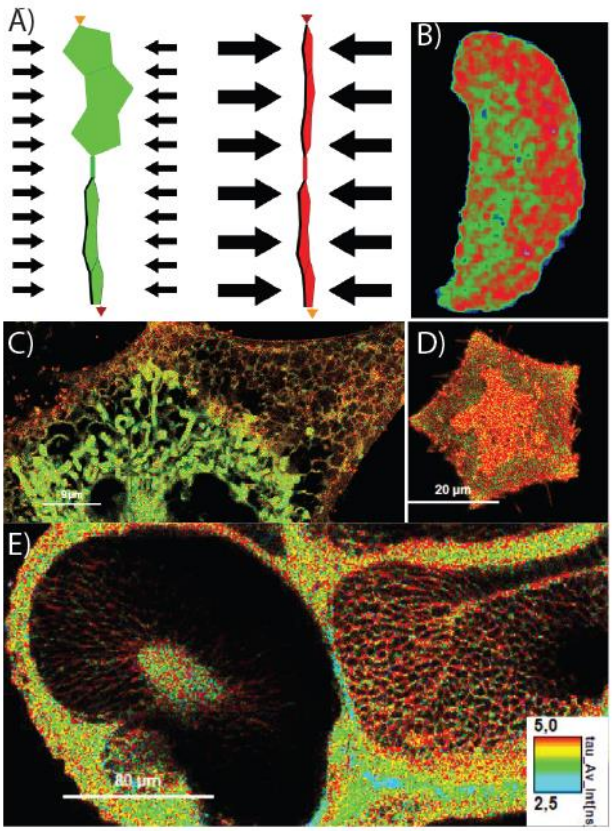

Figure 1. A) Twisted and planar conformation structure of FliptR based in the lateral pressure. B) Cell membrane domain related with tension during cell migration. C) FLIM images of mitochondria and ER organelles, D) cell adhesion surface on pattern and E) zebrafish embryo.

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Characterization of the immunomodulatory properties of graphene-based materials and their application as novel biomedical nanotools

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Abstract

Graphene-based materials (GBMs) are promising nanotools for biomedical application. However, a critical step for any clinical translation of GBMs is represented by the assessment of their impact on the complexity of the immune system.

Encouraged by our previous findings, demonstrating the dramatic impact exerted by the different physicochemical properties of GBMs on the modulation of their immune effects [1-4], here we evaluated the effects of graphene oxide (GO) and amino-functionalized GOs with different lateral size dimensions on a large variety of human immune cells (unpublished data).

Exploiting innovative approaches, such as single-cell mass cytometry, we revealed that the amino decoration of GO increased its immune compatibility and was also able to induce a specific M1 like activation of monocytes and the secretion of interleukin-4 and Granzyme-B from B cells, skewing a cytotoxic-like response.

Moreover, thanks to the combination of GO with inorganic quantum dots containing indium, the detection of GO cell uptake was enabled using single cell mass cytometry. Our results highlighted that monocytes and, intriguingly, B cells, were the main immune cell subpopulations able to internalize GO.

We finally exploited the immune modulatory proprieties of a specific GO on monocytes in combination with the osteoinductive capacity of calcium phosphates (CaP) for the design of a novel biocompatible nanomaterial called maGO-CaP (monocytes activator GO complexed with CaP) [5]. Our *in vitro* and *in vivo* results demonstrated maGO-CaP ability to induce osteoinductive stimuli increasing bone regeneration thanks to its modulation of the immune cell functionality.

Overall, our investigations suggest that well designed and functionalized GBMs allow the modulation of immune cells exploitable for the treatment of several pathologies, paving the way for their future biomedical applications.

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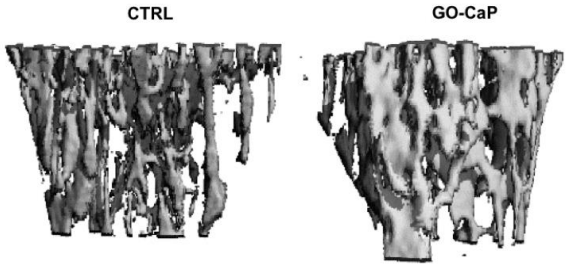

Figure 1. In vivo bone formation. Microcomputed tomography (µCT) image of untreated tibia (left, CTRL) and after one month of maGO-CaP treatment (right, GO-CaP).

Electrochemical nanoreactors and nanomotors for potential biomedical applications

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One of the aspirations of nanotechnology is to become true the long-awaited development of nanomachines which can autonomously move and perform different tasks from sensing to repairing and thus to emulate the complex functionalities of biological systems. Such achievement is expected to have a revolutionary impact in fields such as medicine. Under this context, the engineering of artificial self-propelled micro/nanomachines with multitasking capabilities has become a research line of growing interest since the pioneering studies at the beginning of this millennium.

These self-propelled micro/nanomachines generate local physicochemical gradients to drive their own motion, which is harnessed to perform multi-tasking activities in different locations. Self-propulsion generated by gradients from local electrochemical reactions is a very interesting strategy for autonomous motion. Although there have been many proofs of concept of chemical machines performing different activities, the progress on the comprehension of the physicochemical fundamentals behind the self-generated actuation has been more moderate. In many cases, the precise motion mechanism is still not unambiguously identified, and the key physicochemical parameters are not well-characterized. A complete and deep understanding of such issues would help to improve the control levels for applications and to better assess perspectives and challenges of these self-propelled machines. In this presentation, we will review a combined set of techniques that we have implemented to study chemically propelled micro/nanomotors using pumps as the immobilized motor counterparts (Fig. 1). These techniques have turned out to be very useful for mapping chemical reactions and for extracting physicochemical parameters (e.g. electric fields, fluid flows) and thus to achieve a more complete characterization of the mechanisms driving fluid motion [1-3]. Moreover, the chemical reactions in these motors can not only be used to produce the self-propulsion but also to generate reactive products that can in turn chemically modify the immediate environment on the fly. In this context, we will introduce some promising active micro/nanomachines that could have potential impact on biomedicine.

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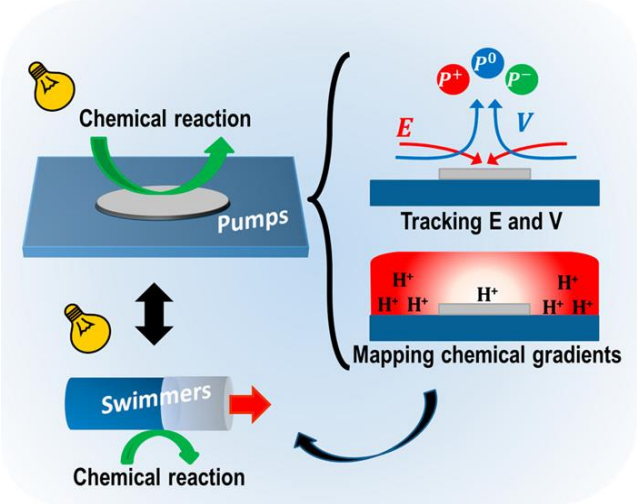

Figure 1. Understanding chemically propelled motors using pumps as the immobilized motors counterparts.

In silico study of protein-nanoparticle assembly and protein aggregation in crowded environments

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A pristine nanoparticle (NP) in a biological fluid is covered spontaneously by absorbed biomolecules forming a corona. Proteins, in particular, are one of the main components of it and can be part of the 'hard' or the 'soft corona', remaining for a relevant time on the NP or dynamically exchanging with those in solution, respectively [1]. Experiments debate if the irreversible protein adsorption can cause loss of specificity in targeting of pre-functionalized NPs [2] or not [3]. Other data show that a minority of the protein's epitopes are appropriately arranged for receptor binding, consistent with a stochastic and irreversible adsorption process in which proteins are, at least partially folded in their native state even when adsorbed in the hard corona [4]. Therefore, the details of the protein-NP assembly are essential to understand the possible nanoMedical applications [5]. Here we perform *in silico* studies to understand how the presence of interfaces or crowding affect the stability of the native state of a protein and its aggregation rate.

We consider several cases of proteins, from those with a unique native state to those intrinsically disordered, by means of a coarse-grain protein model in explicit solvent [6-9]. By Monte Carlo calculations, we show how relevant is the water contribution to protein denaturation and folding [10, 11] and to protein design [12]. We reveal that the hydropathy profile of proteins is a consequence of evolutionary pressure exerted by water in simplified geometries [12] and in bulk [13]. We find that a hydrophobic interface destabilizes the protein native state but can allow it to fold even if adsorbed onto a surface [14].

Furthermore, we study the proteins in a crowded environment, where the aggregation is tuned in a way that enable them to be functional at the concentrations required for optimally efficient performance [15]. We show how the increase of the concentration of individual protein species can induce a partial unfolding of the native conformation without the occurrence of aggregates. A further increment of the protein concentration results in the complete loss of the folded structures and induces the formation of protein aggregates (Fig. 1). We discuss the effect of the protein interface on the water fluctuations in the protein hydration shell and their relevance in the protein-protein interaction [16]. These results can lead the way for engineering proteins and drugs that would be functional at extreme conditions and it is potentially relevant in protein-NP assembly for nano-Medicine applications [17, 18].

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Figures

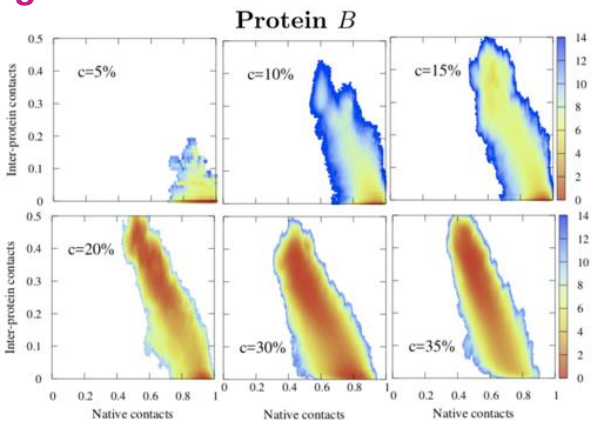

Figure 1. Protein unfolding vs aggregation. Color map of the free energy profile of a selected protein, as function of the native contacts and inter-protein contacts, for different protein concentration *c*. Native contacts and inter-protein contacts have been normalized to 1. Adapted from [16].

Nanomechanical properties of the cytoskeleton influence neuronal function in C. elegans

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Abstract

The millimeter scale mechanics of biological tissues is an emergent character originating from the material properties of the constituent cells and their mutual adhesive interactions. Likewise, the cell is an inhomogenous, non-isotropic and non-linear material composed of thousands of different components that interact transiently and/or permanently. Despite this complexity, previous research has shown that a few dominating elements of the cytoskeleton are largely responsible for the cellular strain response to mechanical stress. Thus, it is plausible that these mechanoresponsive molecules also endow some functionality to the cell - a property that has been largely investigated decoupled from the cellular physiology[1].

During my talk, I will highlight recent progress to understand the interplay between cellular and molecular nanomechanics and their role in regulating cellular physiology[2]. I focus on work on the nervous system of the roundworm *Caenorhabditis elegans*, which is a popular experimental system to combine engineering, biophysics and cell biology, due to its stereotyped body plan, known structural connectome and amenable genetics. *C elegans* uses a set of different sensory neurons to navigate its habitat which endow the animals to process chemical and mechanical stimuli in a changing environment. Some of these neurons respond to minute mechanical forces delivered to the skin of the animal, while others respond to stresses generated within the animals that originate from muscle contraction during locomotion. How these stresses are borne by molecular mechanics, transmitted within biological tissues and activate biochemical pathways is one of the last challenges in mechanosensory biology[2]. This lack of understanding is largely due in part to the absence of appropriate techniques to investigate the effect of mechanical properties of cells and neurons within living organisms at the level of a single molecule. With a combination of genetically encoded reporters for mechanical stress and calcium, together with piconewton force measurements, we investigate the mechanotransmission pathways leading to physiological response to mechanical stress. To our surprise, a single point defect in cytoskeletal proteins can have a large consequence on the stability of neurons against mechanical stresses, leading to a functional deficit[3].
We also introduce novel microfluidic devices (Ref [4] and in prep.) that enables the delivery of precise stresses to immobilized animals while reading out mechanical deformations and physiological functions. Using these technologies, we showed that a ubiquitously expressed protein of the cortical actin cytoskeleton, called β-spectrin, has neuron-specific role on mechanosensation and is subjected to mechanical stress during physiological processes. Mutations in this protein lead to a failure to properly sense forces and consequently severe uncoordination. Strikingly, similar mutations in the linker regions of b-spectrin have been shown to destabilize adjacent domains with consequences on overall cytoskeletal flexibility that lead to hemolytic diseases in humans[5]. Since the molecules are ubiquitously expressed in mammalian central nervous system and highly conserved among different species including humans, the results from our experimental paradigm bridge the gap between tissue culture and the clinics and are thus critical for our understanding of complex mechanical systems such as the brain.

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Versatile Single Chain Polymer Nanoparticles in Drug Delivery and Targeted Imaging

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As the area of controlled drug delivery is increasingly finding application in a variety of diseases, with different targeting sites and drug candidates, there is an urgent need for adjustable nanocarrier systems. Linear biopolymers folded into 3D-structures fulfill a wide array of complex functions and are omnipresent in biological systems; prime examples are proteins, in which the precise positioning of monomeric units (i.e. amino acids) largely determines protein morphology and therefore their remarkable variety of properties.

Due to their chemical diversity and highly modular nature polymers, and polymeric nanoparticles in particular, are popular drug carrier materials. Most polymeric nanoparticles are in the 50-200 nm size range, which is unfortunate, since proteins, enzymes and some viruses are considerably smaller in size.

In order to widen the existing polymeric drug carrier systems to the sub-20 nm size regime, we are investigating single-chain polymer nanoparticles (SCNPs) for controlled drug delivery and targeted imaging. [1] SCNPs are prepared through intramolecular crosslinking of individual polymer chains into individual nanoparticles and thus offer tremendous control over size and dispersity. Through exclusive intramolecular crosslinks, SCNPs are an order of magnitude smaller than conventional polymer nanoparticles, easily accessible in relevant quantities and without the requirement of complex synthetic strategies. Highly modular in nature, these uniquely sized polymer nanoparticles enable encapsulation and controlled release of drug molecules, irrespective of their hydro- or lipophilicity. [2]

Preparation of SCNPs from prepolymers containing pentafluorophenol activated esters, followed by functional amine substitution, allows for careful engineering of the nanoparticle surface, providing control over distribution behavior and enabling the mimicking of proteins in terms of shape, composition and even function. Current efforts focus on evaluation of these promising materials in vivo.

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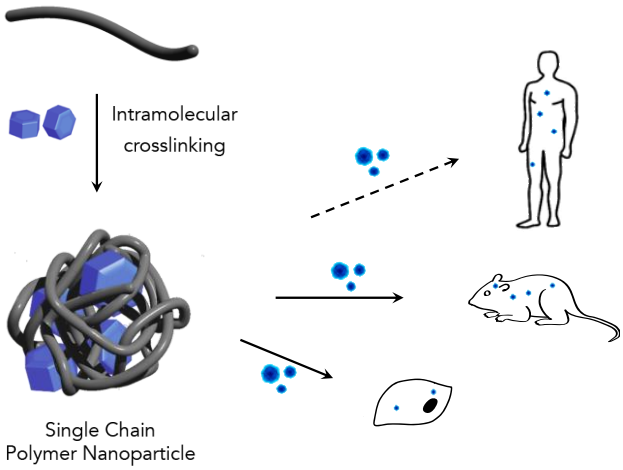

Figure 1. Intramolecular crosslinking of polymers, in the presence of therapeutics, to form single chain polymer nanoparticles for application in nanomedicine.

Super resolution microscopy as a tool for tackling nanomedicine challenges

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Our group uses advanced microscopy techniques to visualize and track in living cells and tissues self-assembled nanomaterials with therapeutic potential. The understanding of materials-cell interactions is crucial towards the development of novel nanotechnology-based therapies for treatment of cancer and infectious diseases.

Many biological structures are made of multiple components that self-organize into complex architectures. Here we want to mimic this phenomenon to develop novel bioactive materials such as nanoparticles or nanofibers able to build themselves (1D or 3D self-assembled nanomaterials). Having the self-assembly motif decorated with different functionalities allows a modular and tunable approach that eases sample preparation.[1]

To study the behavior of such complex nanomaterials in action we make use of a variety of optical microscopy techniques, in particular super resolution microscopy (SRM).[2,3] SRM can achieve a resolution down to 20 nm and represents an ideal tool to visualize nanosized objects in the biological environment. In particular we demonstrate how STORM (Stochastic Optical Reconstruction Microscopy) can be used to image a wide range of nanomaterials beyond the diffraction limit: nanoparticles[4], BTA fibers[5], peptidic nanostructures [6], etc.

Remarkably, STORM allows this observation in the biological environment, thus we are able to follow the journey of nanomaterials inside the body: from protein corona formation[7] to extravasation, targeting and tracking nanomaterials inside the cell.[8-10]

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Curve and cut: membrane trafficking *in sillico*

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Cellular membranes constantly need to be cut and reshaped to sustain inter- and intracellular trafficking and enable life. The cell has developed a wide array of strategies for forming and reshaping its membrane structures, just as pathogens have evolved their own means of breaching the outer cell layer. These are at their heart physical processes, which require mechanical work and involve action across various scales.

Our group develops minimal coarse-grained computer models to investigate membrane remodeling strategies, in close collaboration with experimental colleagues [1-5]. Here I will first present our findings on the evolution of membrane-crossing nanoparticles (unpublished). Then I will discuss our results on how heterogeneity of the membrane composition can increase the membrane selectivity to this nanoparticle uptake [1]. Finally, I will present our recent model on active membrane cutting from the inner side of the membrane neck, driven by ESCRT-III nanomachinery (Figure 1) [2].

The physical principles revealed by our simulations can help us understand how the nanomachinery of life operates and can guide the design of man-made structures to manipulate cell membranes and deliver materials to cells.

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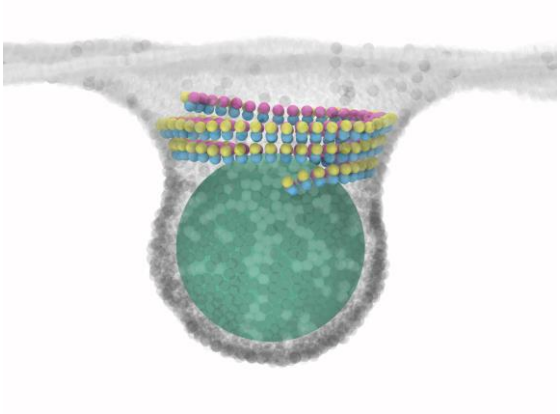

Figure 1. Cargo release by ESCRT-III machinery

Role of surface topography on adhesion of motile bacteria

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Only two decades ago, bacteria were considered as well-characterized biophysical entities, which offered little room for new discoveries. We now know that this picture is truly wrong and naïve. In recent years, bacterial cells have come back to the spotlight due to many bacterial species developing antimicrobial resistance (AMR) even to last-resort drugs. In particular, the way bacterial cells localize near a surface is key to the emergence of several phenomena of biological, ecological and medical relevance, including biofilm formation. Here, I will show how microfeatures on a surface have a non-negligible effect in shaping the propagation and localization dynamics of bacterial cells on the same. Beyond demonstrating the critical role played by surface defects on the near-surface motion of bacterial cells, these results can inspire novel routes to control microbial ecology in natural habitats and devise engineered materials for the control and prevention of bacterial adhesion to surfaces

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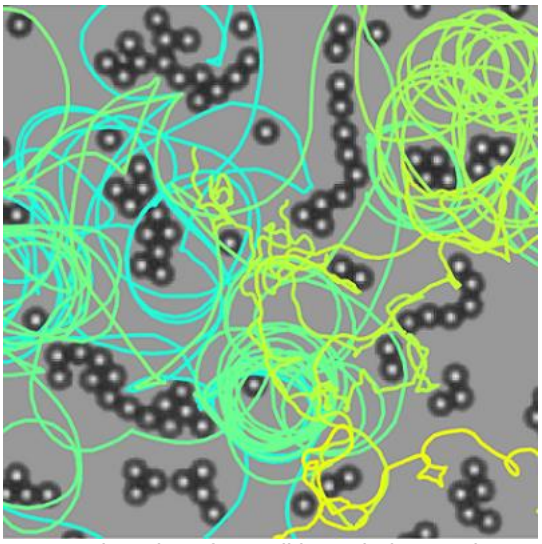

Figure 1. Trajectories of *E. coli* bacteria in complex porous environments.

Orals

Complex Nanoporous Anodic Alumina Photonic Structures for Sensing: Design, Fabrication and Application

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Nanoporous anodic alumina (NAA) is a promising material formed by the electrochemical anodization of aluminum, a cost-effective and fully scalable process compatible with conventional micro and nanofabrication approaches that allow the precise control over the geometry and distribution of the pores [1-2]. NAA has high chemical and physical stability, provides stable optical signals without further passivation; and its surface chemistry can be easily modified with a broad range of molecules.[3] Moreover, the optical properties of NAA rely intrinsically upon its nanoporous architecture. Therefore, to engineer the nanoporous structure of NAA provides novel means of modulating its refractive index in a multidimensional fashion to fabricate advanced materials with unique optical properties to guide, reflect, transmit, emit incident light [4]. Furthermore, the pore geometry can be varied by different methods to obtain different functionalities such as funnels [5], branched pores and even structures with remarkable optical properties such as Distributed Bragg Reflectors, Rugate filters, etc [6-7]

Photonic structures (PS) can be obtained by NAA pore engineering. NAA-PSs are obtained by applying a sinusoidal anodization current, which results in a continuous modulation of the pore diameter along its length. The application of this modulation results in a one-dimensional photonic crystal with a periodic variation of the refractive index along the pore direction and a photonic stop band.[8]. Previous studies have also developed NAA-PSs with different anodization current profiles such as sawtooth-like pulse anodization [9], and square wave [10] to achieve better control over the oxide growth rate and porosity of NAA.

In this work, we propose novel methods to obtain NAA-PSs applying different current profiles. Our study establishes a better understanding of the effect that the different anodization parameters have on the optical properties of NAA-PSs such as their characteristic reflection bands across UV−visible-NIR spectrum. We present a comprehensive study of different NAA-PSs based on single and multiple structures with sinusoidal profiles in an overlapped and stacked configuration. The stacked configuration consists of multiple currents are applied sequentially and the overlapped configuration consists of the average sum of multiple sinusoidal waves into a single complex waveform. Each sinusoidal wave determines the position and the reflectance amplitude of a forbidden band. In this case, the different periodic structures are stacked or overlapped on the same structure. The effective medium of the resulting photonic structures (PSs) is demonstrated to be optimal for the development of optical bio-sensing platforms.

All these photonic structures were prepared by anodization of a high-purity aluminum foils in 0.3 M oxalic acid electrolyte at 5 ºC. Figure 1 shows ESEM top view (a) and cross-section (b) picture in a stacked configuration. The straight pores can be recognized in the cross-section, Figure 2 shows the reflectance spectrum of the sample after removing the remaining aluminum. Figure 2a shows the reflection spectrum of a sample in overlapped configuration with I_0 = 4 mA,I_1 = 2 mA, T1= 100 s, T2= 150 s and T3= 200 s. In figure 2b we can observed the reflectance spectrum in stacked configuration wit I_0 = 4 mA,I_1 = 2 mA, T1= 125 s, T2= 150 s and T3= 175 s. The application of stacked sinusoidal current profile gives rise to the formation the three photonic stopbands. The height of these stop bands reaches 18-37 % while its width was 10-23 nm and the position of the photonic band corresponding central wavelength, λ = 330-500 nm.

In order to evaluate the suitability of these complex NAA-PSs, we assessed shifts in the position of the reflectance band filling the nanopores with different concentrations of aqueous solutions of D-Glucose.
We analyzed changes in the refractive index of the medium filling the pores by real-time spectroscopy. To this end, the sample is mounted in an acrylic cell with a transparent window that permits the measurement of the reflectance spectrum by a fiber mini spectrometer while different fluids are injected to cell and fill the NAA-PSs pores. Figure 3 illustrates the sensing principle of these photonic structures. The position of the reflectance band shifts to longer wavelengths when the nanopores are filled with different concentrations of D-Glucose.

In this work, we demonstrate the possibility to fabricate NAA-PSs by the application of different types current profiles. We also demonstrate the ability of structures to detect a change in the refractive index of the fluid filling the pores by means of real-time spectroscopy. With this method, it is possible to sense glucose in low concentrations. These results open up the possibility of using these complex NAA-PSs for detection of many other molecules for bio-sensing applications.

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Figures

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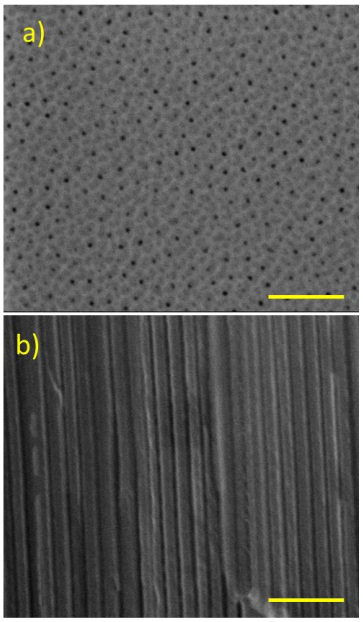

Figure 1. (a) Top view (scale bar 1µm) and (b) Cross-section (scale bar 1 µm) of NAA-PSs (stacked configuration)

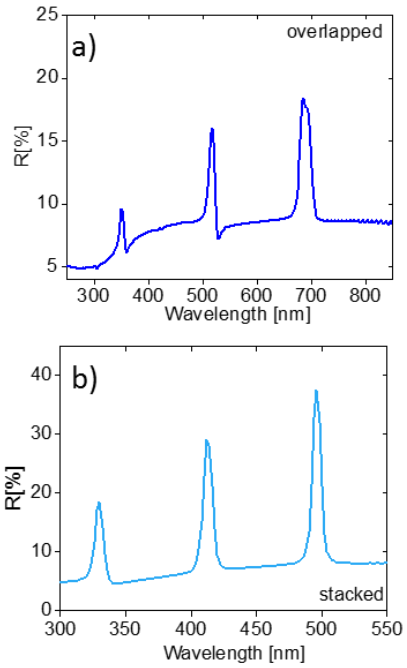

Figure 2. Reflectance spectrum of NAA-PSs (a) Overlapped configuration and (b) stacked configuration

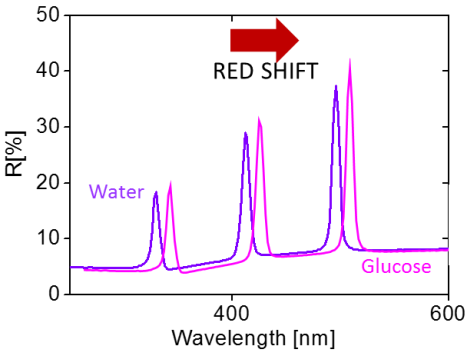

Figure 3. Sensing Principle of stacked NAA-PSs with Water and D-Glucose showing the red-shift in the position of de bands.

