Plasmonic nanoparticle interaction with cell for photoacoustic cancer imaging

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Abstract: Molecular imaging promises to expand existing medical imaging techniques beyond the typical visualization of anatomical features to incorporate functional and pathological information based on contrast agents that include targeting capabilities.

Photoacoustic (PA), imaging a noninvasive molecular imaging modality based on optical excitation and ultrasonic detection has the potential for molecular imaging at high resolution and deep inside the tissue. PA imaging contrast agents using gold nanoparticles (Au NPs) plays a key role in the development of an imaging technology with molecular specificity and sensitivity because the primary advantages of Au NPs lie in their large absorption cross section with unique spectra due to the surface plasmon resonance effect and bioconjugation capability, which means that the gold nanoparticles can be specifically targeted to molecules and/or cells (Thakor *et al*., 2011).

The purpose of our study is to establish deeply penetrating PA molecular imaging technology. We performed a comprehensive PA measurement of various Au NPs to design exogenous imaging agents for enhancing the contrast. The PA signal amplitudes were sensitive to the size and shape of the gold nanoparticles. We also investigated the effects of surface charge of Au NPs on photoacoustic (PA) signal from cultured various cell (Figure 1, Ishihara *et al*., 2014). Our findings are crucial to the design and synthesis of Au NPs as PA imaging contrast agents with maximized diagnostic efficacy.

Keywords: Photoacoustic, Gold Nanoparticle, Polyhedron, Anion, Cation, Localized Surface Plasmon Resonance, cellular uptake, Ultrasound, Pulsed Laser, Tomography, P(VDF-TrFE) film

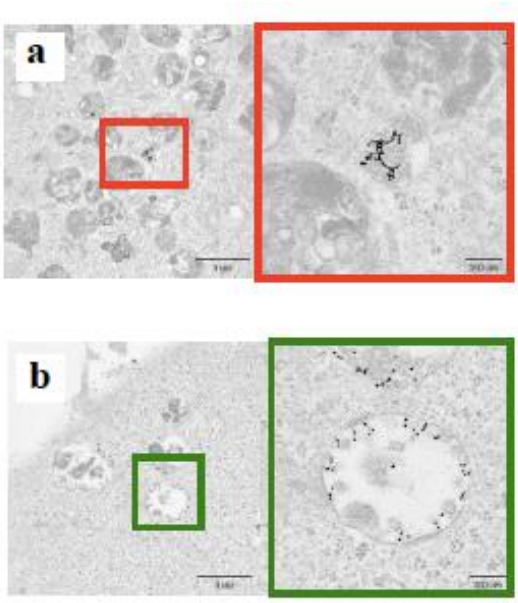

Figure 1: TEM images of different surface charges of Au NPs in cultured A549 cell (3 hours incubation). The cationic Au NPs do not aggregate in the cell (b), but on the other hand, the anionic Au NPs aggregate (a). The uptake of Au NPs by A549 cells (non-phagocytic cells) was highly dependent on the types of surface charge.

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Novel hybrid nanoparticles using upconversion luminescence for *in vivo* imaging

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Abstract: Currently, targeted fluorescent nanoparticles (NPs) have become a major interest in the field of nanomedecine. Especially, upconverting nanoparticles (UCNPs) have attracted much attention due to their peculiar properties, relevant for bioimaging applications in the infrared range. In this work, we developed new multifunctional UCNPs for *in vivo* imaging, particularly in the case of prostate cancer.

These materials permit the conversion of near infrared (NIR) radiations into photons of higher energy (NIR, visible and ultraviolet) via a multiphoton mechanism. In comparison with UV excitation, fluorescence imaging based on NIR light, which is only weakly absorbed by biological tissue, leads to deeper and non-invasive diagnosis. Among the lanthanide doped materials, $NaYF_4$ was reported as the most efficient lattice for UC process. This well-known host was synthetized via a thermolysis process using oleic acid and octadecene in order to obtain spherical and crystallized nanoparticles with a narrow size distribution.

Moreover the UCNPs are functionalized in order to make them biocompatible and decrease their toxicity. To this aim, we have used the ligand exchange method, with two types of molecules: PEG-phosphate and dendronized molecules. In fact these previous molecules are more and more developed for biomedical applications.

Then, the interaction of the water-soluble UCNPs with living cells was investigated. In particular, LNCaP prostate cancer cells were incubated with nanoparticles and have shown low toxicity.

Finally, some *in vivo* tests have been done on mice in order to shown the feasibility of our UCNPs for biological application. Figure 1 presents the *in vivo* whole body images of nude mice with injections of a solution at 1 mg/mL of $NaYF_4$@dendrons (50µL intramuscular –IM- and 20µL subcutaneous – SC). A significant UCL signal was observed for both injections and show the potential of these UCNPs.