All printed environment friendly, biocompatible, sustainable sucrose-based temperature sensor

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Abstract
The in-situ measurement of ambient temperature is significant in our daily life, industrial processes, and environmental monitoring. The thermistor-based temperature sensor is one of the most important candidates for temperature sensing that has outstanding advantages of immunity to electromagnetic interference, simple fabrication, cost-effectiveness, and durability against harsh environments. Thermo resistive devices are extremely sensitive to the external temperature; therefore, by applying thermo-resistive based material a temperature sensor with high sensitivity and practicability can be obtained. Organic and disposable sensors have great potential for applications such as food industry, environmental monitoring and medical industry. Various types of temperature sensors have been fabricated, which include thermocouples [1], nanogenerators based temperature sensors [2], and thermistors [3]. An all printed interdigitated electrodes (IDT) based temperature sensor has been demonstrated in this study. The IDTs were fabricated on a glass substrate by reverse offset printing. The sucrose film was fabricated by spin coating the sucrose solution on the IDTs. The sensors showed stable and close to linear response of resistance change by varying temperature in the range 0°C to 100°C. The resistance of the sensors changed from ~3200 kΩ to ~400 kΩ for the temperature change in the range 0°C to 100°C. This study provides an effective method to fabricate environment-friendly, biocompatible, sustainable and high-performance temperature sensors based on thermistor effect in the future.

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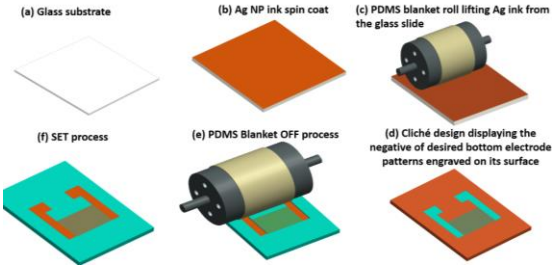

Figure 1 Schematic illustration of Reverse Offset printing of Ag IDTs for cost-effective and biocompatible temperature sensing

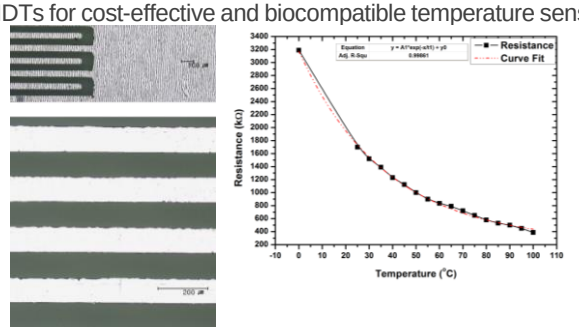

Figure 2 Optical micrographs of IDTs and the response of sensor towards change in temperature

Fundamental behaviour of nanoparticles revealed in zebrafish embryo

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Up to 99% of intravenously administered nanoparticles are cleared by the reticuloendothelial system (RES) cell types in the liver and spleen. The precise biological mechanisms which underpin clearance are not, however, fully understood.

Here, we use the transparent and versatile embryonic zebrafish as animal model to rapidly screen the behavior of nanoparticles *in vivo*. The ability to visualize fluorescent nanoparticles at cellular resolution and across entire living organisms has given us an unprecedented understanding of the fundamental behavior of nanoparticles *in vivo*.

In this presentation, I will demonstrate how key RES cell types of the embryonic zebrafish are genetically and functionally analogous to the mammalian liver sinusoidal endothelium. Furthermore, I will show how we identify *stabilin-1* and *stabilin-2* as the main receptors for scavenging anionic nanoparticles [1] by studying the distribution of various types of nanoparticles in the zebrafish in combination with CRISPR/Cas technology. The ligands that are cleared by zebrafish *stabilin-1* and *-2* receptors include specific (glyco)proteins, polyanionic carbohydrates, endotoxins, viruses and (in)organic nanoparticles -including liposomes used for clinical drug delivery- providing an alternative route to the nanoparticle clearance performed by macrophages.

Importantly, nanoparticle-SEC interactions can be blocked by dextran sulfate, a competitive inhibitor of *stabilin-1, stabilin-2* and other scavenger receptors. The importance of particle size in the nanomedicine field is also highlighted here, showing that small particles are the more convenient to avoid liver sequestration by scavenger receptors and to promote internalization in the cell of interest. Finally, we exploit nanoparticle-SEC interactions to demonstrate targeted intracellular drug delivery resulting in the selective deletion of a single blood vessel.

The identification of *stabilin-1* and *stabilin-2* as mediators in the uptake of anionic nanoparticles, and how to avoid such interaction, is an example of how the zebrafish embryos provides a new and powerful model system to study the mechanism that is required for the role in nanoparticle-mediated drug delivery.

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Figure

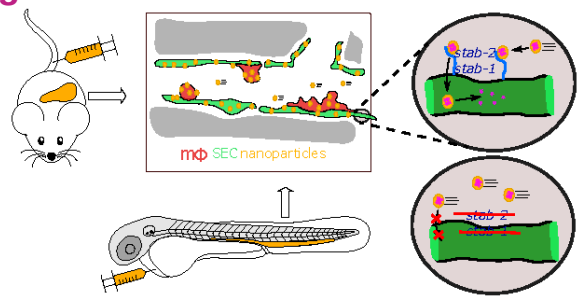

Figure 1. Schematic representation of liver endothelium in mice and zebrafish embryo. Nanoparticles endocytosed by receptor-mediated stab-1 and stab-2 or circulating in the absence/inhibition of stab-1 and stab-2. Modified from [1]

Biodegradable nanofibers scaffolds containing platelet lysate for skin wound healing

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The number of patients suffering from non-healing wounds is reaching epidemic proportions and it is estimated that 1-2% of the population will experience chronic wounds during their lifetime. The prevalence of chronic wounds increases along with vascular diseases and diabetes mellitus, and along with systemic factors such as advanced age [1]. Therefore, it is necessary to develop new drugs and medical devices to improve wound healing. In this regard, nanofibrous scaffolds are promising materials to regenerate the damaged skin, because they can act as a protective barrier against penetration of microorganisms, allow gas exchange, absorb the exudate and mimic the fibrous component of the extracellular matrix. On the other hand, platelet lysate (PL) has been considered to heal skin wounds due the accelerating effect of growth factors and bioactive molecules present in platelets [2]. In the study, polymeric scaffolds containing PL were developed as skin dressings with a controlled release of growth factors and bioactive molecules. Here, we show the *in vitro* effect of two types of biodegradable scaffolds on cell types involved in skin wound healing.

Both polymeric biomaterials, poly(vinyl alcohol) (PVA) and poly(L-lactide-co-ε-caprolactone) blend with poly-ε-caprolactone (PLCL/PCL), were fabricated by electrospinning in order to obtain a mesh of nanofibers. Two different strategies were used to assemble the PL according to the biodegradability of synthetic polymers. In the case of PVA, PL were introduced into the dissolvent to obtain PL-loaded nanofibers. In case of PLCL/PCL nanofibers, the nanmofibers were coated with fibrin assemblies together with PL.
Human keratinocytes (HaCaT), mouse fibroblasts (3T3) were cultured with nanofibrious scaffolds in Dulbecco's Modified Eagle Medium supplemented with 2% of fetal calf serum. Primary human saphenous vein endothelial cells were cultured in EGM-2 medium (Promocel) without growth factors. Cell metabolic activity was quantified on days 1, 3 (4), 7, and 14 days after seeding using MTS assay kit (Abcam). Cell migration was evaluated only for EC and measured by transmigration assay using Corning FluoroBlock cell culture inserts with the pore size of 8 µm. Cell differentiation were detected by immuno-fluorescence staining of specific markers. Basal cytokeratin 14 and differentiated cytokeratin 10 were analyzed for keratinocytes, whereas von Willebrand factor and platelet endothelial cell adhesion molecule (CD31) were analyzed for endothelial cells. For fibroblasts, type I collagen was analyzed.

For PVA samples, cell metabolic activity of keratinocytes, fibroblasts and endothelial cells was increased when PVA contained PL compared with pure PVA. In addition, images of cytokeratin 10 and 14 immunostaining on keratinocytes showed an increased number of differentiated cells positive for cytokeratin 10 on PVA containing PL. Cytokeratin 10 was detected on cells which grew on the upper layers in agreement with other authors that evaluated the presence of different types of cytokeratin according to the stratified layer of skin [3]. Endothelial cells were positively stained for von Willebrand factor and CD31. 3T3 fibroblasts started to produced type I collagen.
Similar results were obtained for PLCL/PCL scaffolds. The higher values of metabolic activity of keratinocytes and endothelial cells were observed on samples containing PL. The number of cells positively stained for cytokeratin 10 was higher on scaffolds with PL. Well-developed cytoskeleton of cytokeratin 10 and 14 can be observed in cells growing on samples (Fig. 1A). Endothelial cells staining for von Willebrand factor and CD31 indicated the presence of both differentiated markers (Fig. 1B). Moreover, endothelial cells migration towards the scaffold was significantly increased when samples contained PL.

The *in vitro* results indicate that both polymers could be considered as candidates for biomedical applications. However, the potential therapeutic application of each scaffold is different due to their physical and chemical properties and biodegradation rate. PVA nanofibers do not allow cells to migrate and grown on samples, so they can be used as dressing for wound healing. On the other hand, PLCL/PCL nanofibres could be used as scaffolds for skin regeneration with cells colonization.

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Acknowledgements

This work has been supported by the Czech Health Research Council, project No.NV18-01-00332.

Figures

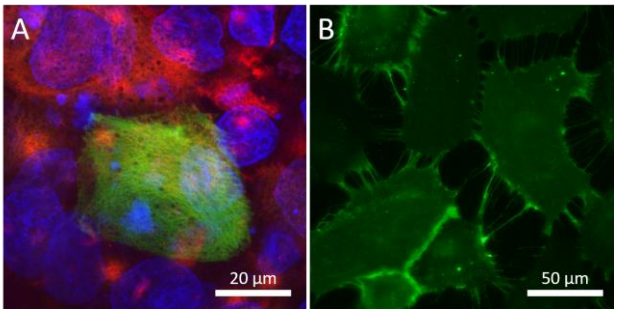

Figure 1. Immunofluorescence staining of cytokeratin 10 (green) and cytokeratin 14 (red) in HaCaT cells 14 days after seeding (A); and immunofluorescence staining of CD31 (green) in endothelial cells 7 days after seeding in medium with PLCL/PCL nanofibers scaffolds containing PL.

Model membranes interfaced with optical tweezers: a versatile microfluidics platform for nanomanipulation and mechanical characterization

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Membranes are the site of vital biological processes, such as sensing, communication and trafficking, involving many of them mechanical forces. But to elucidate the relationship between these membrane processes and mechanical forces it is required the use of tools able to apply and to measure picoNewton-level forces, like optical tweezers. Here, we introduce the combination of optical tweezers with free-standing lipid bilayers, which are fully accessible on both sides of the membrane [1]. In the vicinity of the lipid bilayer, optical trapping would normally be impossible due to optical distortions caused by pockets of solvent trapped within the membrane. We solve this by drastically reducing the size of these pockets via tuning of solvent and flow cell material. In the resulting flow cells, lipid nanotubes are pushed or pulled, and reach lengths above half a millimeter. Moreover, the controlled pushing of a lipid nanotube with an optically-trapped bead provides an accurate and direct measurement of important mechanical properties. In particular, we measure the membrane tension of a free-standing membrane composed of a mixture of DOPC and DPPC to be 4.6 × 10-6 N/m. We demonstrate the potential of the platform for biophysical studies, by inserting the cell-penetrating TAT peptide in the lipid membrane. The interactions between the TAT peptide and the membrane are found to decrease the value of the membrane tension to 2.1 × 10-6 N/m. This method is also fully compatible with electrophysiology measurements, and presents new possibilities for the study of membrane mechanics and the creation of artificial lipid tube networks of great importance in intra- and intercellular communication.

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Figures

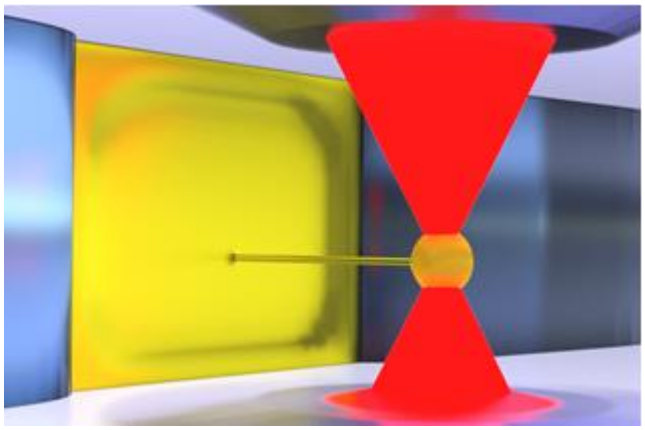

Figure 1. Schematic representation of an optical tweezers (red) pulling a lipid nanotube from a free-standing lipid membrane. The system consists of an artificial lipid bilayer (yellow) assembled on a rectangular aperture (blue) allowing free access to both sides of the membrane.

Lipid-composite particles by liquid-CO_2 cryospraying technology: advantages, challenges and pharmaceutical applications

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In recent years, a new generation of CO_2-based technologies has gained considerable interest in pharmaceutical and biotech industries, offering innovative, organic solvent-free, scaleable and economically viable solutions to address critical issues related to biopharmaceutical properties and drug product development.

Dense or liquid CO_2 has been applied in several processes as an atomization medium for the production of micro- and nanoparticles systems, by exploiting the thermal and mechanical properties of carbon dioxide rapid phase changes [1,2]. Variosol® cryospraying is one of these processes, based on the dynamic interaction between two sprayed fluids in different thermodynamic conditions. A liquid or pasty fluid can be converted into a micro or nano sized solid by the combined thermodynamical (Joule-Thomson effect) and mechanical (atomization) actions resulting from liquid-CO_2 rapid expansion. These properties can be advantageously applied to several classes of excipients, especially lipids or lipids-polymers composite, facilitating in some cases the formation of eutectic blends at low temperatures. Applying appropriate operating conditions, CO_2 expansion allows to eliminate water during spraying at low Tº [3], thus making it possible to spray disperse systems, such as emulsions or microemulsions, to obtain very fine dry particles combining hydrophilic and hydrophobic materials in the same matrix.

Lipid-based formulations (LBF) offer nowadays a wide range of possibilities, from e.g. enabling drugs intestinal solubilization and absorption of oral products [4], to controlling drug release, or improving efficacy in dermal products, facilitating drug interaction with skin components. However, most LBF are liquid or semisolid at room temperature, which may present a disadvantage in terms of stability, processability, product dosing (e.g. necessity of soft gel capsules) and high production costs. In fact, the development of solid forms (especially micro- or nanoparticulates) has been the focus of recent innovation in the field of LBF [4].

The cryogenic effect obtained during liquid-CO_2 expansion can be used to process composites, containing excipients or active molecules in liquid form, and still obtain LBF as fine, solid microparticles. The appropriate choice of materials and processing conditions are key factors to develop micronized and possibly nanosized LBFs for specific application such as improved drug solubilization and bioavailability, controlled or delayed release, drug stabilization, helping to address specific patients needs and targeting different routes of administration.

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Figures

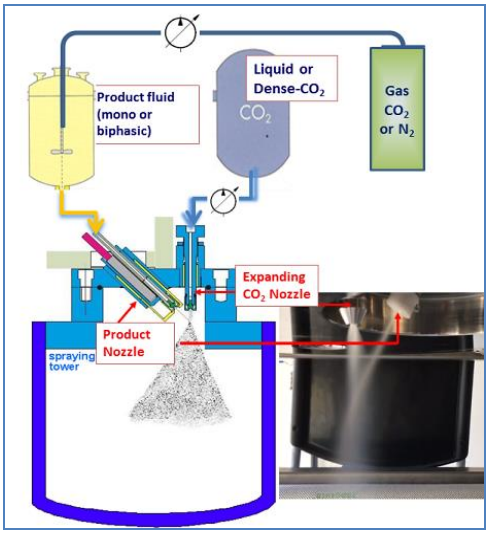

Figure 1. Scheme of CO_2 cryospraying technology

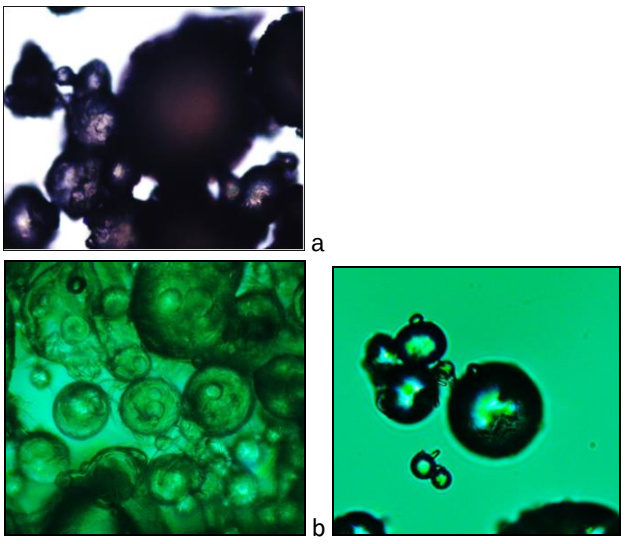

Figure 2. Optical Microscopy Images of dry (a) and water-suspended (b) drug- containing microspheres

Nano harvesting energy applied to cell biology

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Abstract

Our bodies are complex machines whose functioning depends on multiple electrical signals controlled mainly by the nervous system. Afterwards, it is not illogical to think that one day artificial electrical impulses would replace those signals offering support to medical treatments. Nowadays electrical stimulation is used in many therapeutic applications to modulate cellular activity, restore biological lost functions or even improve the performance of certain tissues. However, these systems still carry side effects link to the surgical interventions to place them or place their electrodes, their inherent bulkiness or lack in specificity to target only the cells involved in the condition to treat. The future to transcend these constrains would be possible in the extent that technology ease the path to improve precision, autonomy and miniaturization of the actual therapeutic tools. In this context, micro/nanogenerators play a key role as self-powered devices with high spatial resolution and acute cell specificity.

This work aims to provide micro/nanogenerators to stimulate single cells in its own liquid media. This work explored two technological branches based on photovoltaic and on magnetoelastic (piezoelectric/magnetostrictive) devices to harvest energy. Their fabrication was accomplished through micro/nanosystems technologies and their performance was characterized through several tests to ensure their correct power generation. As these devices were intended to interface biological media, direct cytotoxicity studies were conducted to guarantee their safety. Both branches were biologically validated with *in vitro* models of excitable cells (human osteoblast-like cells) analyzing the electrostimulation effects through morphological changes and through instantaneous ionic responses as calcium signaling.

Here, we demonstrate that the interaction of human cells with piezoelectric nanogenerators (NGs) based on two-dimensional ZnO nanosheets (NSs) induces a local electric field that stimulate and modulate their cell activity. When cells were cultured on top of the NGs, the electromechanical NG-cell interactions stimulated the motility of macrophages and triggered the opening of ion channels present in the plasma membrane of osteoblast-like cells inducing intracellular calcium transients, (activated cells over 62%). In addition, excellent cell viability, proliferation and differentiation were validated [1,2,3].

We will present the technology for microphotodiodes fabrication and the characterization of single microphotodiode characteristics under in vitro conditions. We also demonstrate their capability to harvest energy from light and instantaneously convert it to an electric stimulus. These devices provide a stimulation tool of single cells without the limitation of external cables and electrodes. The electrical stimulation triggered intracellular calcium transients as a response in 46% of the cells. Furthermore, induction of cytosolic Ca2+ transients triggered by the electrical stimuli generated by the microphotodiodes, on which osteoblasts cells were grown, shows the feasibility of this approach towards localized activation of excitable cells in a simple way [4,5].

The results gathered in this research demonstrate the feasibility of these micro/nanogenerators as self-powered electrical stimulators. Furthermore, their reduce size and capability to be suspended in liquid media open the door to further developments towards injected or ingested minimally invasive medical tools.
(*Partial abstract from PhD Thesis of Carolina Vargas-Estévez, Universitat de Barcelona 2019*).

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Figures

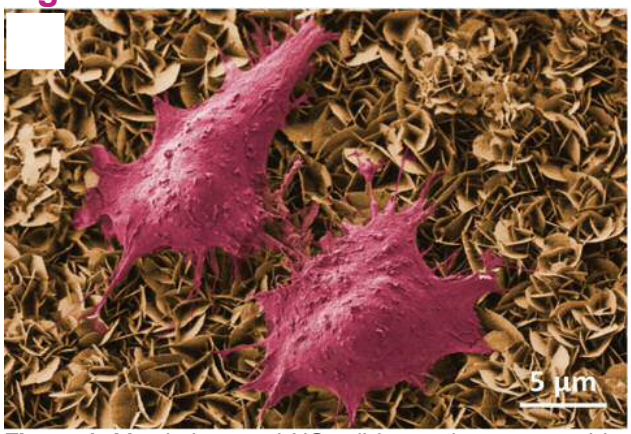

Figure 1. Morphology and NG-cell interaction, assessed by scanning electron microscopy showed that cells were firmly adhered to the nanosheets (NSs) [1]

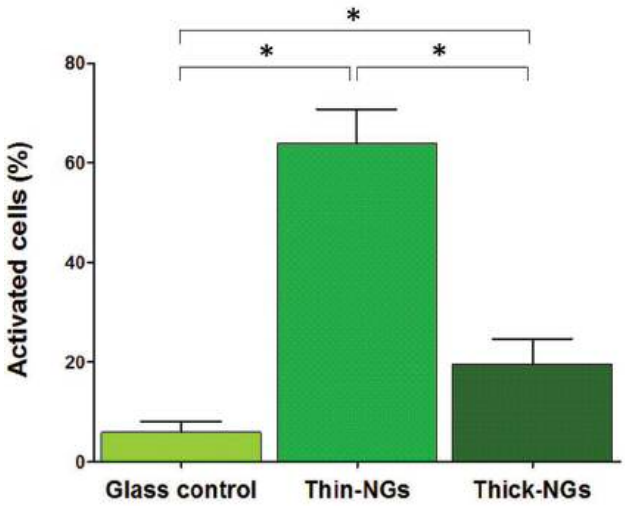

Figure 2. Quantification of Saos-2 cells undergoing changes in Ca2+ concentration (activated cells). Asterisks above the columns indicate significant differences (Kruskal-Wallis test and χ2 test) [1]

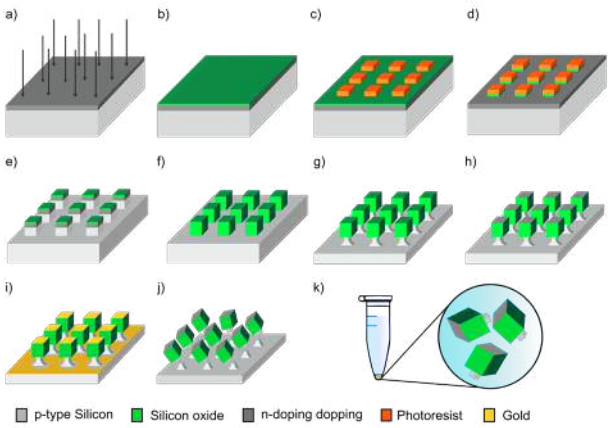

Figure 3. Fabrication process of suspended microphotodiodes [4]

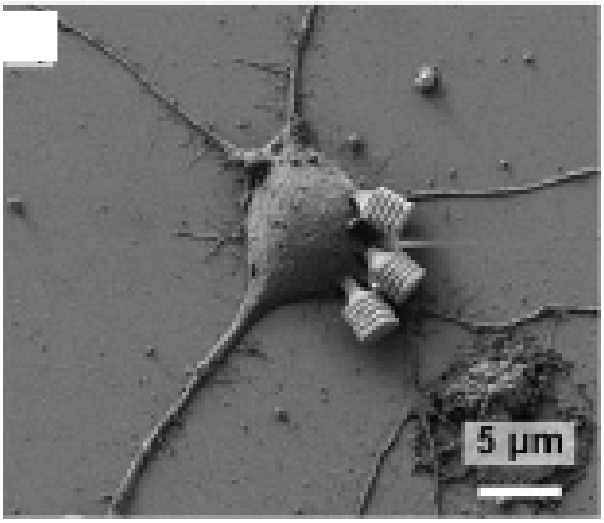

Figure 4. SEM image of neuron with photodiodes attached to soma [4]

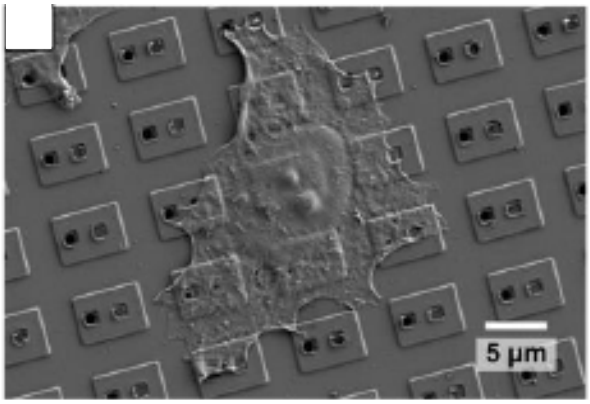

Figure 5. SEM image of Saos-2 cells growing on microphotodiodes array (PVMA). [5]

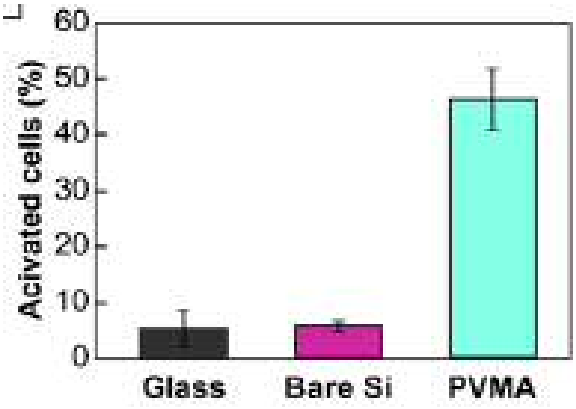

Figure 6. Percentage of activated Saos-2 cells on PVMA and on glass coverslip/bare silicon control for the same light stimulation. [5]

"Three-Bullets" Loaded Mesoporous Silica Nanoparticles for Combined Photo/Chemotherapy

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Multimodal cancer therapy involves the use of two or more treatment modalities with the aim to attack tumour on different sides by acting either on a single oncogenic pathway through different mechanisms or across parallel pathways without amplification of side effects.
In this frame, the combination of conventional chemotherapeutics with light-activated therapeutic treatments is revealing a very appealing approach to potentiate the therapeutic outcome through anticancer synergistic/additive effects. Light represents in fact as suitable and minimally invasive tool that permits the introduction of therapeutic species in biological environments with superb spatiotemporal control [1]. Among the light triggered therapeutic treatments, photodynamic therapy (PDT) is so far one of the most promising to combat cancer diseases [2]. This treatment modality exploits mainly the cytotoxic effects of the highly reactive singlet oxygen ($^1O_2$), catalytically generated by energy transfer between the long-lived excited triplet state of a photosensitizer (PS) and the nearby molecular oxygen.
Another emerging light-activated approach is based on the photoregulated release of nitric oxide (NO) through the use of NO photodonors (NOPD) [3]. Although still confined to the research area, this NO-based PDT, namely NOPDT, has come to the limelight in recent years and holds very promising features in cancer treatment. In fact, apart from playing multiple roles in the bioregulation of a broad array of physiological processes, NO has also proven to be an effective anticancer agent [4]. If generated in an appropriate concentration range this diatomic free radical can act not only as a cytotoxic agent but also as inhibitor of the ATP Binding Cassette (ABC) transporters responsible of most chemotherapeutic efflux and inducing multidrug resistance (MDR) in cancer cells [5]. Noteworthy, both $^1O_2$ and NO are multitarget species that has not a reduced efficacy in multidrug resistant cells due to their short lifetimes, confine their action to short distances from the production site inside the cells (< 20 nm for $^1O_2$ and < 200 μm for NO), reducing systemic toxicity issues common to many conventional drugs. Besides, since NO photorelease is independent of O_2 availability, it may successfully complement PDT at the onset of hypoxic conditions, typical for some tumours, where PDT may fail.
The significant breakthroughs in nanomedicine permit nowadays the development of multifunctional platforms in which more therapeutic agents are entrapped in a single nanocarrier. This offers an unprecedented opportunity to devise a better scheme for precise and controlled delivery of multiple therapeutics in the same area of the body and at predefined extra/intracellular level. In this context, nanoplatforms combining PDT and NOPDT are opening new horizons towards more effective and less invasive cancer treatments entirely based on "non-conventional" chemotherapeutics [6]. Besides, an increasing large number of nanoconstructs in which PDT has been combined with chemotherapy in the management of several cancer types has been reported in literature [7]. In contrast, only recently NOPDT has been successfully used in combination with chemotherapeutics [8]. Based on this scenario, the achievement of a trimodal nanoplatform able to generate $^1O_2$ and NO and, simultaneously, to deliver a conventional chemotherapeutic is a very challenging objective to pursue. Mesoporous silica nanoparticles (MSNs) as very suitable scaffolds to this end due to their high loading capacity, ease of surface functionalization, good biocompatibility and satisfactory light transparency. These inorganic scaffolds have been extensively used as excellent carrier for conventional drugs. Furthermore, it has been used as carrier for PDT agents alone and in combinations with chemotherapeutics, and recently our group demonstrated its versatility using MSNs as carrier for NOPD [9].

In this contribution, we report an engineered MSNs able to deliver "three bullets" for potential anticancer therapy. These MSNs covalently integrate in their molecular scaffold Erythrosine and a nitroaniline derivative as suitable PS and NOPD, respectively, and encapsulate the chemotherapeutic Doxorubicin (DOX) in a non-covalent fashion (figure 1) [10].
These MSNs bind the NOPD mainly in their inner part and the PS in their outer part in order to judiciously exploit the different diffusion radius of the cytotoxic NO and $^1O_2$. We demonstrate that the photochemical properties of the PS and NOPD are preserved in an excellent manner in the MSNs containing all active components. This silica nanoconstuct has been devised in such a way to permit the selective excitation of the NOPD and the PS with light sources of different energy in the visible range window. As a result, the spontaneous

release of DOX under physiological conditions can be combined with the photoregulated generation of $^1O_2$, NO or both by using light stimuli of appropriate energy in the visible light range (figure 2).
Preliminary biological results performed by using A375 cancer cell lines expressing ABC transporters show a good tolerability of the functionalized MSNs in the dark and a potentiated activity of DOX incorporated in our "three bullets" nanoconstruct upon irradiation with blue light, due to the effect of the NO photoreleased.

Figures

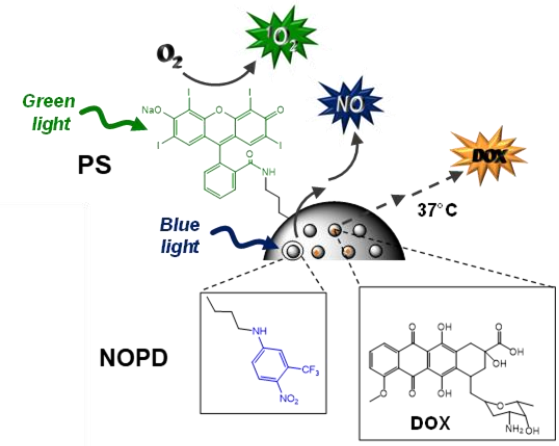

Figure 1. Schematic for the "three-bullets" PS-MSNs-NOPD/DOX and their working principle.

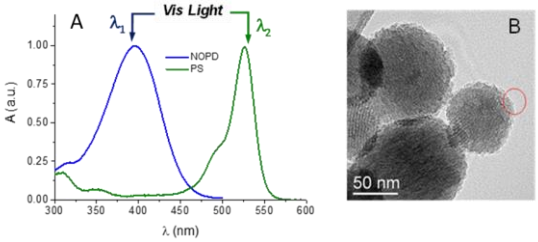

Figure 2. A. Normalized absorption spectra in PBS (10 mM , pH 7.4) of NOPD and PS. B. Representative TEM image of the PS-MSNs.

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Mesoporous silica nanoparticles as dual delivery platform for the efficient drug delivery and CRISPR-Cas9 editing machinery to one-shot treatments

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Abstract

The new CRISPR-Cas9 technology has been established quickly as an easy genome-editing method. This system presents two main components; the non-specific CRISPR associated endonuclease (Cas9) and the guide RNA (gRNA). The Cas9 targets the specific sequence in the genome to be edited guided by the gRNA. The most extended CRISPR application is for knocking out genes, being a potential technology to correct genetic defects in diseases [1]. To take advantage of this technology in therapeutic applications, the development of carriers to deliver the CRISPR-Cas9 system efficiently to human cells represents an important challenge.

Among the delivery protocols to introduce the CRISPR-Cas9 into the cells, the current non-viral used methods are lipofection, microinjection and electroporation.

However, these protocols are complex to apply in *in vivo* settings and the use of nanoparticles for CRISPR-Cas9 efficient delivery has become a research area of great interest. At this respect, new delivery methods has been described using nanomaterials based on polymers, lipid nanoparticles, cell-penetrating peptides and inorganic nanoparticles to deliver the CRISPR-Cas9 machinery [2-4].

From another point of view, several studies showed the use of surface-functionalized mesoporous silica nanoparticles (MSNs) as an efficient and safe carrier for bioactive molecules [5]. Indeed, previous published works described the use of MSNs to deliver nucleic acids into the cells [6]. In this scenario, MSNs could be a potential platform for the efficient co-delivering of the CRSIRP-Cas9 technology, as well as, to deliver an entrapped payload at the same time.

Based on these premises, we described here a novel vehicle that simultaneously delivers the CRISPR-Cas9 technology and an entrapped cargo. As a proof of concept, we used MSNs to edit GFP in human cells and, simultaneously, deliver an entrapped dye. The MSNs were loaded with a dye and then its external surface coated with a polyethylenimine (PEI) layer. Finally, CRISPR-Cas9 system is adsorbed, through electrostatic interactions, onto the PEI layer. This work demonstrated the possible use of MSNs as dual platforms to gene editing and drug delivery. MSNs represent a potential tool in the preparation of advanced nanodevices for one-shot treatments to simultaneously edit genes and release drugs by double-hit strategies, in particular to combine synergistic therapies or to overcome drug resistance in tumours.

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Figures

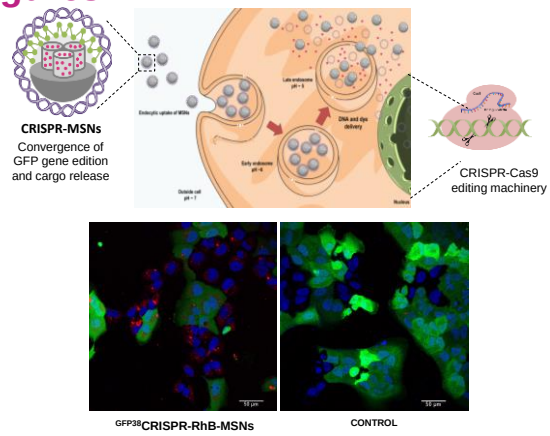

Figure 1. MSNs for the efficient co-delivery of CRISPR-Cas9 editing machinery and an entrapped cargo.

Mesoporous magnetic nanorods for theranostics

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Abstract

The presented work involves developing mesoporous silica nanorods as a platform for production of multimodal agents for diagnostic and therapeutic purposes. The main aim is to demonstrate that such materials can exhibit improved properties compared to the current state of the art, mainly focused on spherical particles.
In this work mesoporous silica nanorods (Figure) have been synthesized and characterized in terms of size, shape, pore size and surface area. In a second step, silica was functionalized by inserting iron oxide nanoparticles into the pores by an in-situ method.
The magnetic properties of the silica nanorods have been investigated and their performance as MRI contrast agents has been studied.
Future work will focus on in vitro and in vivo performance tests as well as on using the nanoplatforms for ultrasound (US) imaging and computerized tomography (CT) imaging.

References

Figures

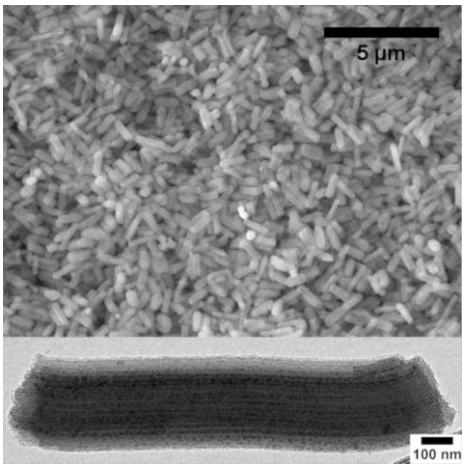

Figure 1. (Top) Scanning electron microscopy (SEM) image of mesoporous silica nanorods synthesized using a templated sol-gel method. (Bottom) Transmission electron microscopy (TEM) image of a silica rod filled with iron oxide nanoparticles.

Polymeric NPs as efficient tool for gene delivery

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Abstract

Gene therapy is described as the direct transfer of genetic material to cells or tissue for the treatment of inherited disorders and acquired diseases, such as cancer. The base of this therapeutic method is to introduce a gene encoding a functional protein altering the expression of an endogenous gene or possessing the capacity to cure or prevent the progression of a disease, or to introduce systems of gene silencing (such as siRNA, etc.) which has the ability to specifically silence target proteins that are crucial for modulating cellular pathways inducing an inhibitory effect on cancer proliferation. There are two types of gene delivery based on the carrier's nature: viral delivery and non-viral delivery. Gene delivery using viral vectors have long been proven to be the most efficient and stable transgene vectors into the cell. In addition, viruses are able to use the host cell machinery for protein synthesis, and some of them are able to stably insert into the host cell genome and provide a long-term transgene expression in transduced cells. Thus, viral gene carriers possess higher gene transfection efficiency than non-viral vectors. Despite some successes in clinic trials, viral vector gene transfer still has some safety issues. In order to limit the use of viral vectors, recent advances in vector technology have improved gene transfection efficiencies of non-viral carriers.
Nanoparticle (NP)-based therapeutic systems developed in recent years have shown efficient delivery of nucleic acids with low toxicity and sustained cargo release.
For gene delivery application, NPs can accommodate large DNA plasmids, RNA or proteins and may be produced at low cost on a large scale. NP-based systems overcome safety problems and limitations of viral vectors. FDA-approved polymers are particularly attractive for *in vivo* drug/gene delivery applications. Recently, we have developed a polymeric system for drug and gene based on pH-responsive and enzyme-responsive, core/shell NPs using the FDA approved PCL polymer [1-4].
Another, attractive polymer for gene delivery applications is polyglycolic acid (PGA), which has been approved by the FDA. PGA degradation is quicker *in vivo* and *in vitro*, and the degradation product, glycolic acid, is non-toxic and it can enter in the tricarboxylic acid cycle.
Our PGA NPs are obtained by nanoprecipitation and desolvation method, and are composed of a core loaded with the acid nucleic molecules and a PGA shell. Prior to particle assembly, active agent is complexed with a pH (as chitosan) or enzymatic (as protamine)-responsive polymer. By combining the sensitivity of the core polymer with the slow degradation of surface PGA, we obtained a simple and easy way to control the release of an active agent and improve its therapeutic efficiency.
The mean size of PGA nanoparticles is 100 nm, with a negative ζ-potential of -12 mV.
Our NPs have a regular spherical shape and no aggregation as observed by AFM and SEM analysis. No cytotoxicity is observed on different cell lines tested.
In addition, our PGA NPs have showed an efficient delivery of cDNA, observing relative numbers and mean fluorescence intensity of transfected GFP-positive cells comparable to those achieved with standard reagents used to promote transfection.
PGA NPs, thanks their properties, as biocompatibility, biodegradability and a good transfection efficacy, can be used as efficient tool of transfection overcome viral vector problems.

Acknowledgement
This study is supported by Tecnomed (FIRS project of nanotechnology, photonics and precision medicine) and AIRC through the grant MFAG n. 16803

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Complex skeletons for improved tracking and motion of 3D printed biorobots

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Nano and microrobots has raised great interest for its potential applications in both environmental and biomedical applications, being traditionally divided in three main categories: synthetic, biological and hybrid microrobots.[1] Among them, biohybrid robots are being explored to exploit and implement some of their unique inherent capabilities, such as adaptability, self-assembly, self-healing, or response to an external stimuli.[2] The development of 3D bioprinting techniques revolutionized the field of advanced biorobots based on living tissues, being especially remarkable the versatility of such approach when cells are directly bioprinted in a rational shape to obtain a defined functionality.

In our specific case, our biological actuator based on skeletal muscle cells is combined with an advanced 3D printed compliant skeleton useful force tracking purposes (Figure 1). Skeletal muscle cells provide a controlled contractile behavior dependent on the applied electrical stimuli, being also of interest for their self-repair capabilities, which could lead to longer operative time in the resulting biobot.[3] On the other hand, a skeleton with a controlled stiffness and a spring-like structure useful for force tracking[4] has been integrated to obtain efficient motion and provide the required stiffness for the optimal cell maturation. The resulting biohybrid robot's capabilities also depend on the performed training protocols, which are already been stablished in the group by applying electric pulse stimulation.[5] Currently, we are not only exploring the effect of such global stimulation, but also how actuation can be modulated by integrating flexible electrodes in the system able to apply local electric field.

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Enzyme-powered nanomotors towards biomedical applications

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Bio-catalytic micro- and nanomotors are capable of self-propulsion in fluids due to the enzymatic conversion of substrates into products. [1,2] These structures have been focus of interest for biomedical applications, such as the active transport and delivery of cargoes to specific locations, as well as to modulate cell targeting and internalization phenomena. Here, we present fuel-dependent anti-cancer drug doxorubicin release and efficient delivery to cancer cells by urease powered nanomotors based on mesoporous silica nanoparticles,[3] as well as their potential to target bladder cancer cells in the form of 3D spheroids by anchoring anti-FGFR3 antibody on their surface. [4] We observed that these nanomotors propel in physiologically relevant fluids, such as PBS. Furthermore, we found a four-fold increase in anti-cancer drug doxorubicin release by nanomotors after 6 hours exposure to urea, compared to static particles. Moreover, active drug-loaded nanomotors present improved anti-cancer efficiency toward HeLa cells, which arises from a synergistic effect between enhanced anti-cancer drug release and generation of ammonia during bio-catalysis. We found that in the presence of urea, a higher content of anti-cancer drug is uptaken by cells after 1, 4, 6- and 24-hour incubations with active nanomotors, compared to static carriers. In addition, we anchored anti-FGFR3 antibody to the surface of urease-powered nanomotors to target 3D bladder cancer spheroids. These nanomotors are able to self-propel in both simulated and real urine. We observed that the internalization efficiency of antibody-modified nanomotors into the spheroids in the presence of urea was significantly higher compared with antibody-modified static particles or non-targeted nanomotors. Furthermore, cell proliferation studies indicated that targeted, active nanomotors induce higher suppression of spheroid proliferation compared with non-targeted nanomotors, which could be due to a synergistic effect between the inhibition of the fibroblast growth factor pathway by anti-FGFR3 antibody and the local production of ammonia by the active nanomotors. Altogether, these results point towards the applicability of urease-powered nanomotors as tools for enhancing disease detection and simultaneous treatment, by combining improved drug release and targeting functionalities.

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Nanomaterial Fate and Exposure Modelling

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Nanotechnology industry is a fast-growing field, but still there is not a robust legislation about how nanomaterials can affect the environment. Anticipating this need, the Biorima (BIOmaterial RIsk MAnagement) project aims at developing a reliable methodology for better risk management of engineered nanobiomaterials. We present here the fate and exposure models developed within the mentioned project.

The nanobiomaterials are engineered nanoparticles (ENP) specifically designed to interact with biological systems with a medical purpose - either in Advanced Therapy Medicinal Products (ATMP) or Medical Devices (MD).

Different fate and behavior models have been developed over the years, being SimpleBox4nano [1] and MendNano [2] two of the most representative ones. These models evaluate the nanomaterials fate from two different points of view: SimpleBox4nano is based on mechanistic formulations of the key processes, while MendNano is based on empirical partitioning of the data.

The fate model needs to know the estimated emissions as an input, coming from a Material Flow Analysis (MFA) in Biorima. As the project is based on the release of nanobiomaterials, they are liberated mainly to water via excretion, hospital waste or residuals, and accidental release. It is also needed to consider the properties of the nanobiomaterials (such as density or diameter), as well as the environmental variables (like mean temperature, volumes, diffusivities…).

From here, a full study of the fate and behavior of the nanoparticles has been done. The main approach is to calculate specific constants, such as removal and transport rates for each component, and use them to calculate the concentration of a nanobiomaterial over time. With the obtained data, it is also possible to calculate the Predicted Environmental Concentration (PEC). Figure 1 is a representation of the model.

Different processes have been taken into account: Sedimentation, resuspension, burial, dissolution and aggregation.

- Sedimentation as a transport process between water and sediment compartments, modelled through a derivation of the Stokes' Law from gravitational settling of particles.
- Resuspension, which is the contrary process of sedimentation, involving the transport of the nanobiomaterial from sediment again to water.
- Burial is a removal process where the nanobiomaterial goes out of the system.
- Dissolution has been considered as a removal process in water and sediment compartiments. It depends on the surface of the particle and the chemical properties of the surrounding material. Noyes-Whitney equation has been applied.
- Both aggregation and attachment to larger particles have been also considered. It highly depends on collision frequencies which are described as a function of the particle size, density, number of concentrations of the particles present and characteristics of the surrounding material.

Biorima fate and behavior model implements all this information and starting from a particle emission, it can calculate the predicted environmental concentration and give it as an output of the model.

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Figures

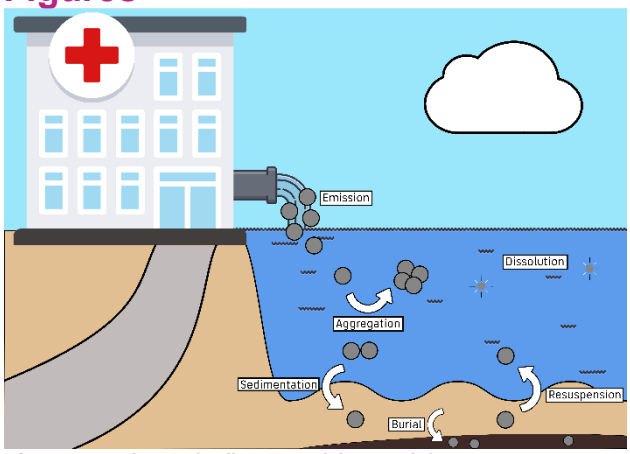

Figure 1. Schematic diagram of the model

Layer-by-Layer decoration of AgNPs with Aminocellulose and Quorum Quenching Acylase for Controlling Bacterial Pathogens and Their Biofilms

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The emergence of drug resistant bacteria and the failure of the existing therapeutics call for development novel antibacterial strategies. Furthermore, the inclusion of pathogens in highly organized biofilms causes difficult to treat infections and lead to poor treatment outcomes.[1,2,3 4] Whereas, the existing antimicrobials and the host immune response can eradicate the planktonic bacterial cells, they become inefficient once the cells establish biofilm structures that restrict the penetration of the antimicrobials. The process of biofilm formation is upon the phenomenon of cell-to-cell communication, known as quorum sensing (QS).

Targeting QS systems in the pathogens is an emerging area of research due to the capability of the anti-QS agents to attenuate bacterial virulence and biofilm formation, making the cells more susceptible to the conventional drugs at lower concentrations. Interfering with bacterial QS may potentiate efficacy of the current antimicrobials lowering the risk of development of resistance.
Herein, we developed novel safe-by-design hybrid nano-entities of silver nanoparticles (AgNPs) decorated with layers of bactericidal aminocellulose (AC) and QS interfering enzyme acylase in a Layer-by-Layer (LbL) fashion. The AC and acylase nanoshell enhanced the antibacterial activity of the AgNPs core towards the common Gram-negative pathogen *Pseudomonas aeruginosa*, lowering their minimum inhibitory concertation by 4-fold and prevented and eradicated the established biofilms with 8-fold lower AgNPs concentration. These nano-sized hybrid entities were able to inhibit QS process by 45% and eliminated the drug resistant biofilms of *P. aeruginosa* from a model surface (Figure 1). Moreover, the surface modification of the AgNPs improved its biocompatibility and did not affect the viability the morphology of human fibroblast cells. In border context, the developed novel NPs could be a promising alternative to combat difficult-to-treat bacterial infections, without creating selective pressure for resistance development.

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Acknowledgments

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Figures

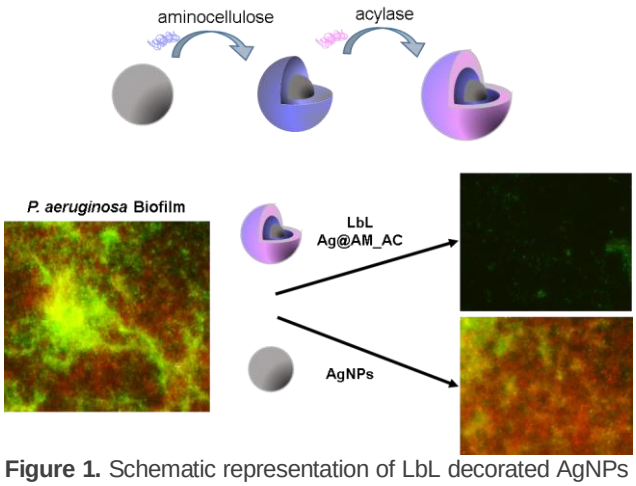

Figure 1. Schematic representation of LbL decorated AgNPs and their exposure to mature biofilms

Characterisation of key bio-nano interactions between organosilica nanoparticles and *Candida albicans*

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Candida albicans (*C. albicans*), a commensal microorganism in healthy individuals, is capable of causing life-threatening infections in immunocompromised patients. Known as the fourth most common hospitable-acquired pathogen, many of these infections are caused by drug-resistant biofilms on implantable devices such as a catheter, which are difficult to treat and diagnose [1, 2]. Mortality rates from systemic infections are between 10-47% despite the availability of antimicrobial therapy and current diagnostic measures [3]. Hence, novel materials need to be explored for the development of new therapeutic, diagnostic or theranostic tools in an effort to combat nosocomial infections caused by *C. albicans*.

Fundamental interactions between mammalian cells and silica nanoparticles focusing on cytotoxicity, cell association and imaging have been investigated for applications such as drug delivery, diagnostics and imaging [4]. While it is widely accepted that a range of inorganic nanoparticles including silver, gold and zinc oxide are inherently cytotoxic to microbial cells [5], the bio-nano interactions between silica nanoparticles and pathogenic fungi have not been well characterised.

In this study, we investigated the interactions between *C. albicans* and organosilica nanoparticles, which are highly tuneable and coated with the antifouling polymer, PEG, to provide an additional functional handle for drug conjugation. We found that size and PEGylation of the nanoparticles had minimal effect on the growth and viability of *C. albicans*. Yet, association between the nanoparticles and the pathogen was found to be size and concentration-dependent. We then employed a whole blood assay to further study how PEGylated organosilica nanoparticles of different sizes and concentrations associate with human immune cells, which are the first cell types nanomaterials are likely to encounter *in vivo* or during an infection. This provided us with an insight into the likely behaviour of the nanoparticles *in vivo*. Lastly, we attached the clinically relevant drug, caspofungin, on the surface of PEGylated nanoparticles to switch the surface interactions between *C. albicans* and organosilica nanoparticles from benign to antifungal, without impacting the viability of primary human neutrophils. Our results illuminate how organosilica nanomaterials could be used as scaffolds for designing biomaterials for detecting or preventing *C. albicans* infections.

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Figures

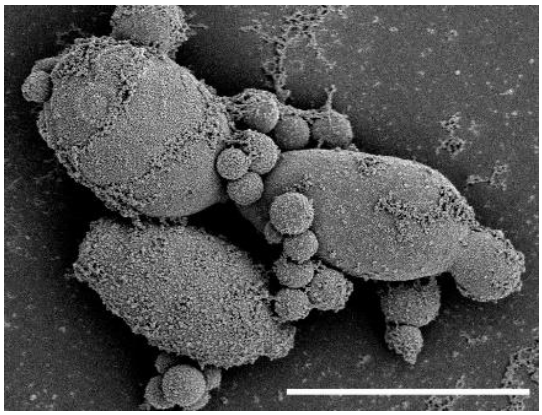

Figure 1. SEM image of ~800 nm PEGylated organosilica nanoparticles associating with *C. albicans*. Scale bar represents 5µm.

Collagen at the solid/liquid interface: Self-assembly, supramolecular organization and interaction with inorganic nanoparticles

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Collagen is the most abundant structural protein in mammals, located in the extracellular matrix (ECM) of connective tissues. *In vivo*, collagen molecules have an outstanding ability to self-assemble and to interact with other entities to form highly organized 3D networks surrounding cells as in hard biomineralized tissues, cartilage, tendon, and skin. These architectures provide a physical support to cells and serves as transducer for biochemical signaling. Collagen is also one of the most common proteins used for the design of biomimetic materials with broad applications in drug delivery and tissue engineering. In vitro, collagen plays a pivotal role to mediate cell-material interaction during in vitro tests. It is, indeed, frequently adsorbed to cell culture substrates to provide a protein coating for cell attachment, as cells lose their normal ECM environment.

Nanostructured collagen layers may be obtained at solid/liquid interface by adjusting the adsorption procedure, the characteristics of the medium (pH, temperature, composition and ionic strength), the properties of the substrates (wettability, surface charge, topography) and collagen conformational states (triple-helix or random coil) and fibrillogenesis [1]. Even so, the self-assembly and organization of collagen at solid/liquid interface remain difficult to predict.

Understanding the behavior of collagen at solid surfaces requires coping with the complexity of the interfaces. Herein, examples are given to illustrate key considerations in situations of biomedical interest:

(i) Mechanism of interaction with spheroid- and rod-shaped TiO_2 nanocrystals. These nanoparticles have, indeed, been the subject of a vast literature regarding their toxicity and their interaction with proteins impacting cell behavior [2], rods being explored for their enhanced photocatalytic properties, however with possible consequence on particle aggregation [3]. In solution, results show that nanoparticles do not alter the conformation of collagen (triple-helix), and slightly delay the kinetics of its fibrillogenesis. By contrast collagen layer is strongly impacted by the presence of nanoparticles in the medium (Figure 1, top).

(ii) Mechanism of interaction with hydroxyapatite (Hap) nanoparticles. A comprehensive understanding of this mechanism is a pivotal step for guiding the design of biologically relevant nanocomposites with controlled hierarchical structure. By using a variety of Hap nanoparticles differing by their shape (rod vs platelet) and their size (~30 vs ~130 nm), we showed that collagen strongly interacts with rod-shaped nanoparticles while only a weak effect was observed with platelets. Interestingly, the use of small rods, typically with ~30 nm of length, leads to the formation of assembled collagen fibrils decorated with Hap nanocrystals which, in turn, self-assemble progressively to form larger fibrillar composite (Figure 1, bottom). Through this study, we showed the possibility to design hierarchical collagen-hydroxyapatite nanostructures which may be relevant for various medical applications.

(iii) Methodological development for probing collagen layers both in the dried and hydrated phases. This includes AFM imaging, force spectroscopy, QCM-D, etc. In particular, we developed a powerful yet straightforward method to exploring the dewetting patterns of the collagen layers using AFM imaging. The method allows the extraction of quantitative parameters describing the collagen networks: density and degree of nodes, area and distribution of holes, physical distance between nodes, etc (Figure 1, top). We showed that information obtained from AFM imaging are consistent with the analysis of the protein layers in the hydrated state, regarding the elastic compliance of the film, i.e. its softness (data from QCM-D), the surface density of fibrils and their “length” (data from AFM force spectroscopy). This constitutes a step forward toward reconciling disparate views of collagen layers’ characterization in the dried state and in the liquid phase.