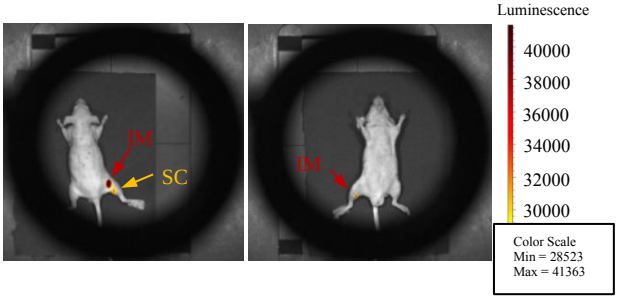

Figure 1 : In vivo imaging of NPs@dendrons injected in mice (power of the laser = 71mW/cm²)

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Efficient and Spatial-Selection Delivery of Quantum Dots in Live Cells by Gold Nanoparticle Medicated Photoporation

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Abstract: Quantum Dots (QDs) as fluorescent probes have attracted considerable interest in many biological and biomedical applications, especially cellular imaging. In comparison with conventional molecular dyes, QDs have several unique advantages such as broad absorption with narrow emission spectra, high resistance against photobleaching, and size-tunable fluorescent emission. To date, QDs have been successfully applied to label fixed and permeabilized cells, or to label membrane proteins in living cells. To extend their use for more general subcellular labeling of living cells, efficient cytosolic delivery into live cells is required, which remains a major challenge. Recently, gold nanoparticle (AuNP) medicated photoporation was shown to be an efficient, non-toxic approach to deliver macromolecules from tens of kDa to hundreds of kDa in live cells. By irradiating AuNPs attached the cell membrane with pulsed laser light of sufficient energy, 'explosive' water vapour nanobubbles (VNB) can be formed around these AuNPs. When the thermal energy of the AuNP is consumed, the VNB violently collapses and causes local damage to the cell membrane by high-pressure shock waves. Using this approach we demonstrated that large macromolecules, such as 500 kDa FITC-dextran, could be introduced into the cytoplasm with negligible cytotoxicity (Xiong *et al.* ; 2014). Considering that the size of PEGylated QDs is similar to the hydrodynamic radius of 500 kDa dextran, here we have evaluated laser-induced VNB mediated membrane poration can also efficiently deliver QDs in cells with low toxicity as shown in Figure 1. Additionally, we also show a spatial-selection delivery of QDs can be easily realized by this AuNP meditated photoporation with laser patterned scanning the cell sample (Figure 2).

Keywords: QDs, spatial-selection delivery, photoporation, vapor nanobubble, gold nanoparticle.

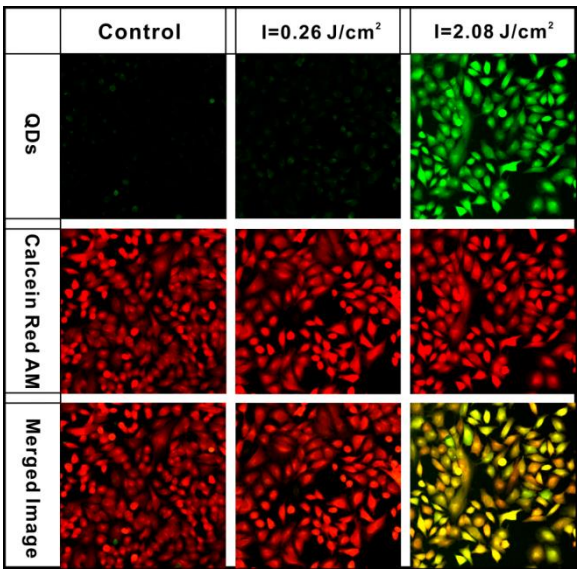

Figure 1: Confocal images showing the delivery of PEG-coated QDs in the green channel (first row) and the viability of HeLa cells labeled with calcein red AM in the red channel (second row). An overlay of green and red images is shown in the bottom row. The first of column is the negative control (cells treated with 2.08 J/cm^2 laser light but without AuNPs), the second and third column show the delivery efficiency of QDs as a function of laser fluence. The field of view is 410 μm by 410 μm.