The approaches described here offers new perspectives to examine the interactions between collagen and inorganic nanoparticles at the solid/liquid interface, particularly at the stage of *in vitro* cell culture.

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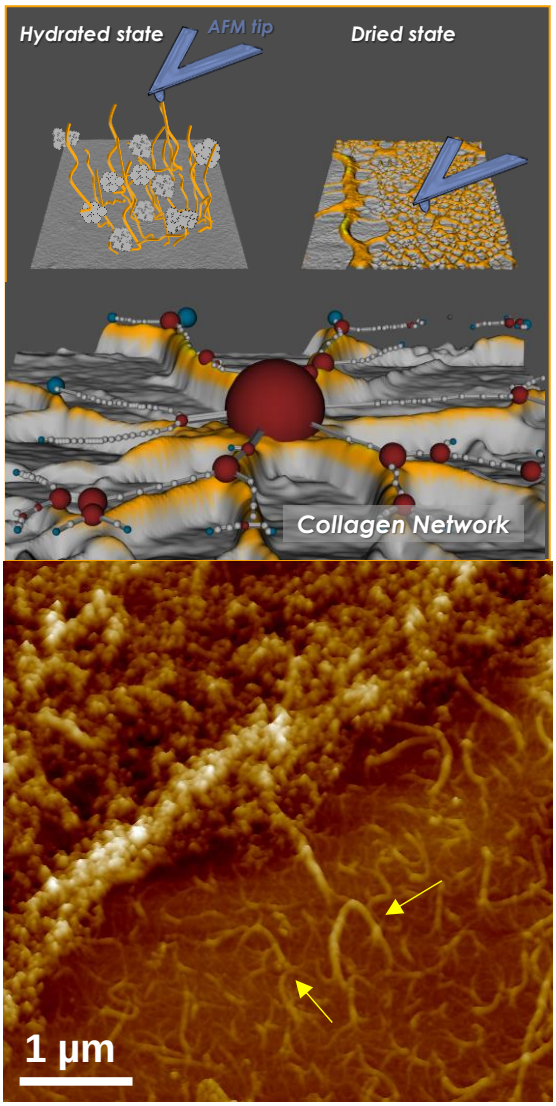

Figure 1. (Top) Schematic representation describing (i) the supramolecular organization of collagen on PS-coated substrate in the hydrated and the dried state in solution containing TiO_2 nanoparticles and (ii) network extraction from AFM images of collagen layers with the degree of nodes (blue = 1 ; grey = 2 ; red > 2). (Bottom) AFM height image showing the formation of collagen-hydroxyapatite composite, and well-defined collagen fibrils (see arrows) remain clearly visible.

Control of particle size and drug release of PLA/PLGA microspheres

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Poly (D,L-lactic-co-glycolic acid) (PLGA) and poly (lactic acid) (PLA) are well known by its biocompatibility and biodegradability in biomedical solutions [1]. The PLGA/PLA microspheres are one of the most successful drug delivery systems (DDS) in lab and clinic [2]. In the present study, microspheres based on blend of PLGA and PLA with ibuprofen as drug and with poly(vinyl alcohol) as emulsifier were prepared by a developed double emulsion oil-in-water-in-water solvent evaporation method and characterized. The blend of PLA and PLGA is formed only by physical interactions between them. This can be seen from the FTIR spectrum which shows both PLA and PLGA component (Figure 1). The effect of added surfactant, PVA [poly (vinyl alcohol)] and PLA/PLGA concentration and emulsion speed stirring on the size of the resultant PLGA/PLA microspheres has been studied. Ibuprofen release profile from PLGA/PLA microspheres with PVA as emulsifier within a phosphate buffer solutions (PBS) is determined and compared to drug release profile of microspheres with different concentrations of PLGA and PLA. This experiment involves the use of PBS as the release medium to regulate the drug release. It was prepared using 0,2 M monobasic and 0,2 M dibasic sodium phosphate to achieve pH 7.4 at 37 ºC [3]. A centrifugation process followed by a drying process at 40 ºC during 4 h was carried out to obtain microspheres ready for drug release experiments. The PLA/PLGA microspheres were characterized by Zeta-sizer Nano ZS to assess their size. FT-IR analysis was used to assess the functional groups present in the PLA/PLGA microspheres. SEM was used to observe the shape and to corroborate the microspheres formation. Thermogravimetric analysis (TGA) was performed from 30 to 700 ºC under nitrogen atmosphere on ibuprofen, PLA/PLGA microspheres-Ibuprofen, PLA/PLGA microspheres-Ibuprofen after the drug release experiment in PBS media and PLA/PLGA without ibuprofen. It was found that the particle size of microspheres could be easily controlled by varying the different parameters ([PVA], [PLA/PLGA] and emulsion speed stirring). It was also proved that drug release could be controlled by varying the particle size. Figure 2 shows the variability of the size distribution with different emulsion speeds (12.000 and 16.000 rpm). Likewise, the comparation of the size distribution by varying the PVA and PLA/PLGA concentration were studied. The overall results of this study showed that the formula using 25 wt.% PLA and 75 wt.% PLGA, 1% w/v of PVA and 12.000 rpm of stirring speed produced the microspheres with the most uniform size distribution of 450-550 nm. Figure 3 shows PLA/PLGA-Ibuprofen drug release profiles determined by UV-Vis spectroscopy. Three types of microspheres were prepared for this study by the same method but with different concentrations of PLA and PLGA. Results indicate that if the size distribution is controlled, better release profiles are obtained. The microspheres with the most uniform size distribution (25 wt.% PLA and 75 wt.% PLGA, 1% w/v of PVA and 12.000 rpm of stirring speed) exhibit lower release rate achieving its full release at about 60 min. Instead, the drug release profile of 50 wt.% PLA and 50 wt.% PLGA microspheres and 75 wt.% PLA and 25 wt.% PLGA microspheres, which have a worse size distribution, achieve its full release at about 10 and 15 min, respectively. In Figure 4, first derivative of TGA (DTG) was applied to obtain degradation curves, which state that after drug release, the ibuprofen degradation stage disappears, which indicates that drug release was completed while PLA/PLGA microspheres with PVA remains stable and exhibits a similar curve to blank PLA/PLGA microspheres. Figure 5 shows the SEM images of PLGA/PLA microspheres with ibuprofen after the drug release experiment in PBS media and the blank PLGA/PLA microspheres, which help us to corroborate that PLA/PLGA microspheres with PVA remains stable after the drug release.

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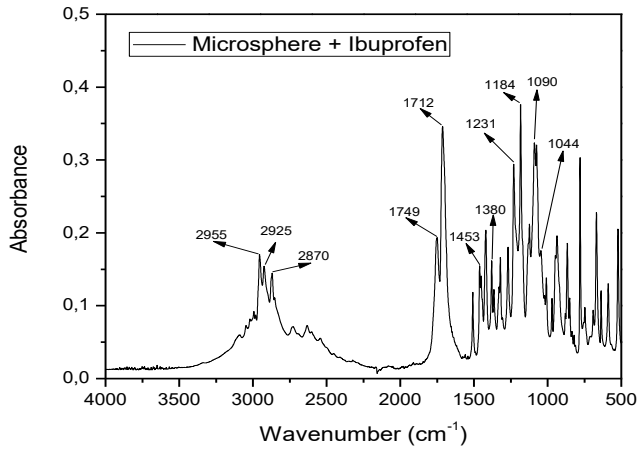

Figure 1. FTIR of PLA/PLGA microspheres

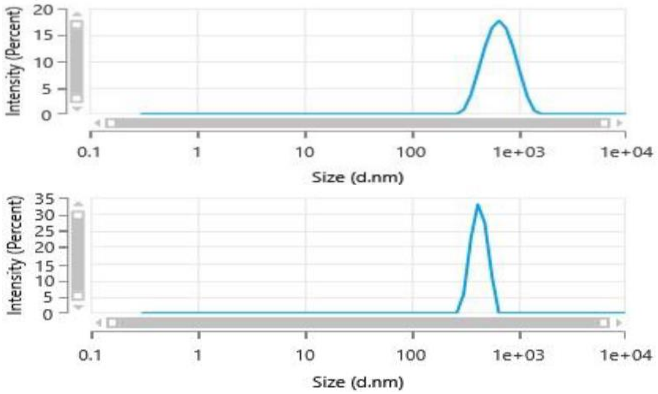

Figure 2. Comparation between the size distribution of two similar PLA/PLGA microspheres with different emulsion speed stirring. **(A)** 16.000 rpm. **(B)** 12.000 rpm.

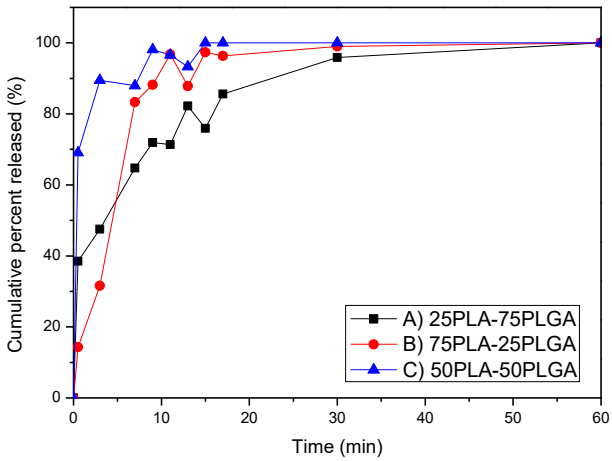

Figure 3. Comparation between the ibuprofen release profiles of PLA/PLGA microspheres with different PLA and PLGA concentrations. **(A)** 25%PLA and 75%PLGA. **(B)** 75%PLA and 25%PLGA. **(C)** 50%PLA and 50%PLGA.

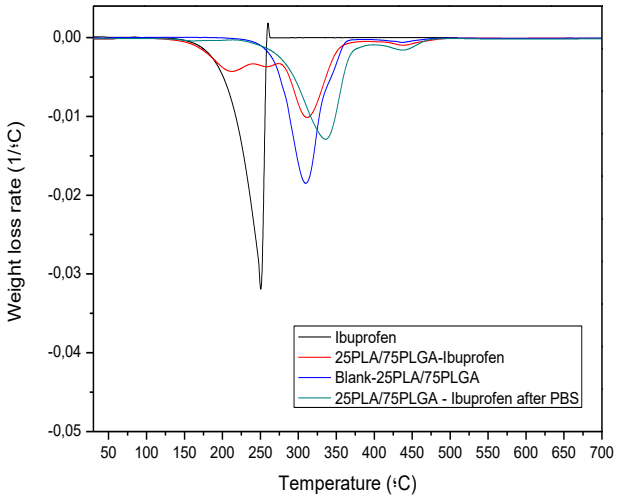

Figure 4. DTG of Ibuprofen, PLA/PLGA microspheres-Ibuprofen, PLA/PLGA microspheres-Ibuprofen after drug release experiment in PBS media and blank PLA/PLGA microspheres.

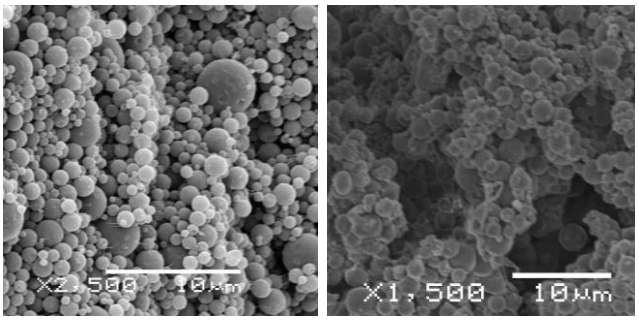

Figure 5. SEM image of blank PLGA/PLA microspheres (left) and SEM image of PLGA/PLA microspheres with ibuprofen after the drug release experiment in PBS media (right).

Magnetite Nanoparticles a Key player in Alzheimer's Disease

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Abstract

The accumulation of iron in form of magnetite nanoparticles with amyloid peptide is a key process in the development of Alzheimer's disease (AD) [1]. However, the origin and role of magnetite inside the AD brain is still unclear [2].

We have investigated the interaction between these important players in AD with superconducting quantum interference, scanning electron microscope, surface plasmon resonance, and magnetic force microscopy [3]. The results support the notion that the magnetite-Amyloid β complex is created before the synthesis of the magnetic nanoparticles, bringing a highly stable interaction of this couple. The capacity of amyloid peptide to bind and concentrate iron hydroxides, the bases for the formation of magnetite, benefits the spontaneous synthesis of these nanoparticles thanks to the catalysis of amyloid peptide [4]. This symbiotic interaction between amyloid and magnetite helps in the Fe^{2+} stabilization as well contributes to the aggregation of amyloid monomers to fibrils. A neuronal culture model was used to monitor the changes in spontaneous activity and effective connectivity in amyloid-magnetite affected cultures. The activity of controls was compared in parallel with magnetite, amyloid and magnetite-amyloid complex. A clear spontaneous degradation of neuronal activity is only observed with the amyloid-magnetite dose, disrupting neuron network communities [5].

Our results demonstrate that magnetite nanoparticles have a more prominent role in this disease, than the previously assigned in the literature, which could help in a better understanding of this neurodegenerative disease and in the development of new AD treatments.
Single-spaced and a single paragraph

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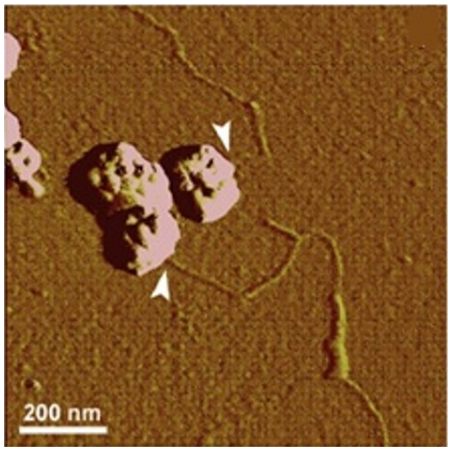

Figure 1. Image from Magnetic Atomic Force Microscope of Amyloid fibril grown up with the generated magnetite nanoparticles [3]

Polyethyleneimine as a versatile coating/reducing cationic polymer for metallic nanoparticle-based cancer theranosis

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Polyethyleneimine has been long known for its efficient DNA/RNA molecule delivery intracellularly due to the so-called ´sponge effect´. However, the potential of PEI as coating and reducing agent in nanoparticle synthesis/stabilization and its implication in nanomedicine in terms of intrinsic biological activities has only been addressed in the last years. Within this scenario, we have thoroughly characterized the chemical, physical, and biological contributions of PEI when coating iron oxide and gold nanoparticles. First, we studied the biological implications of the polymer as coating layer of superparamagnetic iron oxide nanoparticles (SPIONs) by assessing the effect that PEI-coated SPIONs have on mononuclear cells (macrophages), tumor cells, and endothelial cells [1-2]. PEI-coated SPIONs activated macrophages (pro-inflammatory cytokine secretion, pro-inflammatory gene expression, cytoskeleton modulation) mediated by TLR-4 engagement and ROS production, as pro-inflammatory response was inhibited when macrophages were pre-treated with TLR-4 inhibitor and ROS scavengers. In addition, PEI-coated SPIONs skewed endothelial cells gene expression profile toward an activated status. Surprisingly, PEI-coated SPIONs also exhibited anti-migratory effect on pancreatic tumor cells by modulating invadosome dynamics and, thereby, extracellular degradation ability, altogether suggesting an anti-metastatic potential [3]. Likewise, PEI-coated SPIONs impaired endothelial cell migration and invasion by affecting cytoskeleton dynamics, both *in vitro* and *in vivo*, suggesting their use as anti-angiogenic agent in cancer therapy. We then characterized the potential of the cationic polymer as a template for Au^{3+} reduction into gold nanoparticles (AuNPs) with a promising theranostic potential for colon carcinoma treatment [4]. PEI did also proved efficient in reducing and further stabilizing colloidal gold nanoparticles (AuPEI) into quasi-fractal nano-assemblies. These quasi-fractals showed a good plasmon-coupling facilitating NIR photothermal therapy and photo-acoustic imaging of colorectal carcinoma. In summary, PEI proved to be a versatile and promising tool in cancer therapy endowing metallic nanoparticles with intrinsic properties, *e.g.*, anti-migratory/invasion, pro-inflammatory, and anti-angiogenic features.

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Figure

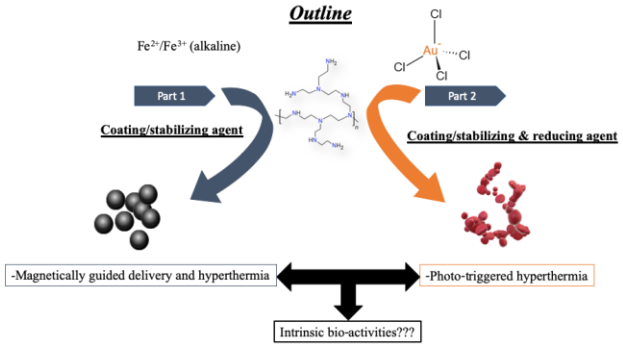

Figure 1. Synthesis routes for polyethyleneimine-based iron oxide and -gold nanoparticles and their exploitation in cancer theranosis

International nanomaterials for biomedicine projects reaching success with support of EU Research and Innovation Staff Exchange (RISE) programme

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What is MSCA and what is RISE?
The Marie Skłodowska-Curie Actions (MSCA) are part of Horizon 2020 (H2020), the European Union's Framework Programme for Research and Innovation (2014-2020).

The **MSCA** support researchers in all scientific domains, promote collaboration between the academic, scientific and business communities, boost the careers of scientists at all stages and develop excellent doctoral training in Europe and beyond through international and intersectoral mobility.

The current budget (2014-2020) under Horizon 2020 is 6.2 billion euro and represents around 8% of the overall H2020 budget. Since 1994, the programme has trained around 130,000 researchers — including 35,000 doctoral candidates —throughout their career [1].

RISE stands for "Research and Innovation Staff Exchange" and it is part of the Horizon 2020 family of **MSCA**. The budget for the next call (opening Dec 5th, 2019) is set for 80 million Euro.

What does RISE offer?
RISE funds short-term international and inter-sectoral exchanges of staff members involved in research and innovation to develop sustainable collaborative projects between different organisations. It helps people develop their knowledge, skills and careers and ensure the transfer of knowledge.

RISE enables staff mobility and also contributes in a flexible way to research and administrative costs. The administration is quite simple for our coordinators as the programme works by "unit costs" (Figure 1).

Who applies?
International networks of research organisations from the academic and non-academic sectors.
A RISE consortium consists of at least three independent participants in three different countries (two must be EU Member States or Associated Countries).

Who is funded?
Researchers, technical administrative and managerial staff of the participating organisations, of any nationality and at all career levels.

What type of research project proposals?
The work can be basic research and/or applied research leading to publications, patents, software tools, training materials, prototypes ready for market, etc....

Exchanges (secondments) are open to all countries and nationalities. Within Europe, the special feature of RISE is that exchanges eligible for funding need to be between academia and industry.

Proposal applications are made within one of eight specific panels (i.e. Physics, Mathematics, Life Science, Engineering, Chemistry, and Economic and Social Sciences).

Within the research projects proposals the applicants also show how their work will meet the more general objectives of MSCA and RISE [1]

Which types of organisations?
All organisations involved in research and innovation activities, academic and non-academic (e.g. SMEs, NGOs, museums, hospitals, etc.), are potential participants.

RISE funds exchanges of research staff between academic and non-academic organisations (inter-sectoral secondments) and between EU Member States / Associated Countries and other organisations located in the rest of the world (international secondments). The projects are normally lasting 4 years with a maximum number of exchanges of ~540 person months (~2.5 million EUR).

Examples of Success Stories in field of nanomaterials and biomedicine?
Three recently funded MSCA-RISE projects [2-4] will be highlighted in the presentation. The projects are in under the "Physics" panel and focus on nanomaterial applications in biomedicine (dental implants and cancer detection and therapies). The consortia consist of ~33 organisations from Austria Czech Republic, Estonia, France Italy, Latvia, Lithuania, Poland, Spain, Portugal, United Kingdom, Argentina, Chile, Belarus, Brazil, Ukraine, United States. One third of these organisations are SMEs.

The science is exciting! From the perspective of the Marie Sklowdowska-Curie programme what is also important about these projects is the difference they make to the career development of the researchers involved and the special collaborations between

industry and academia, within Europe and across the globe.

These projects are a proof of concept that innovation comes from mobilizing scientists between countries, between sectors, and between the fields of physics, chemistry, biology, and medicine.

How to apply?
Submit your application in response to a call for proposals through the Funding & Tenders Portal – where you will find the Work Programme and the Guide for Applicants. Designated National Contact Points are there to support you to make a successful application [6]

Discussion for NanoBio&Med participants
In this presentation, Dr. Ozin-Hofsaess – MSCA RISE Research Programme Administrator/ Panel Coordinator for Physics and Mathematics projects - will share her experience working with these scientists to reach their goals and the aims of the MSCA RISE programme.

She will inform and inspire the audience how they can make a strong application in the next and last RISE call - *opening Dec 5th and close date April 28th, 2020* - of the programme as part of the Horizon 2020 framework programme of the European Commission.

Also the other MSCA opportunities for funding (Figure 2). A preview of what is in store for RISE in the next framework programme, Horizon Europe, will also be presented.

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[6] National contact points https://www.net4mobilityplus.eu/msca-ncp/

Figures

Figure 1. Summary of budget for MSCA RISE projects. For each month that the researcher staff is mobilized they received funds for travel/accommodations, research, training, and networking and also management overheads.

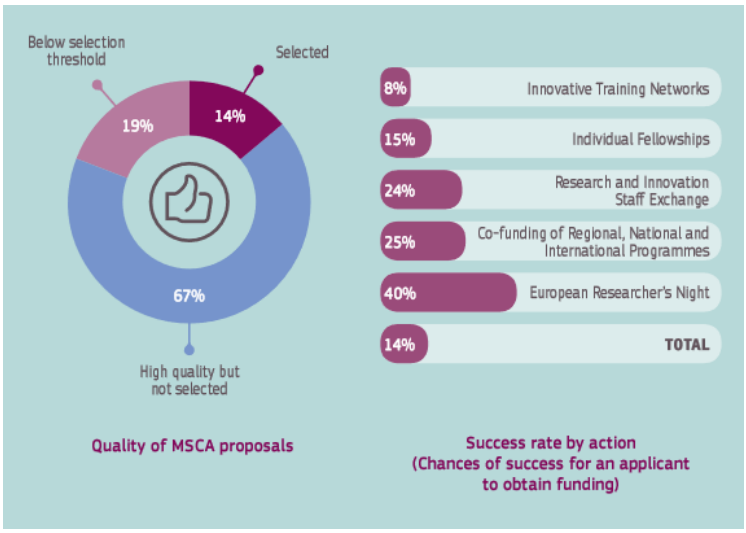

Figure 2. The MSCA are highly relevant and attractive, as shown by a growing number of high-quality proposals. However, many cannot be funded due to a lack of funding: proposals submitted under Co-funding of Regional, National and International Programmes; Research and Innovation Staff Exchange and European Researcher's Night are most likely to be funded, whilst there is a high demand for Individual Fellowships and Innovative Training Networks [1].

3D multiplex, paper-based rapid diagnostic tests using plasmonic gold nanoprobes

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Abstract

Plasmonic properties of gold nanoparticles are a promising tool to develop sensing alternatives for the traditional, but disadvantageous, enzyme catalyzed reactions and other biological recognition elements in biosensors. The need for sensing alternatives, especially in under developed areas of the world, has given rise to the application of non-enzymatic sensing and quantification approaches to biochemical analysis. Because of environmental and cost disadvantages of enzymatic reagents, metallic nanoparticles, with higher focus on the gold ones, have been synthesized and modified with the purpose of achieving sensitive probes for relevant health biomarkers over the years. The integration of these new recognition elements into versatile platforms, such as paper, gives the opportunity for the development of new, easy-to-use, low-cost, sustainable diagnostic tools. More specifically, the pairing of paper µfluidics and gold nanoparticles is a promising approach for the development of point-of-care devices, which can be used together with information technologies, such as smartphone embedded cameras and cloud computing, to develop complete systems to be used in under developed areas, for various health applications. Here, we present three individual, low-step, wet-chemistry assays, for each of the target health markets (free cholesterol, glucose and uric acid), and the fabrication and calibration of a µfluidic, multiplex, paper-based device, to serve as a platform for said assays. We found that the optical properties of differently produced AuNPs are influenced by the presence of the target analytes, by changes in size, conformation and interparticle distance, both in colloidal gold mixtures and in AuNPs immobilized in a chromatography paper substrate. The direct influence of glucose, in different quantities, towards the reduction of a gold salt, in alkaline medium, is used as a sensing approach [1], while for uric acid and cholesterol sensing, the introduction of molecules with thiol moieties, for surface modification and functionalization, was the approach. The use of digitonin [2] and 2-thiouracil (2-TU) [3], which present affinity towards cholesterol and uric acid, respectively, allows us to tune optical properties of AuNPs, depending on analyte concentration. In the case of glucose and uric acid, the interaction between nanoparticles and increased concentrations of the analytes cause a blue-shift, related to diminishing sizes of AuNPs, resulting in optimal sensitivity ranges of measurement (1.25 – 50 mM for glucose, 0 - 5 mM for UA, both including the clinical measuring range), while for cholesterol, interactions produce an increase in interparticle distance and consequent decrease of optical density. UV-Vis spectrophotometry, dynamic light scattering (DLS) and transmission electron microscopy (TEM) were used to determine the influence of the biomolecules in the plasmonic properties of AuNPs and the changes in size and conformation, in addition to applying Raman spectroscopy and other characterization techniques to study AuNP properties.
The studied AuNPs were posteriorly translated to the chromatography paper substrate, by two different approaches, for the creation of lab-on-paper applications: (i) consecutive deposition of reagents for direct synthesis of AuNPs into the paper substrate (for glucose sensing); (ii) direct deposition of surface modified and functionalized AuNPs, to serve as gold-based probes (for free cholesterol and uric acid sensing). We found that for the sensing of glucose, direct synthesis of AuNPs result in increasing color intensity of pink/red AunPs, because different amounts of particle are produced. This color intensity can be correlated to glucose concentrations, using digital, colorimetric calibration employing different color spaces, mainly RGB. For uric acid sensing, 2-TU functionalized AuNPs deposited into the paper substrate are influenced by the introduction of UA by color change indicative of anti-aggregation effects (from blue/purple to red), resulting in digital color calibration with a linear correlation and a sensitivity range between 71/87.5 µmol/L (depending on the color space used for calibration) and 1 mmol/L, which includes the clinical range of measurement. For cholesterol sensing, digitonin functionalized AuNPs (DAuNPs) interact with cholesterol in the paper substrate, to cause a decrease in color intensity, correlated to cholesterol concentrations using the HSV color space.
With the purpose of developing a platform to host the paper-based, colorimetric assays, a µfluidic, multiplex device is presented, for simultaneous, multiparametric sensing of the target health markers, resulting in a low-cost, low-complexity tool for diagnostics in low-resource areas of the world.

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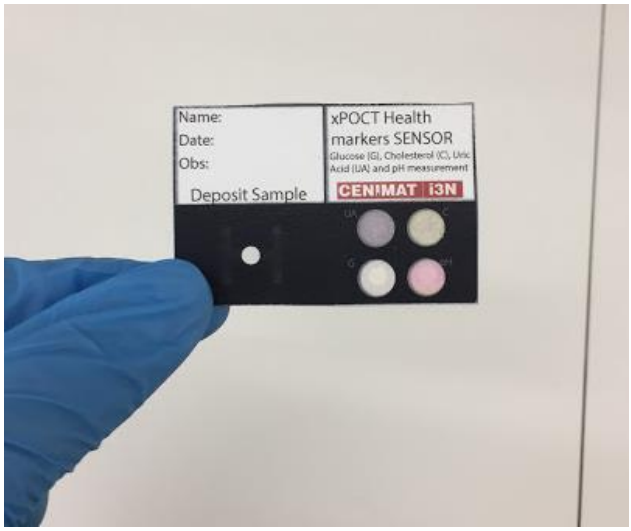

Figure 1. Real-size image of paper-based, 3D multiplex platform for screening of glucose, cholesterol and uric acid.

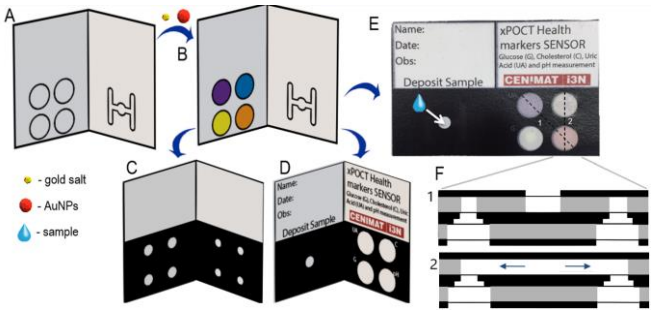

Figure 2. Schematic Illustration for assembly and operation of 3D µPAD.

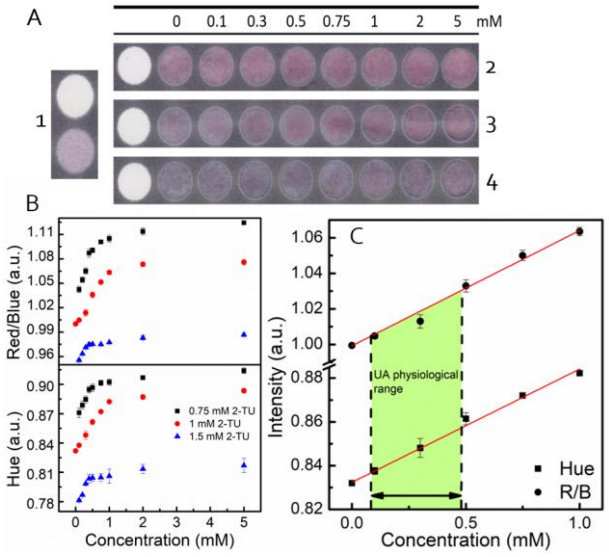

Figure 3. Paper-based assay of uric acid colorimetric behavior and color calibration.

Ultra-sensitive and multiplexed detection of protein biomarkers using Mecwins technology

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AVAC technology is an innovative biosensing platform for the ultra-sensitive and multiplexed detection of protein biomarkers. The fundamental principle of this innovative technology is based on the optical detection of plasmonic nanoparticles amplified by the multi-dielectric substrate underneath [1,2].

The immunoreactions take place on a multi-dielectric substrate coated with antibodies specific to the biomarker of interest. Once the sample has been incubated, a specific antibody tethered to the surface of plasmonic nanoparticles recognizes the biomarkers of interest (see Figure 1). As each plasmonic nanoparticle binds specifically to the biomarker of interest, counting the number of nanoparticles allows to quantify the number of biomarkers immobilized on the surface.

The nanoparticle detection is performed with the AVAC scanner, a proprietary platform that allows the simultaneous measurement and analysis of plasmonic nanoparticle with ultra-high throughput [3,4]; the complete measurement and analysis of a 96-well plate takes less than 5 minutes. The plasmonic nanoparticles are first optically identified, and then the scattering spectrum of each individual nanoparticle is analyzed in order to characterize, classify, and finally count the particles. The combination of different nanoparticle parameters such as brightness, spectral information or polarization state allows a very high specificity, and results in a very low limit of detection in the femtogram range, and provides the capability of detecting several biomarkers within the same sample (multiplexing). In combination with the biosensing platform, Mecwins has developed a cartridge prototype, which is compatible with sample handling systems routinely used in hospitals and analytical laboratories.

Our proprietary detection technology is up to one million times more sensitive than ELISA technology, which has been the standard for protein detection for over forty years. This innovative AVAC technology provides a solution for those diagnostic situations where ultra-sensitive detection is needed; moreover, this technology is also suitable for point-of-care (POC) applications. AVAC technology has proven extraordinary sensitivity with many established biomarkers in clinical use for diagnosis, monitoring, and prognosis, with applications in oncology, myocardial infarction, rheumatoid arthritis, and infectious diseases. An example of the extraordinary capabilities of the technology is demonstrated with the p24 immunoassay (Figure 2), which exhibits a sub-femtogram sensitivity in human serum samples.

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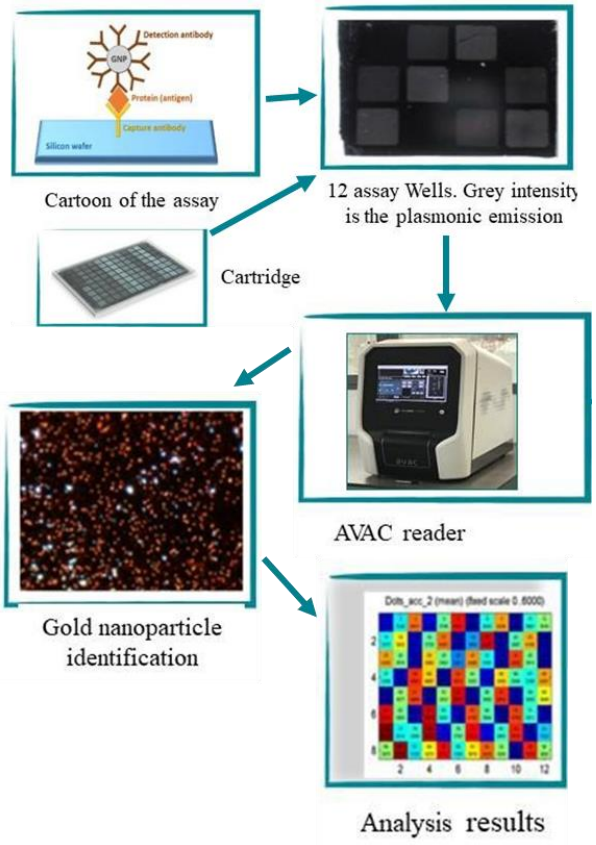

Figure 1. Workflow of the entire AVAC assay process.

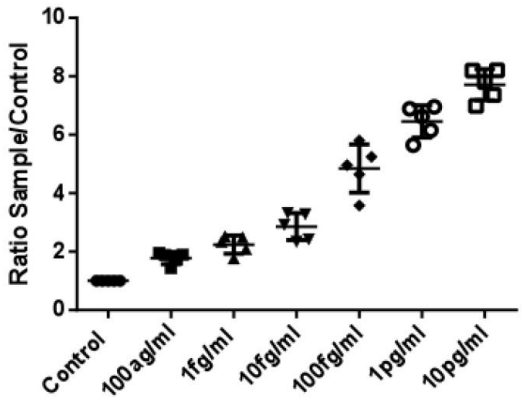

Figure 2. Calibration curve for Mecwins' p24 immunoassay in human diluted serum; data are represented as ratio sample/control.

In vivo biodistribution of siRNA-supported by polymer nanoparticles using Positron Emission Tomography (PET) imaging

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After the emergence of nanotechnology, many different types of nanoparticles (NPs) are being used in the biomedical field to encapsulate drugs that are not suitable for direct delivery. Several strategies have been used for the encapsulation of drugs using NPs, where the drugs can be linked to the core or surface of the NP by covalent, electrostatic or hydrogen bonding interactions. In recent years, small interfering RNA (siRNA) has emerged as a promising treatment strategy for various genetic diseases. However, siRNA is readily degraded after administration into the blood stream. Such degradation can be retarded by using appropriate drug delivery systems. One possible strategy is the use of NPs based on the self-assembly of polyamines and phosphate ions. Here, we describe the use of poly(allylamine hydrochloride) (PAH) as a carrier of siRNA and the application of radiollabelling followed by Positron Emission Tomography (PET) imaging to study its biodistribution *in vivo*. The ^{18}F-labelled SiRNA was prepared by the reacting the amino group-modification of siRNA with 6-[^{18}F]fluoronicotinyl-2,3,5,6-tetrafluorophenyl ester ([^{18}F]FPy-TFP), which was prepared as previously reported [1]. *In vivo* PET Imaging studies in rodents after intravenous administration clearly showed a different biodistribution between free [^{18}F]-siRNA, which was rapidly eliminated via urine, and PAH/[^{18}F]-siRNA nanoformulations, which showed significant accumulation in the liver, the spleen and the lungs (Fig. 1).

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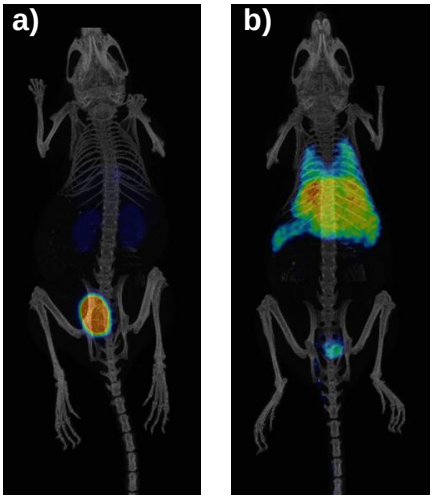

Figure 1. In vivo biodistribution studies of a) [18F]siRNA, and b) PAH/[18F]SiRNA in healthy mice using PET-CT. Images show the biodistribution after intravenous administration at time 30 min.

Photometric-based biosensor for PKU and control unit miniaturization for point-of-care diagnostics

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Phenylketonuria (PKU) is a metabolic disease resulting from a deficient enzyme (phenylalanine hydroxylase) and consequent build-up of dangerous levels of an amino acid, L-Phenylalanine (L-Phe), in the blood.[1] If left untreated, PKU can lead to neurodegenerative symptoms, which can be prevented by following a diet low in L-Phe.[2] Thus, early detection of PKU in newborns is essential. Screening and monitoring of the disease is currently centralized at the site of patient care, which generally requires specialized and costly equipment, as well as qualified personnel to perform the diagnostics.[3,4] In this work, we present an enzyme-based qualitative photometric strategy to accurately diagnose PKU. It consists of an assay performed in a 96 well microplate, previously functionalized to specifically detect L-Phe. Measurements are performed by a microplate reader, which allows the samples of up to 48 patients to be analyzed simultaneously within a matter of hours and at the site of patient care. The measurements are fast, versatile, low-cost and easy to carry out. The presented diagnostic system has been validated with anonymous real plasma samples provided by the hospital Sant Joan de Deu (HSJD), accurately discriminating healthy from diseased patients.

Moving forward, a more portable point-of-care (POC) device is also envisaged which aims to miniaturize the control unit for electrochemical detection or integrate a smartphone with the photometric sensor. With this goal in mind, we are developing a compact stand-alone benchtop photometric sensor with wifi and bluetooth connectivity, allowing real-time plotting on a mobile phone. In addition, a low-cost miniaturized potentiostat has been designed and assembled for a more wearable/portable electrochemical sensing platform with state-of-the-art performance.

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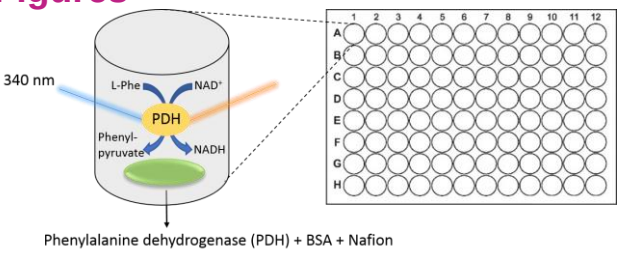

Figure 1. Schematic representation of the detection mechanism of the photometric platform for phenylalanine detection. The bottom of the microplate wells is modified with a mixture containing phenylalanine dehydrogenase (PDH), an enzyme that catalyzes the conversion of phenylalanine into phenylpyruvate in the presence of NAD^+ cofactor. The production of NADH (the reduced form of the cofactor) is monitored by measuring the absorbance at 340 nm.

Natural proteinaceous nanoparticles for theranostic purposes

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Natural protein nanoparticles are presented as a great alternative for the development of tools in the biomedical field, providing advantages in terms of biosafety and biocompatibility. This kind of These nanomaterials has been are being used as diagnostic tools, drug delivery systems, vaccination vehicles, among others; so they are considered as a new set of theranostic tools. In this context, viral-derived nanoparticles, specifically those derived from plant viruses, offer an extra of biosafety because they are not human or animal pathogens, as well as interesting structural characteristics for tool development, and also cost-economy by plant molecular farming. This is the case of Turnip mosaic virus (TuMV), a potyvirus with whose particles exhibit a flexuous elongated structure which allows the development of functionalized nanoparticles with up to 2000 copies of different compounds with a biomedical interest.

Derivatization by chemical conjugation to different amino acid residues, located in specific regions of the particle surface [1], has allowed a designed functionalization of these particles depending on the application. This functionalization pathway strategy has allowed to conjugate different compounds, such as:

- Peptides with a diagnostic value, in this case related with an autoimmune inflammatory disease.
- Small proteins, like PruP3, a peach allergen with a molecular weight of 9 kDa [2].
- Fluorophores, for bioimagen diagnosis.
- Natural compounds with biomedical properties: antimicrobial, antitumoral and/or antiviral.

These functionalizated VNPs would be are used in different applications, such as antitumor treatments, antimicrobial systems, diagnosis of autoimmune diseases, or theranostic tools for food allergies.

Other approaches consisted of functionalization through genetic fusion of peptides to the virus coat protein (CP), rendering peptide-coated nanoparticles, deployable as tools for immunization [3], or for high sensitive diagnosis [3,4]; or the development of nanonets with immobilized enzymes, which has been shown as a possible potential useful tool in the pharmaceutical industry [5], allowing greater yields, for example, in racemic mixtures resolution.

All these advances are opening doors to the multifunctionalization of viral nanoparticles, where different functionalities are presented together and simultaneously in the same nanoparticle.

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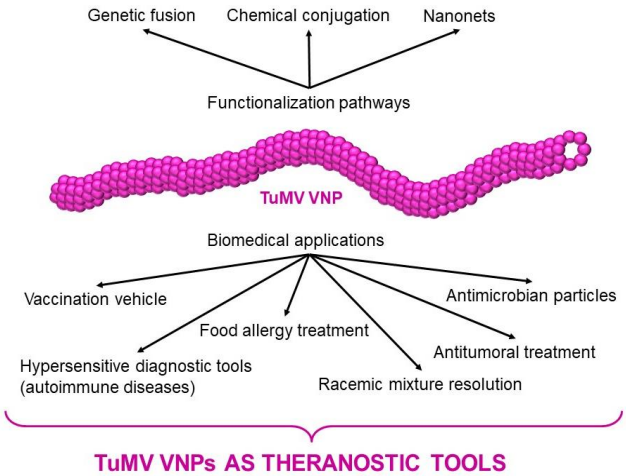

Figure 1. Functionalization and application of TuMV VNPs in the biomedical field as theranostic tools.

Posters

Mechanical properties of aligned PCL nanofibers made by electrospinning

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Abstract

The importance of mechanical properties of artificial scaffolds for cell growth is particularly obvious in tissues such as for bone, cartilage, blood vessels, tendons and muscles. Polycaprolactone (PCL) is a very convenient polymer for such purpose due to its low degradation rate and good mechanical properties.[1] Here we report on the preparation and mechanical characterization of aligned PCL nanofibers produced by electrospinning stabilized with a co-flow jacket of solvent vapor.[2] The electrospinning setup is shown in Figure 1. By using aligned nanofibers,[3] mechanical properties of a single fiber were computed. The dissolution of PCL was done in a chloroform-methanol (3:1,v/v) mixture.[4] The average diameter of the PCL nanofibers was determined from their sizing in Scanning Electron Microscopy (SEM) images. Dynamic Mechanical Analysis (DMA) was carried out on samples containing thousands of identical nanofibers. As the fibers are aligned and identical, the mechanical tests performed on each sample can be used for computing single fiber average properties. The aligned fibers and the methodology which was followed are shown in Figures 2 and 3. The elastic modulus was obtained by computing the stress for a single PCL fiber. The average tensile elastic modulus and ultimate tensile strength for our neat-PCL nanofibers (Figure 4) was determined as 139 and 41 MPa, respectively. This elastic modulus value lies between that for bulk PCL (343.9 to 363.4 MPa) and the value previously reported for electrospun PCL nanofibers (27.3±3.1 MPa).[1],[5]
Given the broad material versatility of electrospinning, the proposed method can be extended to many other nanofiber compositions.

Acknowledgements

This project has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No. 713679, from the Catalan Government (2017SGR1516), and from the Universitat Rovira i Virgili (URV).

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Figures

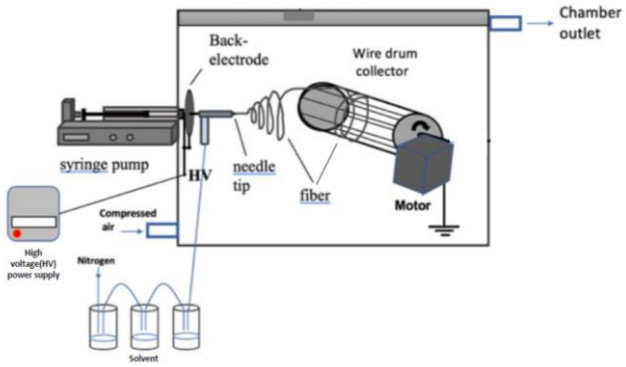

Figure 1. Electrospinning setup

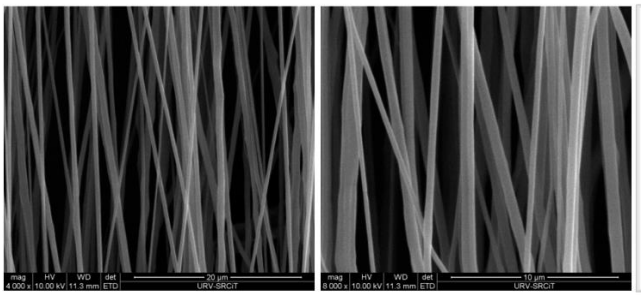

Figure 2. SEM images of aligned PCL nanofibers.

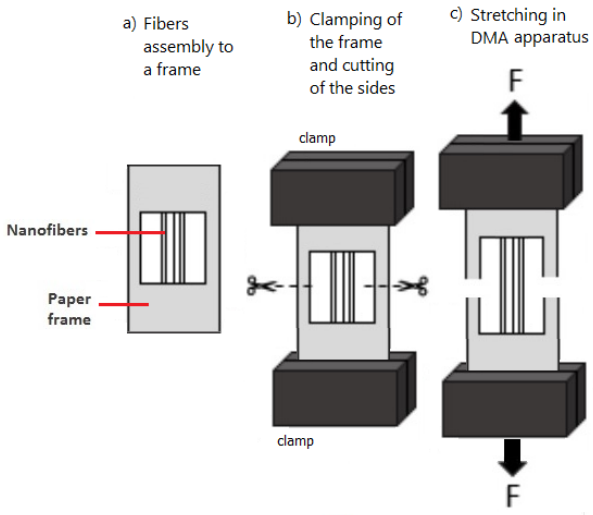

Figure 3. Mechanical testing of the aligned nanofibers. Assembly on DMA clamps (a) and (b) ; DMA test (c).

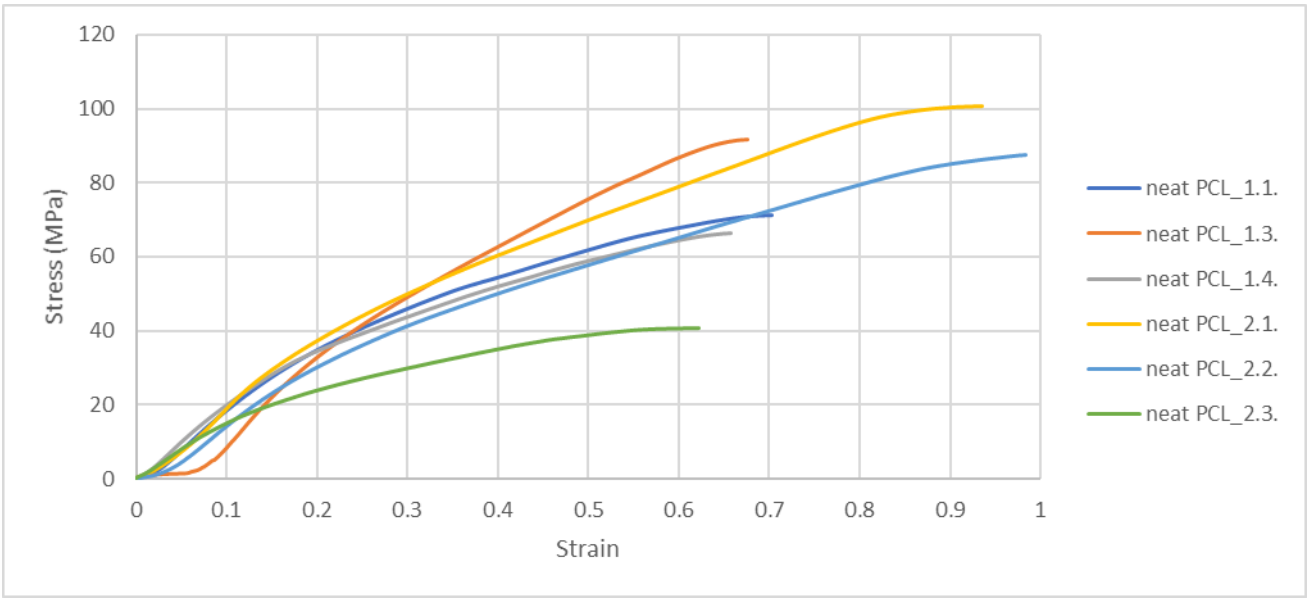

Figure 4. Tensile Test Curves of aligned PCL fiber mats.

Design of multivalent polymersomes for range-selective binding

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The success of nanomedicine is nowadays strictly associated to the ability of selectively targeting the site of interest: the more specific a therapy is, the more likely it is to be efficient.

The use of multiple ligands, i.e. multivalency, with low affinity for their receptors has proved to successfully address this 'selectivity' requirement [1]. In fact, thanks to the ligand-receptor simultaneous interactions, the collective binding (avidity) compensates for the low affinity and generates a switch-like behaviour where the binding occurs only above a certain receptors density [2].

This mechanism, widely spread in nature for the mediation of several aspects in cell biology [3,4], has recently inspired the design of several drug delivery polymeric nano-constructs functionalized with suitable ligands to guide the carriers according to the level of receptors expression and to deliver their cargos where most needed [5].

Polymersomes, self-assembled amphiphilic diblock copolymer vesicles, have been widely used as nano tools for drug delivery purposes thanks to their versatility and increased stability within biological environments. A recent research carried out on pegylated polymersomes has shown, through cellular uptake studies, that these nanovesicles can display a superselective behaviour by tuning the effective contribution, in terms of chemical potential, of different parameters including the polymer brush length, particle sizes, ligands number [6].

Taking inspiration from these results, the main purpose of the present study was to explore a new type of selectivity where multivalent polymersomes only bind targets when the receptor density is within a certain range. To this aim, a statistical mechanical modelling study was firstly carried out in order to characterise the region where to expect the range selective targetin. Then, Angiopep2-decorated polymersomes were prepared by a solvent switch approach. The formulations were characterised by DLS and TEM and binding studies between functionalized polymersomes and highly-expressing LRP1 FaDu cell line were carried out through fluorometer and laser confocal scanner microscopy.

Understanding the behaviour of the modelled system will significantly improve the design of precise nano-devices with the ability of performing *ad hoc* selective transport and site targeting within cellular environment.

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Antifungal effect of pegylated graphene oxide and silver nanoparticles against candida albicans

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Abstract

Due to the global concern over antimicrobial resistance,[1] advanced materials are being studied for their possible antimicrobial properties. Nanoparticles (NPs) have been shown to have antimicrobial effect against certain pathogens. In our study, an initial antimicrobial screening of 13 different NPs standard materials against a series of harmful micro-organisms was carried out. Along with Copper (Cu) and Silver (Ag) NPs, certain Graphene oxides NPs were found to effectively counteract a fungal species, called *Candida albicans*.[2] With an aim to improve colloidal stabilities of this Graphene oxides (GO), hence to provide better opportunities to microbial exposure, surface modification of GO NPs was performed. As shown in **Figure 1**, new PEGylated products GO-PEG was synthesised *via* amide bond formation after the coupling reaction of GO and 4-arm-PEG5K-NH_2 in the present of 1-ethyl-3- (3-dimethylaminopropyl)carbodiimide hypochlorite (EDC HCl).

The physiochemical properties of both GO and GO-PEG were investigated using pH, zeta potential and Nanoparticle Tracking Analysis (NTA).[3] During the initial antifungal screening, all treated *C. albicans* cultures were visualized using an inverted microscope with an aim to capture interactions between the cells and nanoparticles. Biological staining and fixation techniques were applied prior studying morphological changes of affected cells using Scanning Electron Microscopes (**Figure 2**).

MIC values were obtained using broth dilution method where each absorbance was measured at λ 490 nm (XTT) in 96 well plates. Four different ratios of GO-based and Ag NPs combinations were used to determine synergistic anti-fungal effects. The results obtained shown the antimicrobial effect depend of GO-based materials /different Ag NPs ratio.

In addition, Molecular Dynamic (MD) simulations of the adsorption of Ag clusters on pristine GO and GO-PEG surfaces were carried out. The calculated adsorption energies from these models demonstrated that the introduction of PEG chains on the GO NPs strengthens the interaction between the surface and the Ag cluster.

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Figures

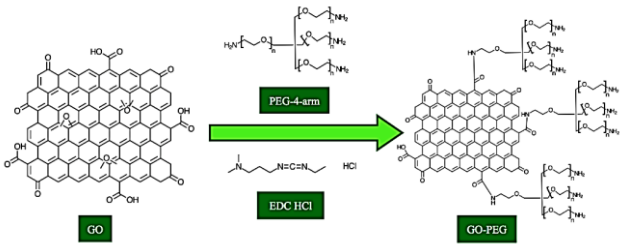

Figure 1. Synthesis of GO-PEG via coupling reaction

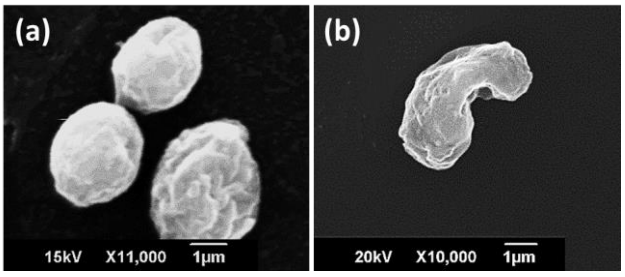

Figure 2. SEM images of (a) C. albicans control and (b) affected cell after NP treatment.

Intrinsic Enzymatic Properties Modulate the Self-Propulsion of Micromotors

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Bio-catalytic micro- and nanomotors self-propel by the enzymatic conversion of substrates into products.[1] Enzymes offer a combination of biocompatibility, bioavailability and versatility, making it a promising tool for certain biomedical applications.[2] Despite the advances in the field, the fundamental aspects underlying enzyme-powered active motion have rarely been studied, and need to be addressed to make implementations more feasible. We focus our research on the study of the fundamental aspects that rule the active motion of enzymatic micro- and nanomotors,[3] such as the role of the enzyme intrinsic properties.[4]

We explore the versatility of such systems by studying the powering capacity of a library of enzymes to propel silica-based micromotors: urease, acetylcholinesterase, glucose oxidase and aldolase. We study how their turnover number and conformational dynamics affect the self-propulsion, combining both an experimental and molecular dynamics (MD) simulations approach. Results show that the motion behavior is strongly dependent on the enzyme type.

We conclude that the conformational changes are a precondition for urease catalysis, and that the rate of catalysis is essential and directly related to active motion. Future research will be focused on studying the effect of extrinsic parameters such as the media properties and composition.

Figures

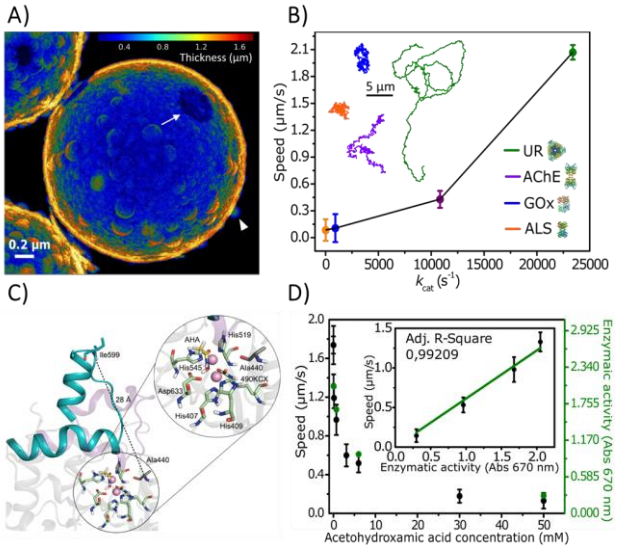

Figure 1. (A) TEM micrograph of a microcapsule colored depending on thickness. Hole detection (arrows) and silica bulks (arrowheads) detection. (B) Speed vs. turnover number (k_{cat}) of different enzymatic micromotors (urease, acetylcholinesterase, glucose oxidase and aldolase). Inset: Trajectories of the enzymatic micromotors. (C) Molecular dynamic simulation (MDS) of urease with acetohydroxamic acid (AHA) stabilizing the flap in wide-open state (teal). (D) Speed of UR-HSMM and enzymatic activity (Abs 670 nm) vs AHA concentration in 500 mM urea. Inset: linear correlation of speed and enzymatic activity.

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Curcumin-loaded PVP particles produced by electrospray

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Electrospray, also known as electrohydrodynamic atomization (EHDA), is a robust technique for producing uniform polymeric particles with controlled morphology and size. In this technique, a liquid pumped through a nozzle is electrically charged by applying high voltage. The electrified liquid adopts a conical shape (called Taylor cone) with a thin jet at its end. This jet then breaks up into highly charged droplets, which form a charged spray plume as they drift towards an oppositely charged substrate, where they are collected as solid particles after the solvent has evaporated from them. One of the advantages of EHDA over other liquid atomization methods is its ability to produce much smaller particles (in the few-microns and nano-metric size ranges) [1].

From the point of view of biomedical and pharmaceutical applications, it is desirable to produce size-monodisperse particles. Such particles are used as vehicles for drug delivery, where the release profile of an encapsulated drug is strongly influenced by the particle size; thus the importance to control particle size in the production method [2]. Whereas it is possible to produce homogeneous particles by electrospray, the droplets in the spray often undergo Coulombic instabilities, resulting in particles with non-ideal shapes, commonly elongated or pointed particles with nano-filaments attached [3].

The objective of this work is to produce homogenous polyvinylpyrrolidone (PVP) particles by electrospray. Particles with well-controlled morphologies and sizes of up to a few microns were imaged by scanning electron microscopy (SEM) (Figure 1).

We observed that the particle size is influenced strongly by the infused liquid flow rate. The initial polymer concentration and polymer molecular weight (MW) remarkably affect the morphology of the particles. With Mn 360 kDa MW, as the polymer concentration in the solution increases we observed a progression in particle morphology from filamented particles (due to Coulombic instabilities) to non-filamented particles, to filamented morphology (due to incomplete jet breakup).

The solvent system can also affects the particles morphology and size. In the case of PVP 40kDa, less spherical (more corrugated) particles with larger sizes were obtained when we used a solvent mixture of solvent (ethanol/acetone 50/50) than when we used ethanol as solvent.

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Acknowledgements

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Figures

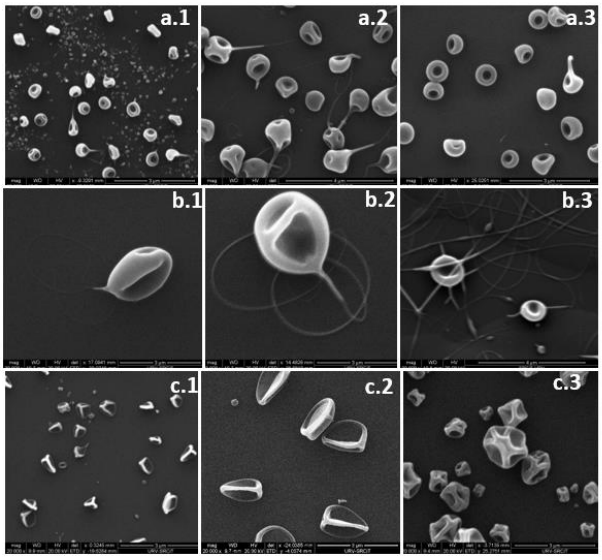

Figure 1. SEM images of PVP-curcumin microparticles of different morphologies collected on a silicon wafer.
(a) PVP 40 kDa and curcumin (0,1 %w) in EtOH at different polymer concentrations (by wt.): 1% (a.1), 5% (a.2), 10% (a.3).
(b) PVP 360 kDa and curcumin (0,1 %w) in EtOH, at polymer concentrations (by wt.): 1% (b.1), 1.5% (b.2), 3% (b.2).
(c) PVP 40 kDa and curcumin (1:1) in ethanol/acetone (50/50 v/v) at polymer concentrations (by wt.): 0.6% (c.1), 5% (c.2), 10% (c.3).

Water elimination by CO_2 cryospraying technology to obtain dry microparticles from biphasic, lipid dispersed systems

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Introduction

Variosol® cryospraying technology exploits the physical properties of rapidly expanding liquid or dense CO_2 [1] to perform as cryogenic agent (Joule-Thomson effect), while providing mechanical energy through a rapid pressure drop. This rapid expansion can induce phase change, solidification and atomization in a sprayed fluid, forming a dynamic mixture with the expanding CO_2 and transforming the liquid product into a micronized, dried powder. The main advantage of this technology is that it operates at relatively low temperatures and pressures, and does not require supercritical CO_2, thus reducing the use of energy-consuming elements, and making the process simple and efficient.

One peculiar feature of the expanding CO_2 cryospraying is the capability to eliminate significant amounts of water from biphasic disperse systems, during fluid atomization at low temperatures. This "dewatering" effect occurs when the product is sprayed under certain conditions of CO_2 pressure and temperature [2]. Presumably, the water contained in the atomized fluid product is rapidly cooled down and micronized with very high specific surface, and subsequently eliminated in the surrounding atmosphere (phase equilibria) leaving the spray tower with the exhaust gas. Therefore, it is possible to obtain solid, dry microparticles with a residual moisture of about 0.5 to 3%, and diameters below 100 µm, after atomization under dense CO_2 expansion [2,3]. This feature offers the possibility to formulate and produce lipophilic or amphiphilic micromatrices containing hydrophilic, water soluble molecules. The technology can also directly encapsulate natural extracts containing significant amounts of water, transforming them in formulated, micronized powders [4], without the need to reach supercritical CO_2 conditions, as it occurs in PGSS-drying technology [5].

The main objective of this study is to evaluate the impact of different process parameters on particles size, morphology and residual water content. Parameters such as pressure applied to sprayed fluid, nozzles type, diameters and geometry were investigated using aqueous disperse systems of lipidic excipients with amphiphilic properties. The results obtained could be used to explain and understand the key features of particles formation and dewatering phenomenon by CO_2 cryospraying technology. This will help us to optimize the physical properties of the microparticles and process water elimination capability.

Materials and methods

Mono, di- and triglycerides with medium to long chain fatty acids, and their amphiphilic derivatives (e.g. poly-hydroxylated fatty esters) were used for these studies. These materials were selected because of their known function in pharmaceutical preparations as solubilization or absorption enhancers, and as controlled release excipients. In addition, their relatively low melting point (typically ranging from 35 ºC to 75 ºC), combined with the rapid solidification properties at low temperatures, makes them suitable to formulate thermolabile molecules, and processable with the CO_2 cryospraying technology. Different operating conditions were applied in these studies. Gas pressure applied to sprayed material ranged between 1 to 4 bar, and fluid product temperatures were maintained between 55 ºC and 85 ºC, depending on the formulations. Nozzles producing capillary flow, with diameters between 0.36 to 0.85 mm were used to spray the fluid product. Two different types of dense CO_2 nozzles - full cone flow geometry or swirling flow pattern, were tested and compared. Dense CO_2 pressure was maintained between 48 and 55 bar. Microparticles were characterized by particles size analysis, Optical Microscopy, SEM and residual moisture content by Karl-Fisher Analysis or TGA.

Results and discussion

Spherical or spheroidal microparticles, with a matrix-type morphology were obtained at all the conditions studied. Depending on applied process conditions, the average diameters varied between 20 to 100 µm approximately, with narrow particle size distribution and good powders flowability. Optical Microscopy and SEM observation indicated that different surface features could be obtained, depending on excipients combinations and on applied processing conditions. Porosity and water residual areas observed by SEM suggest that the "dewatering" phenomenon is likely generated from water rapid freezing and subsequent evaporation during the spraying in the presence of CO_2.

Applying different conditions of pressure, temperature, nozzles diameter and geometry, we observed that effective water elimination to obtain dry microparticles can be achieved when the formulations to be sprayed contain up to about 35% water. Therefore, it is possible to produce dry microparticles by spraying, for instance, drug – containing

emulsions, microemulsions, and other complex systems such as fractions of natural extracts. Water elimination capacity and residual moisture in the particles also depend on the nature of the sprayed materials and their affinity to bind and retain water molecules or moisture. In fact, we observed how microparticles containing more hydrophilic excipients (e.g. polyhydroxylated – lipid derivatives) tend to retain more moisture. The dewatering effect may be enhanced by generating high surface area within the sprayed material, during the liquid CO_2 expansion. This can be achieved by introducing modifications in equipment design, while formulating disperse systems with smaller inner-phase droplets.

Conclusions

Cryospraying technology exploits the physical properties of rapidly expanding liquid-dense CO_2 to perform as cryogenic agent while providing mechanical energy through a rapid pressure drop. Such features allow to produce dry microparticles from biphasic dispersed systems, facilitating the elimination of water directly during the atomization at low temperatures.
Formulations including up to about 35% of water can be sprayed using the existing equipment design. It is thus possible to manufacture amphiphilic, composites microspheres containing combinations of different drugs or moieties, in a simple, one step process.

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Figures

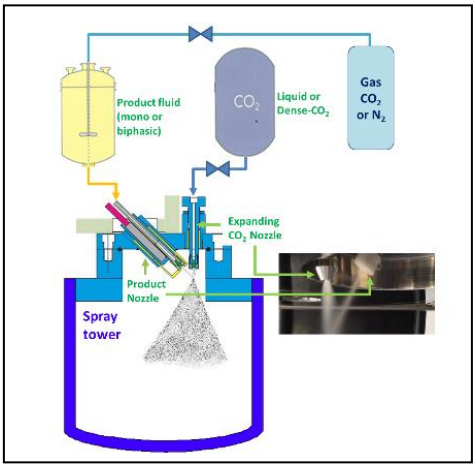

Figure 1. Scheme of dense CO_2 Cryospraying technology

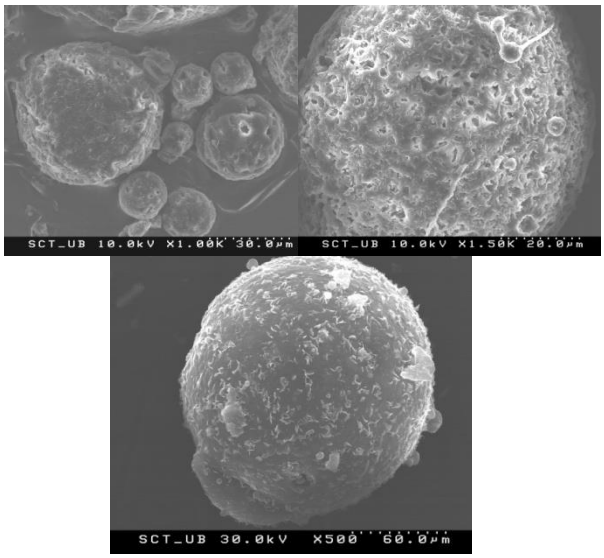

Figure 2. SEM Images of formulated microparticles with porous and not porous morphology

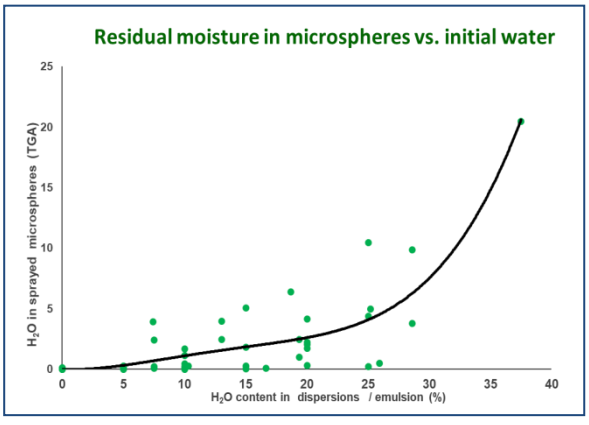

Figure 3. Residual moisture (%w) in microspheres (TGA) vs. water content in formulated emulsions before spraying

Coarse-grained model for water and water-protein interfaces.

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We study, by Monte Carlo simulations, a coarse-grained model for nanoconfined water that includes many-body interactions associated to water cooperativity, originally introduced by Franzese and Stanley (FS) [1]. The FS model is computationally efficient [2] and allows us to equilibrate water at extreme low temperatures *T* in a wide range of pressures *P* [3,4]. Moreover, it can be easily extended to include proteins, so we can analyze the behavior of the water-protein interface and how the solvent affects the protein configuration [5,6]. Here, we focus on the phase diagram of the FS model, showing that it reproduces, at least qualitatively, the experimental water phase diagram both in confinement and in bulk [7], a prerequisite for using it as a proper solvent in protein solutions. Our results compare well with atomistic simulations and show, for both confined (Figure 1) and bulk water, the presence of a low-density liquid and a high-density liquid water phases, separated by a liquid-liquid phase transition ending in a liquid-liquid critical point. Our results clarify fundamental properties of hydration and bulk water and are potentially useful for better understanding the effects of *T* and *P* on nano-bio-interactions among proteins and other species or interfaces.

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Figures

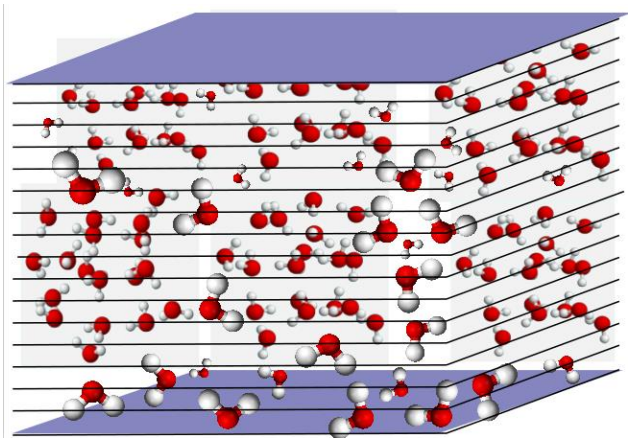

Figure 1. Schematic representation of water multilayers under nanoconfinement. We consider a system of 20 layers corresponding to a separation between walls of 6 nm.

Silver foams with hierarchical pores for antibacterial activity

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Filters are mainly used to physically block objects or substances, to remove impurities such as dirt, bacteria or oil from the flow. Since open-celled foams have high specific surface area and permeability property, they are often preferred for flow-through applications as filters in where these internal surface properties can be exploited. Foams can be used in virtually any filtration field as wet or dry filters thanks to their excellent active surface area and porous structure**.** [1, 2] It is very important that the foams have good mechanical properties as well as they allow for a highly stable flow during the filtration. [3] Especially open cell metallic foams, have received considerable attention in recent years as materials that find use in various engineering applications. They are also suitable for use in the field of filtration to reduce the concentration of undesirable ions dissolved in water. [4] In applications intended to reduce the concentration of dissolved ions in water, open cell metallic foams with high liquid / gas permeability are used which allow liquid flow through micro and macro pores. [2, 3] Foams have been regarded as a convenient candidate for air filtration, drinking water purification, antibacterial purposes and for industrial and environmental filtration applications and previously many studies have been made for these purposes. [5] Microbial contamination from microorganisms causes various problems in human life. It is impossible to create a completely sterile environment to prevent infections, but it is possible to take precautions to prevent the increase in the number of microorganisms. Bacterias are the most common and most serious infections of microorganisms. As is known, ions of metals such as copper, zinc and silver have a strong antibacterial effect. [6] In order to combine and take benefit of these advantages of ionic silver and cellular structure of foam, researchers have also been focused on studies to obtain materials which have uniform microstructures with micro and macropores and large internal surface area. Since the cellular structure of the foam can be controlled by porosity, there are several methods that can be used for the fabrication of open celled silver foams having micro and macro pores throughout the foam. [7, 8] The primary objective of this study is to produce antibacterial effective foam structures with replication method following by chemical dealloying. For this purpose, production was started with alloying aluminium and silver metal, and then it was used to manufacture foams. Two different route were used as 5% HCl and 20% NaOH solution in order to observe the effect of dealloying environment on the microstructure and pore size distribution [9]. The codes were used to define samples as: AgAl is the foam resulting from infiltration of a packed bed of preforms with liquid AgAl alloy, after dissolution of the NaCl particles (with no further chemical treatment); Ag-HCl is the foam after HCl treatment; Ag-NaOH is the foam after NaOH treatment; Ag-sint is a foam resulting from sintering. Foams were analyzed by SEM, interconnected-bicontinuous structures with hierarchical pore sizes ranging from nanoscale to micron scale were obtained with both dealloying route. XRD analysis was carried out to determine phases the samples contain after the dealloying processes. With the detailed pore size distribution study, the contributions of the different scale pores to the foam porosity which sourced from alloy microstructure, infiltration process and the dealloying process were determined. Nitrogen adsorption/desorption isotherms were used to determine surface area, pore volume and nano pore size distribution. Multi point BET method was used to measure surface area of the samples. Pore volume and nanopore size distribution was carried out by Quantochrom software. In addition to characterisations were mention above, to evaluate the performance of the foams as a filter, antibacterial efficiency was investigated according to ASTM E2149 against Gram Positive (S. aureus) and Gram Negative (E. coli) bacterias. The effect of dealloying process and pore size distribution on antibacterial performance of the foam were investigated. After 24 hours, the foam dealloyed with NaOH exhibited a superior performance compared to the other foams, providing 99.99% reduction in both bacteria species. These promising results have shown that this process combination allows to tailor the pore size, distribution and porosity according to the intended area of use.

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Figures

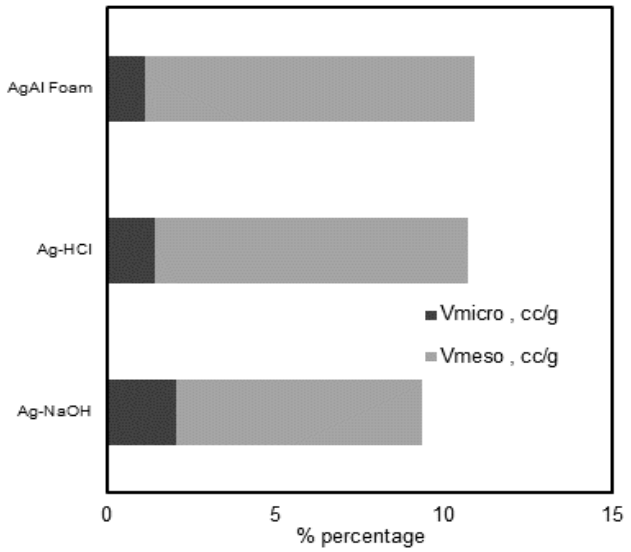

Figure 1. Contribution of micro and meso pores to total volume

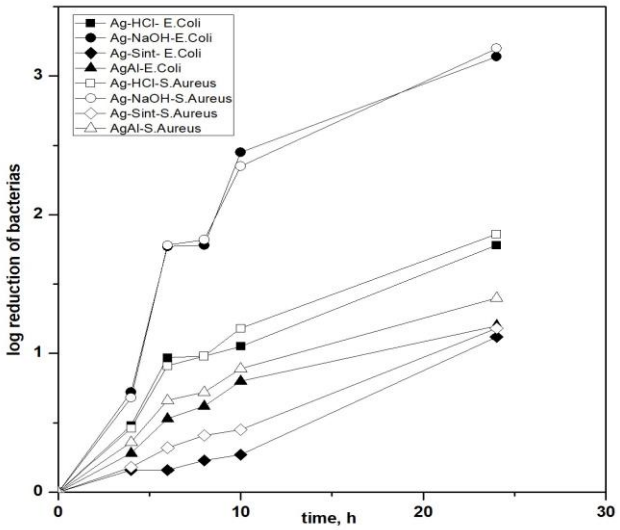

Figure 2. Experimental results for antibacterial activity were displayed as log reduction versus exposure time for AgAl, Ag-HCl, Ag-NaOH and Ag-Sint samples against E.Coli and S.Aureus.

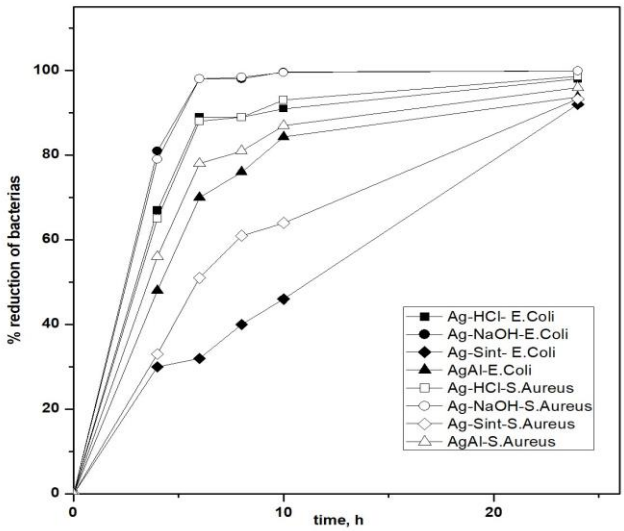

Figure 3. Experimental results for antibacterial activity were displayed as % reduction versus exposure time for AgAl, Ag-HCl, Ag-NaOH and Ag-Sint samples against E.Coli and S.Aureus.

Insights into the synthesis of Bi_2S_3 nanostructures for Computed Tomography

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Computed tomography (CT) is an X-ray based whole body imaging technique widely used to enhance the contrast among human body tissues because it allows deeper tissue penetration and higher-resolution imaging [1]. Currently, clinically approved CT contrast agents are iodinated molecules or barium suspensions, but to provide a good contrast large doses are needed, and their short circulation time limits their applications. Nanoparticles (NPs) show several advantages in comparison with these small molecules such as high residence times, potentiality for cell-tracking and targeted imaging applications due to their functionalizable surface [2],[3]. In particular, Bi_2S_3 NPs are a good choice because bismuth shows a large X-ray attenuation coefficient which enhances the contrast for small variations of the X-ray voltage. In addition, it is less expensive and also exhibit lower toxicity than other metals with similar X-ray attenuation coefficient. However, there is a lack in the literature of methods enabling a good control over the NP structure.

Here, we show a 2-step method to obtain Bi_2S_3 NPs with tunable shape and size based on the high temperature decomposition of bismuth (III) neodecanoate using different high boiling-point solvents, 1-octadecene and benzyl ether [4]. We have monitored the particle size and shape by tuning the duration of the last step of the reaction from 1 to 120 minutes. After that, we have stabilized the samples in aqueous media by a ligand exchange process.

After the first reaction stage, crystalline 5 nm spherical nanoparticles are obtained. Rod-like-shaped NPs can be grown by further increasing of the reaction time, which yields elongated NPs with 5 nm in width and from 5 nm to 50 nm in length. Despite the good control found in the particle size distribution, particles show poorer crystalline quality as they become elongated, which may be due to a faster growth process. By EELS analysis, we have discarded the presence of any parasitic phase of bismuth oxide. These nanoparticles may pave the way to enable the combination with other materials to achieve multifunctional systems for diagnosis or theranostics.

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Figures

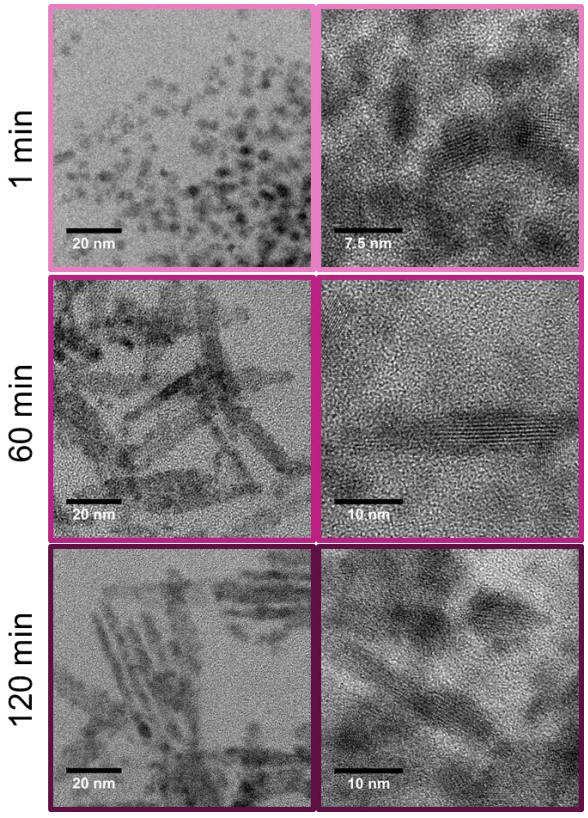

Figure 1. High-resolution transmission electron microscope images of the NPs obtained changing the duration of the last step of the reaction from 1 min to 120 min.

Metal-enzyme nano-composites with bactericidal and antibiofilm efficacies

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Antimicrobial resistance (AMR) is causing 50 thousand deaths per year in Europe and North America, with a concomitant heavy economic burden. Bacteria have developed different specific mechanisms conferring antibiotic resistance altering or destroying the antibiotic molecules, decreasing the membrane permeability to the antibiotic or expulsing it through efflux pumps.[1] Besides these resistance mechanisms, the ability of bacteria to proliferate adhered to inert surfaces or living tissues forming biofilms aggravates the appearance of AMR. Bacteria encased in these structures avoid the immune system response and makes them highly tolerant to high concentrations of antimicrobials.[2] Furthermore, conventional antibiotics do not remove biofilms thus, despite a successful antibiotic treatment; remaining sessile cells may lead to an infection relapse.

Metallic nanoparticles (NPs) are increasingly used to target pathogenic agents as an alternative to antibiotics. Metallic NPs offer a platform against bacteria, viruses, fungus and protozoa, these nanoparticles present multiple biocidal mechanisms that occur at the same time[3] (oxidative stress induction, metal ion release, or non-oxidative mechanism) reducing and hindering the appearance of the microbial resistance. On the other hand, among anti-biofilm strategies, different enzymes can be applied to prevent the biofilm formation or its eradication once established.

In this study, we integrate metallic particles with antibiofilm enzymes in the same nano-entity to combine synergistically different antimicrobial agents. The enzymatic component disrupts the bacterial communication pathways, through the hydrolysis of small molecule signals that govern the biofilm formation.[4] On the other hand, the biocidal activity of the nano-composite is supplied by the metallic particles while functions as an active template for the grafting of the enzyme. This antimicrobial approach could be applied in the form of coatings on surfaces such as hospital textiles, water treatment membranes and implantable medical devices, ensuring a safer environment for both patients and healthy population.

Silver-chitosan NPs (AgCh NPs) were synthesised by silver ions reduction, using chitosan as a reducing and capping agent. After washing the particles, UV-vis spectrometry reveal the pick at 420 nm corresponding to the formation of Ag NPs. TEM images revealed spherical NPs of 25-30 nm of diameter. After that, the AgCh NPs were functionalized with the quorum quenching enzyme (QQE) acylase I. The TEM analysis showed that the AgCh NPs were embedded in a protein matrix-like structure forming a silver-chitosan-acylase I (AgChAcyl) composite. The particles containing acylase were able to hydrolyse N-acetyl-L-methionine during the ninhydrin assay, indicating that the enzyme maintained part of its activity after the cross-linking between the chitosan and the acylase I.

The antimicrobial efficacies of AgCh NPs and AgChAcyl NPs were tested towards *Psuedomonas aeruginosa*. Silver and chitosan are well-known antimicrobial agents.[5] However, the antimicrobial assay showed that the presence of acylase I enhanced the antimicrobial activity of AgCh NPs (Fig. 1A). In addition, the time-killing assay reveal that AgCh NPs and AgChAcyl NPs displayed a rapid antimicrobial effect. After 20 minutes incubation of the NPs with *P. aeruginosa*, the bacterial concentration was drastically reduced. Nonetheless, this reduction was faster when the nanoparticles contained the QQE enzymes acylase I (Fig. 1B). Acylase I by itself did not presented any antimicrobial effect, which indicated that there is a synergistic effect between the QQE and the antimicrobial agent.

P. aeruginosa was exposed to AgCh NPs and AgChAcyl NPs to test the capacity of composites to disrupt the biofilm formation. The biofilm was washed to remove the NPs and the non-attached bacteria. The establishment of the extracellular matrix was assessed by crystal violet assay and live-dead staining. Both assays confirmed that the presence of acylase I in the nanoparticles inhibits the formation of biofilm (Fig. 2). Acylase I is able to hydrolase N-butyryl-l-homoserine lactone, a quorum sensing molecule of *P. aeruginosa*, hindering the quorum sensing and affecting the processes that are related with this communication, such as the production of virulence factors and the biofilm formation.

Finally, the biocompatibility of the particles was assessed using different human skin cell lines. The toxicity towards these cell lines was reduced when the enzyme was present on the surface of the NPs reduced the toxicity of the composites towards mammal cells.

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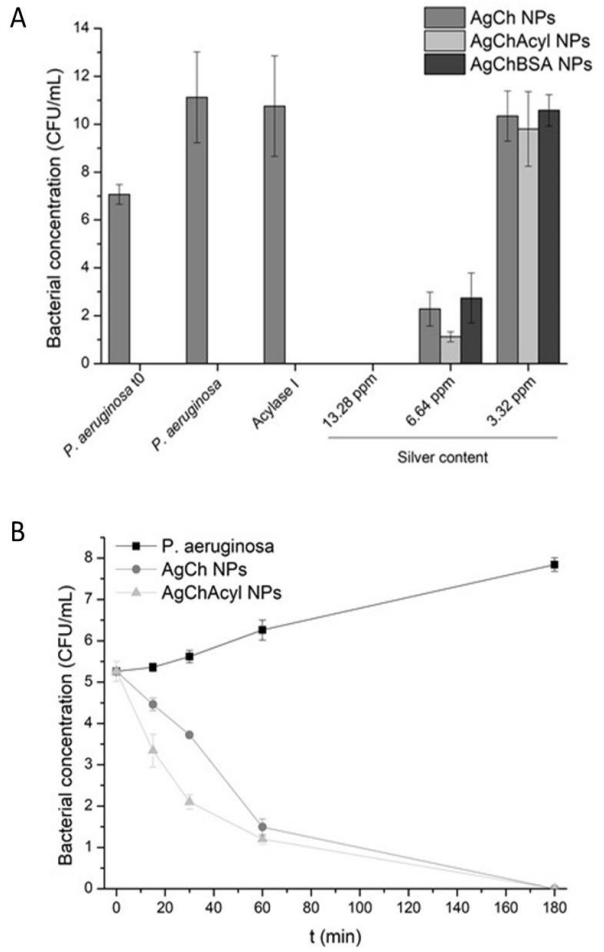
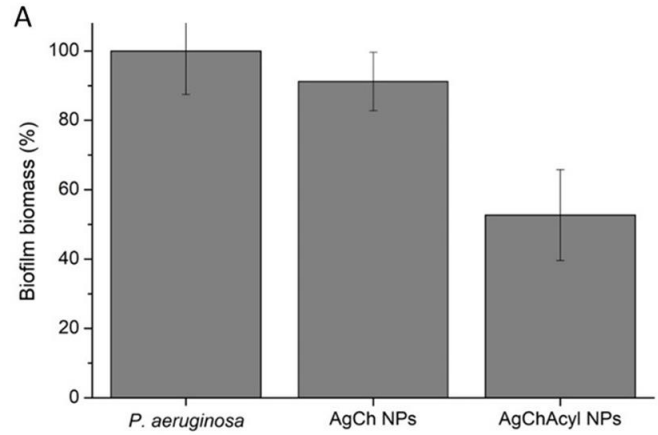


Figure 1. A) Evaluation of the antimicrobial activity of AgCh NPs, AgChAcyl NPs and AgChBSA NPs. The particles were tested using the same silver content. Acylase I was tested at the initial concentration of the synthesis of the particles. *P. aeruginosa* t_0 indicates the initial bacterial concentration of the test and *P. aeruginosa* value indicates the final concertation of bacterial without treatment B) Time-kill curves of AgCh NPs (dark grey) and AgChAcyl NPs (light grey) towards *Pseudomonas a*eruginosa. *P. aeruginosa* was incubated with 13. 28 ppm of silver and the bacteria surviving the treatment were estimated after 15, 30, 60 and 180 min.



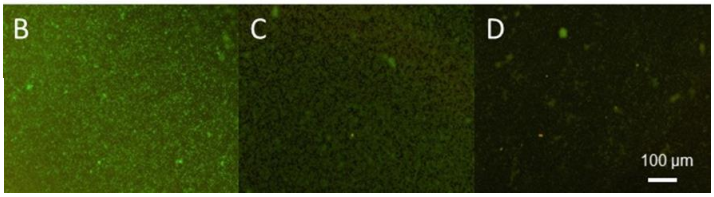


Figure 2. Evaluation of the biofilm inhibitory activity of AgCh NPs and AgChAcyl NPs. A) Assessment by crystal violet assay of *P.aeruginosa* biofilm inhibition, incubated for 24 h with AgCh NPs and AgChAcyl NPs. B) Fluorescence microscopy images of *P. aeruginosa* biodfilm after the treatment with C) AgCh NPs and D) AgChAcyl NPs.

When and Where: Microfluidic Cancer-on-a-chip platform for real-time imaging of drug delivery systems stability and extravasation

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Drug delivery vehicles confront multiple physiological barriers after injection into the human body. Their performance strictly depends on the stability in that complex environment. Effective nanocarriers should be stable enough to successively overcome the encountered barriers avoiding premature drug release, while being smart enough to free the cargo once the target is reached. Ideally, the performance of a drug nanocarrier should be evaluated in an environment mimicking human physiology, to reduce and refine the number of preclinical and clinical trials. Commonly used 2D cell culture models do not reflect the dynamic and complex organization of a human body, meanwhile animal models are ethically arguable, expensive, time-consuming and still lack direct translation due to the differences between species. Those challenges in drug delivery screening, together with the emerging era of microfluidic technology are the driving forces for the creation of new solutions [1,2].

In our work we present a perfusable 3D cancer-on-a-chip platform, where we study time- and space-resolved stability of potential drug delivery nanocarriers. In the microfluidic chip we recreated a part of tumor microenvironment, where we focus on essential barriers challenging drug delivery systems stability (Figure 1). The microfluidic model recapitulates present *in vivo* perfusable blood vessel lined with organized Human Umbilical Vein Endothelial Cells (HUVECs), that create the cellular wall between the vessel lumen and the extracellular matrix, in which 3D HeLa cancer cells spheroids are distributed [3].

From the different drug delivery systems, we chose to evaluate supramolecular structures. We perfused the microfluidic platform with three amphiphilic PEG-dendron hybrids (previously studied in 2D cell culture), that change their fluorescent properties upon assembly into micelles [4]. Thanks to the compatibility of the microfluidic model with confocal spectral imaging we could register real-time stability and extravasation of the introduced nanostructures interacting with the 4 defined barriers (blood vessel, endothelial wall, extracellular matrix, tumor spheroid). This special property of the micelles allows us to follow their stability in correlation with the barrier they encountered, by real-time monitoring of changes in their fluorescence emission.

We observed a difference in extravasation of nanostructures depending on the leakiness of the endothelial barrier (studying healthy blood vessel and tumor blood vessel models), what correlates to the Enhanced Permeability and Retention (EPR) effect found *in vivo*. Interestingly, we could observe how the number and proximity of cancer cells affects the integrity of endothelial wall. Furthermore, we were able to identify most and least stable formulations by following their fluorescence emission. We registered decreased micelle internalization comparing to previously investigated 2D cell culture.

Our results demonstrate the applicability of the cancer-on-a-chip microfluidic platform in bridging the gap between 2D and *in vivo* studies. The vision of fast screening of a drug nanocarrier candidates in a 3D cell culture that could use patient derived cancer cells provides knew knowledge in the field of

nanomaterials' performance and brings us one step closer to the personalized nanomedicine.

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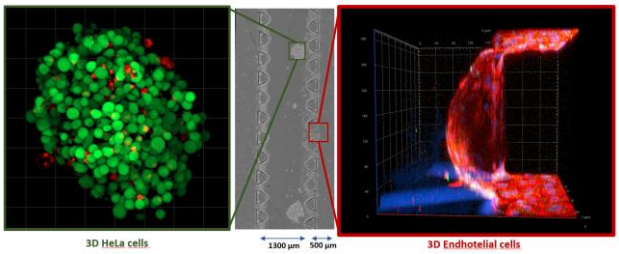

Figure 1. Cancer-on-a-chip platform. From left to right: A. HeLa cells spheroid confocal microscopy image (Green: live cells stained with Calcein, Red: dead cells stained with Propidium Iodide), spheroid diameter in vertical line: 240 µm. B. Transmission image of the cancer-on-a-chip platform. C. 3D reconstruction of HUVECs monolayer creating an interface between the extracellular matrix and the perfusable vessel lumen. Image acquired using confocal microscopy in Z-stack mode, in red: Phalloidin, blue: Hoechst, image height: 280 µm.

Hybrid fibrous microenvironments for muscle tissue engineering

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To mimic the natural environment of tissues, in-vitro, support structures are necessary in order to allow for healthy cell and tissue development. Hydrogels have become popular materials to develop these support structures due to their substantial water content and the simplicity in tailoring their physical, biological or chemical characteristics. However, hydrogels alone cannot provide all the necessary stimuli and microenvironment for cell development and, therefore, hybrid materials are currently being developed.

Cells and tissues also require more than a suitable biochemical environment to successfully develop and differentiate [1]. Specifically, electrical and mechanical stimuli are extremely important for tissues such as skin, cartilage, bone and muscle. Scaffolds can be functionalized with nanomaterials, so they can deliver these stimuli to improve the viability of the new tissue [2].

Muscle is an electromechanical material, highly responsive to these stimuli [3], and it is known that negatively charged surfaces improve cell adhesion and proliferation and that the directional growth of the myoblast cells can be achieved by using aligned fibers [4].

Therefore, incorporating electroactive polymers in a hydrogel scaffold, and stimulating these polymers externally so that they can in turn stimulate the incorporated cells, allows for in-vitro mimicry of the normal conditions of muscle cell growth and differentiation [5].

In this context, the present work focuses on the development of electrospun electroactive polymer fibers [6], to fabricate functionalized hybrid hydrogel scaffolds for muscle tissue engineering.

Oriented and non-oriented fibers, based on polymers that are electroactive, biocompatible, biodegradable and biostable, have been processed and characterized. They were further modified with ionic liquids and magnetic particles to allow electro and mechanotransduction to be applied to the cells. This resulted in fibers with diameters between 0.5-3 µm, crystallinity between 45-60%, β-phase content around 90%, and magnetic properties suitable for use in a magnetic bioreactor, with the thermal stability of the polymer being unaffected by the inclusion of these materials.

Funding by Spanish Ministry of Economy and Competitiveness (MINECO) through project MAT2016-76039-C4-3-R (AEI/FEDER, UE) (including FEDER financial support). Financial support from the Basque Government under the ELKARTEK, HAZITEK and PIBA (PIBA-2018-06) programs is also acknowledged.

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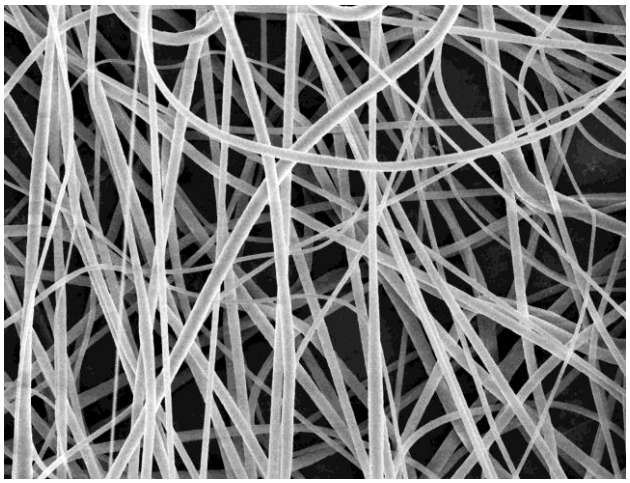

Figure 1. Non-aligned PVDF fibers

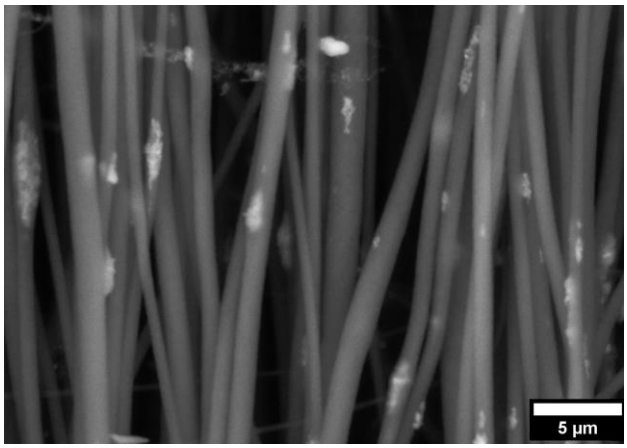

Figure 2. Aligned PVDF fibers with magnetic particles

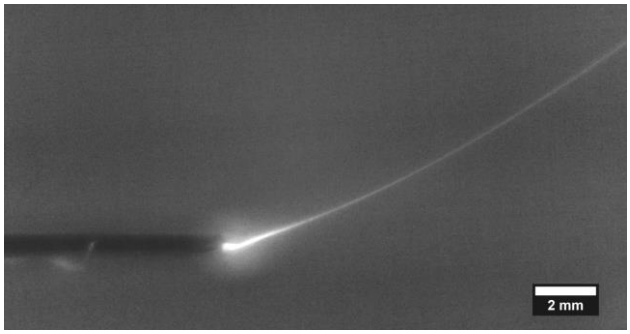

Figure 3. Photo of a fiber at the start of the electrospinning process

Controlling Cellular Trafficking by Nanoparticle Avidity: From Endocytosis to Transcytosis

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Drug delivery to the brain is hindered by the presence of the blood-brain barrier (BBB), which consists in specialised endothelial cells that line brain capillaries restricting movement of molecules from blood-to-brain. Nonetheless, brain endothelial cells (BECs) rely on endogenous transport mechanisms (such as, receptors) that allow certain molecules to transverse the BBB. By using Angiopep-2(AP2)-decorated pH-sensitive polymersomes, we previously demonstrated the ability to specifically target lipoprotein receptor-related protein-1 (LRP-1) at the surface of BECs, and trigger transcytosis allowing the delivery of macromolecules into the brain parenchyma [1]. Here, we explore the mechanism of transcytosis of the polymersomes as a function of the number of AP2 ligands (i.e., avidity) using an established *in vitro* BBB model as well as *in vivo*.

AP2-poly(oligo(ethylene glycol) methyl ether methacrylate)-block-poly((diisopropylamino) ethyl methacrylate) (POEGMA-PDPA) polymersomes showed binding to LRP-1 in BECs, and significantly higher levels of apparent permeability (2.5-folds) compared to unfunctionalised polymersomes. When decorated with different numbers of AP2 ligands (I = 16 to 82), the rate of transport across a bEnd3 monolayer is affected, with the highest efficiency observed for 16 AP2 per polymersome. At the density of I = 16, the overall affinity of the nanoparticle triggers transcytosis by the formation of tubular structures, as demonstrated by live cell imaging and molecular dynamic modelling simulations. Cellular trafficking of AP2 polymersomes, I = 16 and I = 82, illustrated that, depending on the number of ligands, polymersomes undergo distinct intracellular sorting in BECs. After a tail vein administration of the polymersomes, 3.82% of the injected dose was obtained in the brain for the polymersomes with I = 16 which is significantly higher than unfunctionalised polymersomes, I = 80 AP2-functionalised polymersomes and AP2.

Together, these results elucidate the impact of the density of AP2 ligands on the intracellular trafficking of POEGMA-PDPA polymersomes at BECs. Hence, our work offers a new insight into the mechanism of transcytosis that could be explored when engineering nanoparticles for drug delivery to the delivery.

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STORM characterization of enzyme powered micro- and nanomotors

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Self-propelled enzyme micro- and nanomotors are promising in the biomedical field as a drug delivery platform. This kind of self-propelled motors make use of enzymes to transform the environmental chemical energy into active motion. Despite the recent advances in the field, a deeper understanding of fundamental aspects is required for an efficient implementation in biomedicine. For example, in order to achieve active motion, an asymmetry on the distribution of the particles has traditionally been claimed. Herein, we study the quantity and distribution of urease molecules around the surface of our different motors, including mesoporous silica nanomotors, polystyrene and core-shell polystyrene silica micromotors, in order to understand key parameters such as the enzyme coverage, the number of enzymes needed to power motors, or the impact of an asymmetric enzyme distribution to the motion. Moreover, given the applicability of nanomotors in the nanomedicine field, we also provide a study of the interactions between the nanomotors and biological fluids. The motion of the motors was measured with optical tracking software and the distribution of single molecules (urease and other physiologically relevant molecules) around them was observed and quantified using Stochastic Optical Reconstruction Microscopy (STORM). Interestingly, we observed an asymmetric distribution of enzymes around the motors surface regardless of particle composition and size, suggesting the stochastic asymmetric distribution is enough to break force symmetry and produce motion. These results provide deeper knowledge on the fundamental aspects underling enzyme-powered motors and might help to establish more efficient designs for better applicability in the near future.

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Figures

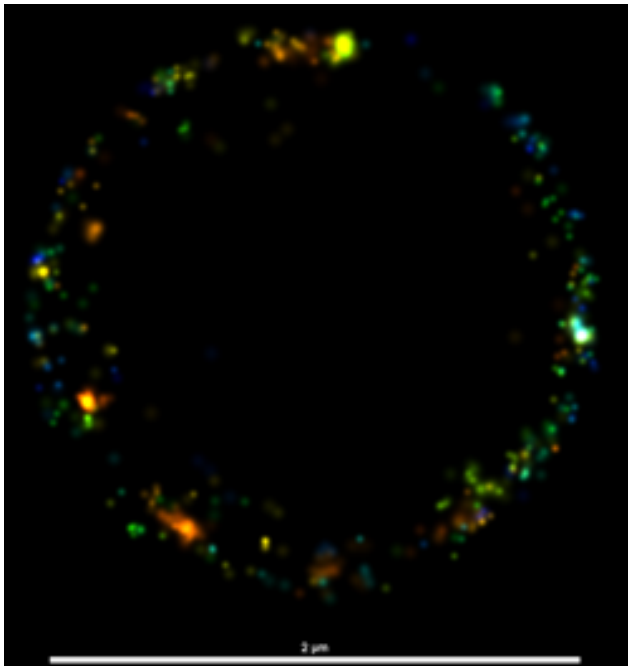

Figure 1. Urease molecules conjugated to the surface of a micromotor viewed with STORM. Scalebar is 2 µm.

Label-free nanosensing platform for breast cancer exosome profiling

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Cancer constitutes the second leading cause of mortality worldwide, only surpassed by cardiovascular disease. Based on GLOBOCAN estimates, about 18 million new cancer cases and 9.6 million deaths occurred in 2018 worldwide. Breast cancer is the most frequently diagnosed form of cancer and the leading cause of cancer death among females worldwide. It totalizes 11.6% of the global registered cases in both genders. The diagnosis of breast cancer in the current scenario is made by mammography and its spectroscopy, and breast biopsy test. All these detection techniques are usually expensive, invasive and time-consuming. Thus, the development of simple, reliable, cost effective and non-invasive detection methods for early cancer diagnosis and posterior follow-up is particularly important, due to the disease's prevalence and potential lethality.

Exosomes are small (50–100 nm in diameter) extracellular vesicles secreted by all cells into body fluids such as blood, saliva and urine. These extracellular vesicles show potential for cancer diagnostics, as they transport several molecular contents of the cells from which they originate. Additionally, exosome' analyses are minimally invasive and afford relatively unbiased readouts of the entire tumour burden, less affected by the scarcity of the samples or intra-tumoral heterogeneity. The essential first step of current exosome analysis is purification by ultracentrifugation. Afterwards, exosomes may be analysed using western blot, enzyme-linked immunosorbent assay, flow cytometry and other analytical methods. Though robust and effective, these strategies are expensive, time-consuming and rely heavily on the sample handling skills. Thus, the development of sensor technologies for rapid, inexpensive, simple and on-site exosome screening is necessary. Some exosomes-based sensors have already been reported, such as label-free exosome assay utilizing transmission surface plasmon resonance (SPR), mass-sensitive sensors aptamer-based sensors magneto-electrochemical sensors, among others. [1,2,3]

Surface Enhanced Raman Spectroscopy (SERS) has emerged as a highly sensitive and rapid analytical technique with wide application regimes, from biological analysis to environmental monitoring. At the most basic level, SERS is a way to significantly increase the signal from the weak yet structurally rich technique of Raman scattering. A SERS-active substrate is generally based on a platform coated with a roughened or nanostructured metallic surface (silver, gold, etc.) that enhances the Raman signal due to the Localized Surface Plasmon Resonance (LSPR). However, the commercially available SERS-active substrates come with a high cost and low shelf-life. Cellulose is the most abundant polymer on earth and meets several interesting properties. Being a biocompatible, biodegradable, flexible, recyclable and low-cost material draws the scientific community attention in several applications, from biosensors to electronics. In this work [4], low-cost SERS-active substrates were produced based on silver nanoparticles (AgNPs) grown *in situ* into bacterial cellulose (BC) coming from commercial *nata de coco,* providing a low-cost and simple alternative to the conventional production methods (Figure 1). The AgNPs were grown by hydrothermal synthesis assisted by microwave radiation, that allows well-controlled and fast synthesis. Two routes were followed: one using silver citrate as precursor and another using silver citrate and ammonium citrate dibasic, to induce alkaline environment. A systematic study on the precursor concentration, synthesis time and temperature was performed in order to obtain a good SERS enhancement factor (EF). Ultimately, it was possible to achieve EF from 10^4 to 10^5, detecting rhodamine 6G (R6G) with concentrations as low as 10^{-11} M, which was used as model-molecule. Finally, biological tests were performed on the optimized SERS substrate, with exosomes samples coming from MCF-10A (non-tumorigenic breast epithelium) and MDA-MB-231 (breast cancer) cell cultures lineages and the obtained SERS spectra were subjected to statistical Principal Component Analysis (PCA), to analyse variances in each collected spectrum and group the similar ones. This analysis tool was chosen since exosomes segregated by the same cell may have different Raman fingerprints: this is probabilistic and is related to the way the exosomes may be positioned in the SERS substrate. Since exosomes have several surface's proteins, different positions of the exosomes in the SERS platform will show different Raman spectra, thus inducing some intra sample variation. So, by combining PCA with Raman intra and inter variability in exosomal samples, it was possible to differentiate tumoral and non-tumoral exosomes, with a data grouping of 95% confidence. The obtained 95% confidence ellipses (Figure 2) are especially useful in this work final objective, since they can be used as a form of diagnostic: if an analysed sample Raman spectrum is located inside any of the presented ellipses, it's safe to assure with 95% confidence that the sample belongs to the

respective group. As a proof-of-concept, a close-to real time diagnosis was performed. For this, tumoral and non-tumoral sample SERS spectra were collected and analysed in the previously obtained score plots. It was registered that both samples fell within the respective ellipses, proving PCA as a valuable diagnosis tool, being able to distinguish between exosomes segregated by tumoral and non-tumoral cell lineages trough SERS while using innovative substrates, with an overall cost of 0.39 € per membrane, which can provide several dozens of different measurements. This approach rendered a simple, label-free and easy-to-perform method for exosomes profiling and shows promising results as far as breast cancer diagnostic by SERS is concerned. Moreover, we believe that this novel exosome's profiling method can provide precious information on both the prognosis of the disease and the predictive outcome of a given therapy.

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Figures

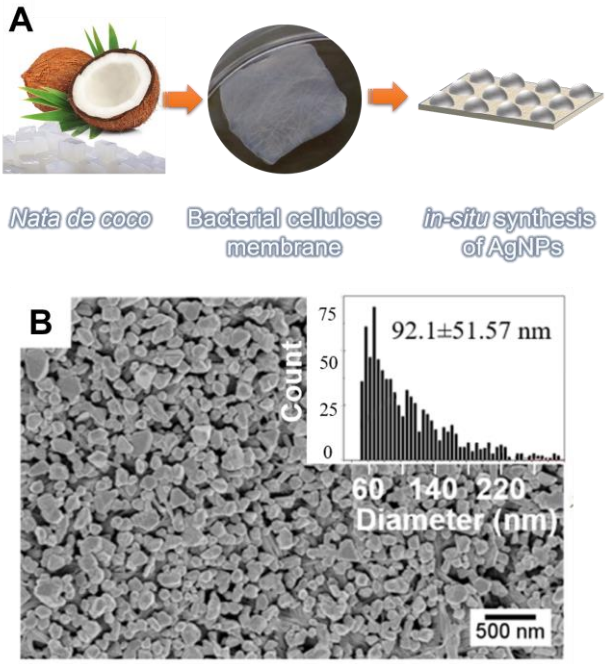

Figure 1. A: Schematic workflow of the active-SERS substrate's production: from the source (*nata de coco*) to the final BC/AgNPs platform; B: SEM imaging and AgNP's diameter histogram of the optimized active-SERS platform using BC/AgNPs composites.

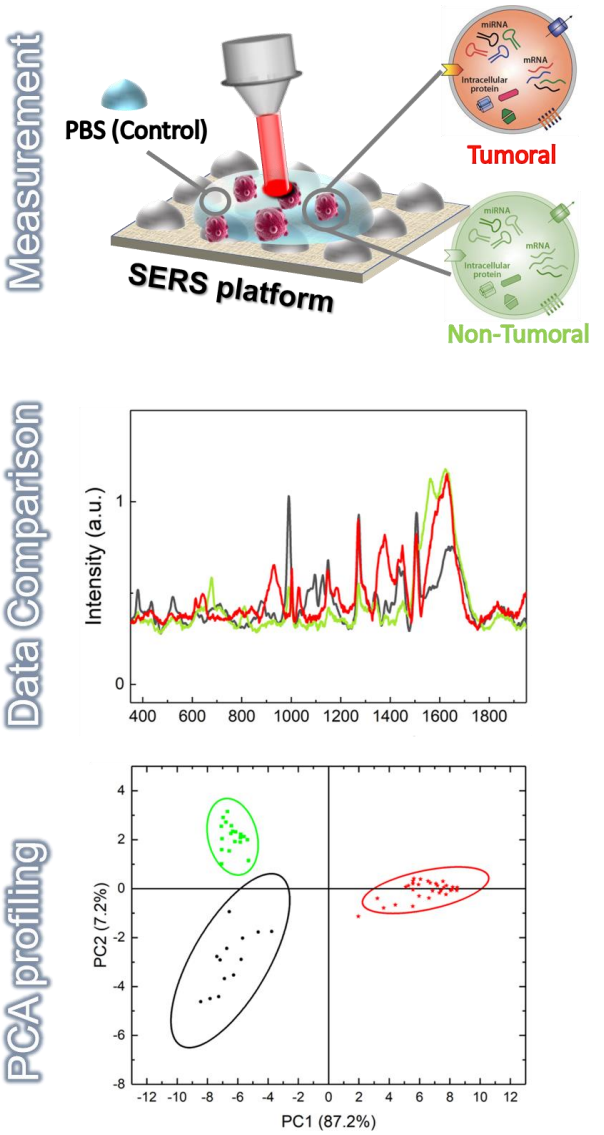

Figure 1. Schematic representation of the detection system used in this work. Measurement of exosome's samples, spectra data comparison and PCA profiling: PCA resultant score plot using PC1 and PC2 as the analysed principal components: PBS (control) in black, MCF-10A (green) and MDA-MB-231 (red) exosomes sample analysed.

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SRs and Cd81 Receptors-mediated endocytosis of PMPC-PDPA polymersomes via dynamin II-independent manner

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Abstract

Polymersomes have been revealed to be an ideal tool for the efficient intracellular delivery of a large diversity molecules. However, the molecular pathways regulating polymersomes uptake by cells remain still poorly investigated, especially regarding the role played by surface membrane receptors such as scavenger receptors and tetraspanin family proteins. This subject has crucial implications in biomedical research applied to payload delivery by polymersomes. Here, we investigated the mechanisms involved in polymersomes interaction with specific cell receptors. PMPCx-PDPAy-based self-assembly of polymersomes was carried out using the pH-switch method. After this, polymersomes were purified through a hollow fiber (tangential flow filtration system) followed by step-gradient centrifugation to isolate several subpopulations of particles having homogenous sizes. Polymersomes concentration was quantified by HPLC, while the size characterised by DLS and TEM analysis. The screening of specific receptors guiding the uptake of polymersomes was evaluated by western blot and immunofluorescence. The cytofluorometric method was used to study the kinetics of polymersomes uptake. siRNA and/or shRNA was used to knockdown the expression of endocytosis proteins and manipulate the uptake of polymersomes by carcinoma cells. The results showed that changes in the polymer lengths of PMPC affected the rate of vesicles uptake. Moreover, we deciphered an essential correlation between the degree of polymerisation(i.e., the polymer lengths) and the kinetics of polymersomes uptake. We confirmed a key role played in PMPC25-PDPA70 polymersomes uptake by scavenger receptors (SRBI and CD36) and CD81 receptor in cancer cells. Silencing of SR-BI gene using siRNA in FaDu cells decreased PMPC-PDPA polymersomes uptake by 50% in a dynamin-independent manner. Here, we highlighted the dynamics of interaction between polymersomes and plasma membrane receptors, SRs and CD81. We have shown that polymersomes uptake guidance is dynamin-II-independent.

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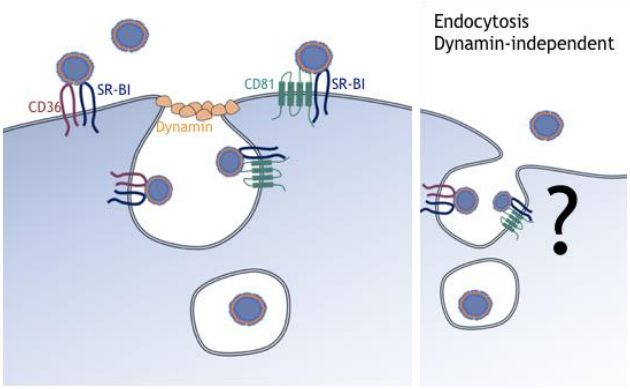

Figure 1. The SR-BI/CD36 and the tetraspanin CD81 are natural receptors for cellular uptake of $PMPC_{25}$-$PDPA_{70}$ polymersomes via dynamin-II independent manner.

Novel 3d Printed Capacity and pH Buffer Sensor

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Abstract

This work presents a novel 3D-printed micromixer capable to not only measure pH also molarity of the buffer. Buffer molarity control is especially sensitive in biological processes[1]. The design takes advantage of the diffusion of the solutions (buffers) and analyzes interaction area and the color change experienced by bromocresol purple (pH indicator). The performance of the proposed solution was evaluated in three different buffer molarities (0.1, 0.5 and 1.0 M) with three different pH values (6.0, 7.0 and 8.0). The microfluidic channel was designed for a Reynolds number of 1, therefore the diffusion was occurring progressively from the inlet to the outlet. Molarity measurements were done at the intersection of the inlets, while pH measurement (that required complete mixture) was done at the outlet. The results showed that not only the current setup is successful at measuring the buffer molarity and pH, but also the fabrication method is more rapid and flexible in creating microfluidic devices.

Methodology

The microfluidic device shown in Figure 1 (a) and (b) was manufactured using a 3d printer based on Digital Light Projection Technique (DLP) [1] . Briefly, the geometry from Figure 1 is modeled in 3D using @Solidworks software and exported to *.slt format to be introduced to the @MiiCraft Plus printer.

The channel's height is also set to 500 µm and the top layer to 300 µm to ensure that the channel is fully sealed and the chip is printed uniformly.

The pH indicator is Bromocresol purple (5',5"-dibromo-o-cresolsulfophthalein), @sigma Aldrich. (4-(2-hydroxyethyl)-1-pi-perazineethanesulfonic acid) HEPES, from @Sigma Aldrich, was used as buffer at different molarities and pH [28].

During the assay the main channel injects the Bromocresol and the buffers are injected into the device, through lateral channels. The injection is done by another syringe pumps (kdScientific 410-CE- @kdScientific, Holliston, MA, US).

Conclusions

According to the results, the DLP 3D printed microfluidic mixers can be employed as a flexible process to manufacture pH and molarity buffer sensor. The use of 3D printed microfluidics provides portability to pH measures and the opportunity to control the buffer capacity measuring its molarity/concentration. In the current study HEPES buffer of 3 different concentrations were evaluated showing clearly that BCP advanced further into the lateral channels when the concentration was increasing. In the same magnification scale, BCP penetrated up to 39 pixels in buffers with 0.1 M of HEPES, see Figure 2. Whereas, the indicator marked a 147 pixels advance for the buffers with 1.0 M of HEPES, showing a 2.77 times increase in the diffusion width, while the inlets' discharges were kept constant. Besides, pH measurement, which required a homogeneous mixing, was achieved as well at the outlet of the mixer, See FIGURE 3. This study showed that the 3D printed device could be successfully employed in order to detect buffers in the pH range of 6.0 up to 8.0 as the investigation showed a 21.7% mean blue color difference between buffers with pH 6.0 and 7.0. Although, a 11.2% difference between the buffers with pH 7.0 and 8.0 was reported, but the analysis showed a 35.3% change when buffers with pH 8.0 and 6.0 were compared. Furthermore, the assay showed that the setup could be used as a self-referencing sensor, when two unknown solutions are being injected into the lateral channels. The results showed that, not only the 3D printed device reproduced the numerically predicted trend, but also it provided 76.3% of homogeneity, which is only 1.3% off the numerically predicted efficiency.

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Figures

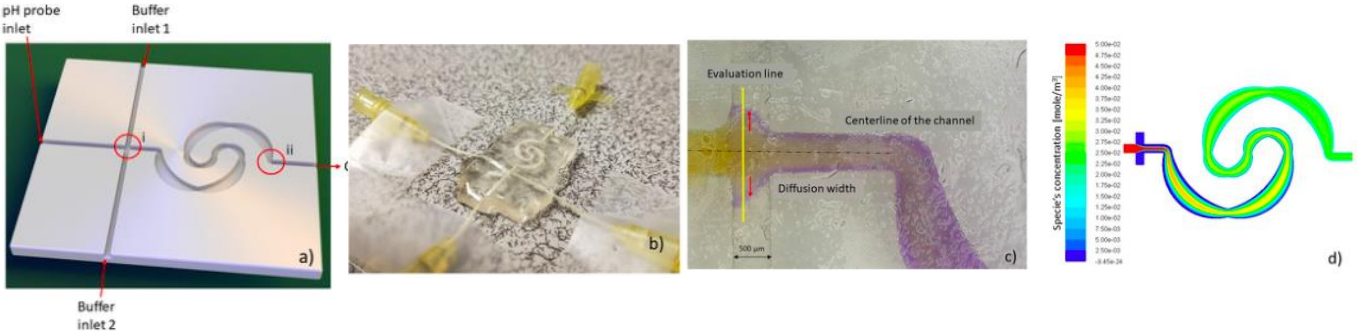

Figure 1. a) shows a 3D view of the investigated device, inlets and measurement zones. i) illustrates the buffer measurement zone and ii) displays the pH measurement zone. b) Inlets, outlet and the channel of the 3D printed device is shown. c) Displays the diffusion phenomena at the intersection of the inlets. The evaluation line, where the buffer diffusion is measured over, is also displayed. d) Shows the numerical simulation of diffusion in one loop mixer with 10% expansion parts, assuming that 0.05 M of a specie is injected into a device while the fluids flow at Re=1.0.

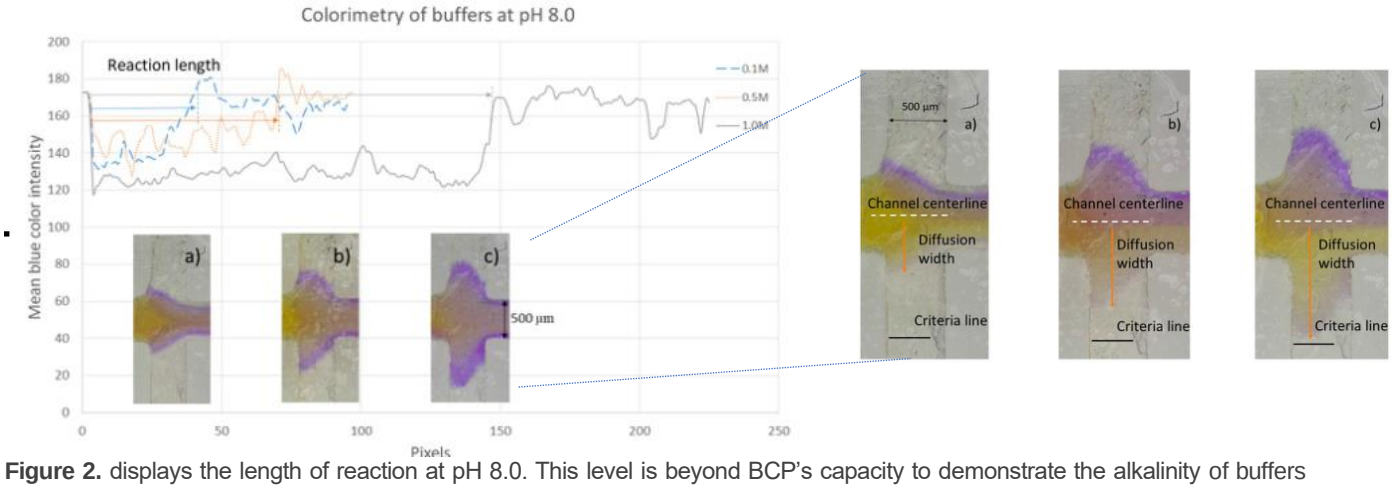

Figure 2. displays the length of reaction at pH 8.0. This level is beyond BCP's capacity to demonstrate the alkalinity of buffers and the color change and reaction lengths are at their maximum. Reactions of BCP and buffers with 0.1, 0.5 and 1.0 M of HEPES are represented in pictures (a), (b) and (c), respectively. Lateral (a), (b) (c) shows the reaction of BCP and buffers with different pH levels at the intersection of the inlets. The upper inlet was dedicated to the buffers with pH 8.0 and through the bottom inlet, the buffer with pH 6.0 was injected. Pictures (a), (b) and (c) show the reaction of buffers with 0.1, 0.5 and 1.0 M of HEPES, respectively.

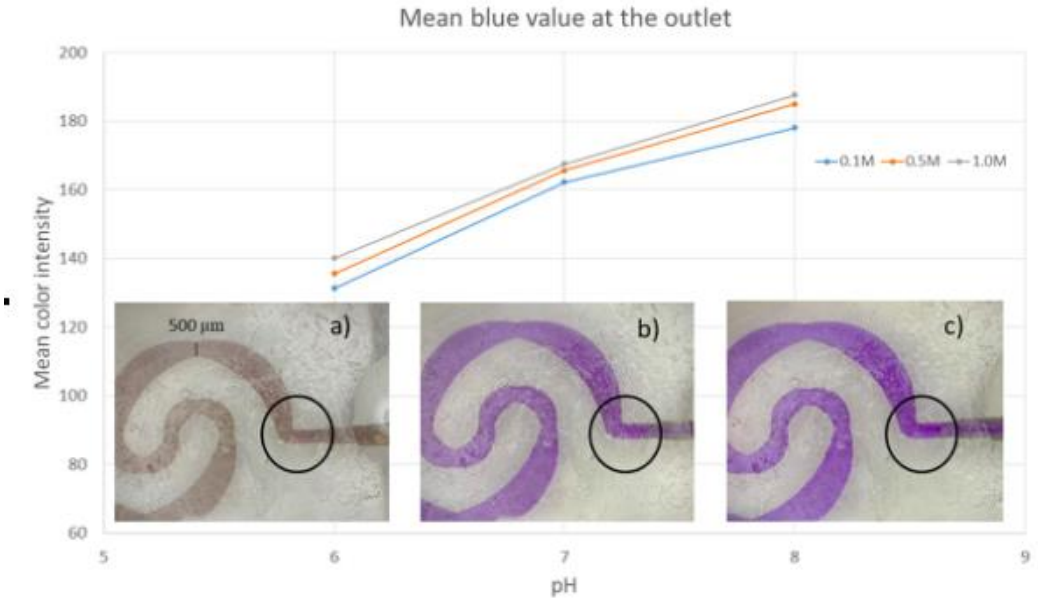

Figure 3. shows the analysis of the mixture of the BCP and the buffers at the outlet. The transition of color in the range of pH 6.0 to 7.0 is quite obvious, but for the range of pH 7.0 to 8.0 further investigation and accurate measurements are necessary. Pictures (a), (b) and (c) show the final mixture of the BCP and buffers with 0.5 M of HEPES at pH 6.0, 7.0 and 8.0, respectively

Modification of the magnesium corrosion rate in physiological saline 0.9 wt % NaCl via chemical and electrochemical coating of reduced graphene oxide

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Abstract (Arial 10)

Different metallic materials have been used as biomaterials for the manufacture of medical implants. Commonly used metallic biomaterials include stainless steel, pure titanium, titanium-aluminum-vanadium-based alloys and cobalt-chromium-molybdenum-based alloys [1–5]. The advantages of biodegradable Mg-based implants [6–8] lie in their mechanical and electrochemical properties. Mg is a lightweight metal with a density of 1.74 g cm −3 versus 7.9 g cm −3 for Al and 4.5 g cm −3 for Ti. Moreover, Mg presents an elastic modulus and compressive yield strength closer to those of natural bone [9]. In addition, Mg is a biocompatible material naturally found in the human body (approximately half of the total physiological Mg is stored in the bone tissue) [10]. Setbacks when using Mg as metallic material for biomedical applications are related to its low corrosion resistance under the physiological conditions [9] and the excessively rapid production of hydrogen gas during the in-vivo corrosion [11]. The first issue could lead to both a rapid loss of its mechanical properties and severe problems in tissue regeneration, and the second, to harmful effects during the tissue healing process. One of the most recent studies dedicated to slowing down the dissolution of magnesium in saline conditions was performed on samples of magnesium foam manufactured using the replication method from carbon spheres as a template. The heat treatment in air flow at 540 °C applied to burn the template particles generated a layer of oxide on the surface of the foam which notably slowed down its dissolution at 37 °C in an aqueous solution containing 3 wt % NaCl that had a pH of 7.4 (a pH closed to that of the human body) [12]. Other surface modifications that were proved to be successful in slowing the corrosion rate of magnesium were fluoride conversion coatings, phosphate treatments or chemical deposition of hydroxyapatite and octacalcium phosphate [13].
Moreover, it has been proved that both graphene oxide (GO) and reduced graphene oxide (RGO) show anti-corrosion properties when coated onto metal substrates [14–19]. The syntheses of the different graphene-metallic substrate specimens were carried out following both electrochemical [20–22] and chemical [23,24] methods.

The synthesis of reduced graphene oxide onto magnesium discs by electrochemical and chemical methods is presented in this work. The surface morphology and atomic composition were investigated using field emission scanning electron microscopy and energy dispersive X-ray spectroscopy. The corrosion rate of different samples was analyzed in physiological saline 0.9 wt % NaCl solution by potentiodynamic polarization, electrochemical impedance spectroscopy and scanning electrochemical microscopy. As a result of the different treatments, a progressive decrease in the corrosion rate of the magnesium disc in the corroding environment was obtained, reaching up to 80% of reduction for the chemically modified sample.

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Development and permeability evaluation through BBB-on-a-chip model of Gold nanorods with therapeutic potential for Alzheimer's disease

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Alzheimer's disease (AD) is a chronic neurodegenerative disorder characterized by a progressive loss of cognitive capacity and memory. AD is mainly associated to two neuropathological processes: hyperphosphorylation of tau protein (P-tau) and the accumulation of toxic aggregates of amyloid β peptide (Aβ) in the brain. In pathological conditions, Aβ peptide is synthetized from a transmembrane protein (APP) which is cleavage by the beta and gamma secretase enzymes [1]. Then, the Aβ peptide is aggregated in oligomeric species, fibers and amyloid plaques which produce oxidative stress and neurotoxicity [2].

In the last years, multiple efforts have been performed in order to develop new molecules for AD's treatment based on the disaggregation of Aβ cumulates [1]. However, most of them do not reach the action site due the strict permeability in the brain by the blood brain barrier (BBB). By the way, nanotechnology is an attractive field that offers several alternatives for the treatment and diagnosis of AD. Specifically, a previous work reported gold nanorods (GNRs) functionalized with polyethylene glycol (PEG), a peptide that acts as a β sheet breaker (D1 peptide) and other one to allow the shuttling through the BBB (Angiopep-2) by LRP1 receptor present in the brain endothelium. The results revealed that the nanosystem (GNRs-PEG-Ang2/D1) performed Aβ growth inhibition *in vitro*. Remarkably, this effect was enhanced by irradiation due GNRs present photothermal properties. In addition, the nanosystem decreased the toxicity of Aβ aggregates in a *Caenorhabditis elegans in vivo* model [2].

Therefore, promising therapy/diagnostic agents are being developed and need to be evaluated quickly and easily for the early provision of new alternatives for AD. BBB-on-a-chip is an interesting alternative due their versatile, controlled, repeatable and lower cost design to mimic both *in vivo* physiological and pathological conditions for the study of drug permeability, disease progression, efficacy of treatment and others [3].

In the present work, we proposed the development of GNRs-PEG-Ang2/D1 and evaluate its permeability in a BBB-on-a-chip model which allows a 3D arrangement closer to the biological structure and tuning different flow conditions.

For this, we synthetized GNRs by a seed-mediated growth method and then were conjugated with PEG, Angiopep-2 and D1. These nanosystems were characterized by dynamic light scattering (DLS), zeta potential, electron microscopy (TEM) and UV-Vis-NIR spectroscopy. On the other hand, for the construction of the BBB-on-a-chip we will use brain endothelial cells and pericytes; therefore, the cytotoxicity of the nanosystems was determined by Annexin V/DAPI assay. Then, we expect to assess the cell uptake by flow cytometry. Finally, we will evaluate the permeability of the GNRs at different flow conditions in BBB-on-a-chip by atomic absorption and Nano tracking analysis (NTA). Also, the integrity of the BBB after GNRs administration will be assessed by immunofluorescence and trans-endothelial electric resistance (TEER).

From UV-vis-NIR spectroscopy of GNRs and its conjugates showed two absorption peaks about 520 and 710nm, directly regarding to this type of nanoparticles. Also, DLS and zeta potential revealed that the GNRs surface functionalization caused an increase in the hydrodynamic diameter and electrostatic change from positive to negative charge (+40 to -5 mV). Finally, electron microscopy displayed the shape and size of GNRs of length 40nm and 10nm width. On the other hand, these nanosystems did not show cytotoxic effects over endothelial cells and pericytes between 0.05 to 0.4nM for 24 hours.

We conclude that obtained GNRs-PEG-Ang2/D1 in a reproducible manner and they were not toxic for the endothelial and pericytes cells for BBB-on-a-chip. In future, we will evaluate the permeability of the GNRs at different flow conditions in BBB-on-a-chip and the integrity of the barrier after GNRs exposure.

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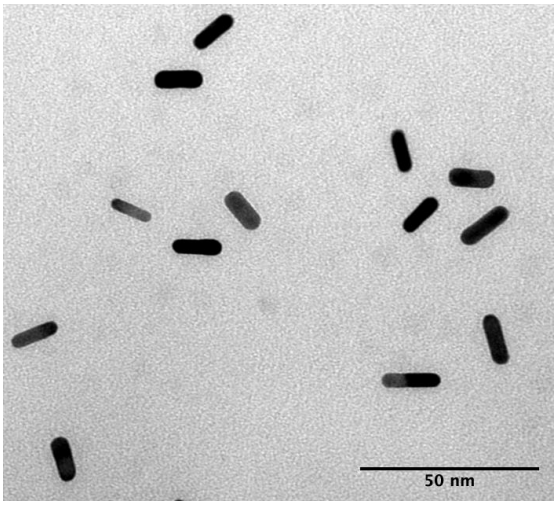

Figure 1. Electron micrograph of GNRs-PEG-Ang2/D1.

BBB-targeting liposomes : Design, characterization and *in vivo* evaluation

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The blood-brain-barrier (BBB) remains a formidable obstacle for the treatment of brain diseases. Current technologies, characterized by high complexity, rarely achieve delivery of the drug in the brain beyond 1% of the total injected dose [1] and therefore prognoses for brain diseases remain extremely poor.

By using embryonic zebrafish (*Danio Rerio*) as a model to assess nanoparticle behavior *in vivo* [2], we report a simple liposome formulation composed of just two lipids, with a significant specificity for the brain endothelium. We demonstrate how the highly unusual morphology of these liposomes, characterized by phase-separation and a single protrusion per liposome, is essential for BBB targeting, as well as our current understanding of the mechanism behind this biological response. Finally, we show the incorporation of a pH sensitive lipid-dye and of a single gold nanoparticle per liposome, which enables the elucidation of the liposome fate using high-resolution imaging techniques.

Ultimately, the unprecedented specificity of this liposomal formulation for brain endothelial cells and our comprehension of its *in vivo* mechanistic behavior, may lead to the development of a novel powerful tool for drug delivery to the CNS.

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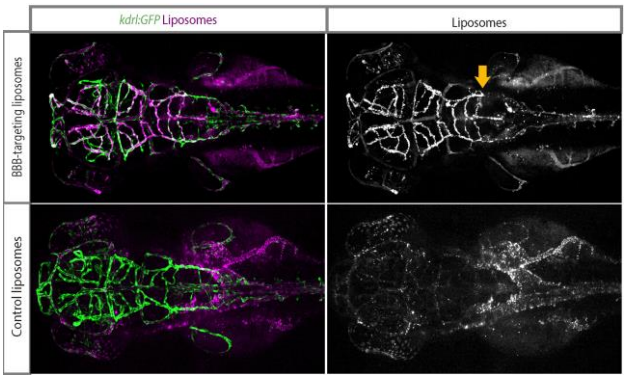

Figure 1. *10x fluorescent images of the embryonic zebrafish head (dorsal view), following intravenous administration of BBB-targeting liposomes and control liposomes (liposomes in magenta (left) or white (right)). Arrow indicates the specificity of the liposomes for the BBB, as targeting starts where the brain endothelium begins. Zebrafish embryo is at 3 days post fertilization (3dpf), expressing green fluorescent protein throughout its vasculature (kdrl:GFP).*

Affinity-Targeted Silver Nanoparticles as a Research Tool and a Drug Carrier

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ABSTRACT

We have developed silver nanoparticles (AgNPs) as a model carrier system to study tumor homing peptide-mediated targeting and cellular/tissue interactions of AgNPs *in vitro* and *in vivo*. AgNPs loaded with fluorescent dyes can be tracked in cells and tissues by ultrasensitive optical imaging, as the silver cores of the AgNPs plasmonically enhance the fluorescent signal [1]. To allow quantitative internally-controlled cellular uptake and tissue biodistribution studies using AgNPs, we have developed isotopically barcoded AgNPs and optimized their detection by inductively coupled plasma mass spectrometry (ICP-MS) and laser ablation ICP-MS. This approach allows ultrasensitive parallel auditioning of peptide-guided and control AgNPs in the same biological test systems [2,3]. Furthermore, peptide-targeted AgNPs can serve as a carrier for cytotoxic payloads to receptor-positive cancer cells [4].
In addition to the synthesis and characterization of the nanoparticles, we show that AgNPs loaded with a potent anticancer drug, monomethyl auristatin E (MMAE), and targeted with Neuropilin-1-targeting C-end Rule peptide, RPARPAR, accumulate in prostate cancer cells overexpressing the receptor protein and cause selective toxicity in these cells through the release of MMAE by lysosomal cathepsin B (Figure 1). Importantly, the specific cytotoxic activity of RPARPAR-MMAE-AgNPs (vs. non-targeted MMAE-AgNPs) can be potentiated by dissolution of the extracellular nanoparticles with a mild biocompatible etching solution [1]. We demonstrated that the binding and subsequent internalization is Neuropilin-1-dependent (Figure 2), and quantified the uptake of MMAE, which was delivered to cells on AgNPs, by HPLC-MS. RPARPAR-MMAE-AgNPs were also tested in a mixed culture of target and control cells, where specific elimination of target cancer cells was clearly evident. Several assays were used to evaluate the cytotoxicity of these nanoparticles.
These studies suggest that the AgNP platform can be used for quantitative nanoparticle biodistribution studies and as an anticancer drug carrier *in vitro*, and that elimination of extracellular AgNPs by exposure to etching solution can be used as an endocytosis research tool and as a means to improve the therapeutic index of AgNPs loaded with anticancer payloads. Validation of peptide-targeted MMAE-AgNPs as an effective drug carrier *in vivo* are currently under way.

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