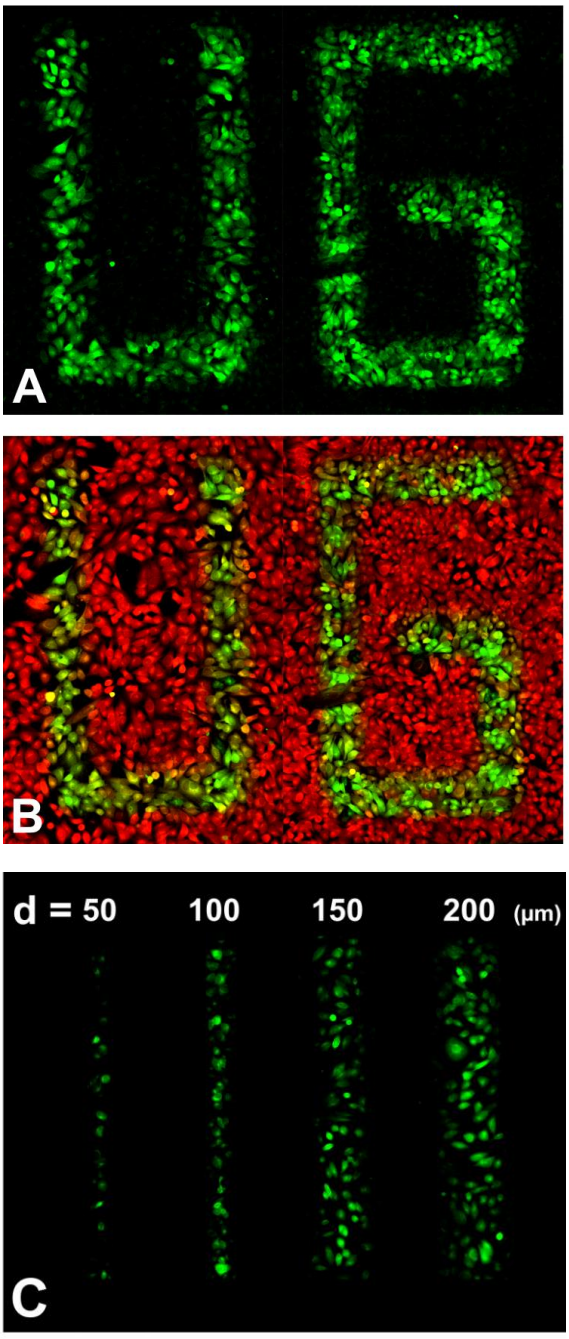

Figure 2: Spatial-selection delivery of QDs into live cells by VNBs inducing photoporation. A. Confocal image in green channel shows the QDs were selectively delivered into the cells in the patterned region by letters of 'U' and 'G' after VNBs inducing photoporation treatment with laser fluence of 2.08 J/cm^2. B. An image merged red channel of cell viable fluorescence of Calcein red AM with the channel of green fluorescence of QDs. C. Linearly patterned scanning the cell samples to estimate the delivery resolution of spatial selection by adjusted the laser beam size from 50 μm to 200 μm at a fixed laser fluence of 1.81 J/cm^2 The size of letters 'U' and 'G' in A and B is 800 μm by 400 μm.

Reference:

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Nanoparticles functionalized with an antibody: toward an specific contrast agent of brain tumors by MRI

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Abstract: Endothelin receptors are clearly identified and related with initiation and progression of certain type of cancers[1]. Glioma, the most common primary brain tumor in humans, is associated with a deregulation of the endothelin axis, leading to an overexpression of endothelin B receptor (ETBR)[2,3]. Targeting glioma cells with the monoclonal antibody rendomab B1 (RB1) directed against ETBR[4], is a promising therapeutic strategy[5]. Antibodies are playing a central role as targeted therapeutics for oncological applications[6]. Concurrently, iron oxide nanoparticles (NPs) are promising tools for molecular imaging applications, including magnetic resonance imaging (MRI), exploiting their superparamagnetic behavior. This study aims to develop an experimental multimodal nanoplateform : iron oxide nanoparticles functionalized with a specific antibody and fluorescent probes for the diagnostic by MRI and the guided surgery.

In this project, iron oxide nanoparticles surface was directly coated with caffeic acid and functionalized with antibody. *In vitro* relaxometry measurements were performed to assess longitudinal r_1 and transverse r_2 relaxivities at 7 T. *In vivo* experiments were carried out on Swiss mice by acquiring T_2*-weighted images before and after intravenous injection of NPs and computing 3D angiograms to reveal vasculature enhancement.

We obtained the first imaging tracer based on iron oxide NPs functionalized with fluorescent dye-labelled RB1 antibody leading to a bi-modal contrast agent. This new tracer conserved the recognition properties for ETBR for both affinity and specificity and a fluorescent visualization was obtained with the functionalized NPs, as demonstrated by flow cytometry experiments. Additionnaly, the iron oxide nanoplatform exhibited highly efficient MRI contrasting properties to be detected in mouse brain when acquiring *in vivo* images at 7 T.

In conclusion, the main outcome of this work is a fully functional tracer targeting the membrane protein ETBR overexpressed in the glioma and playing a key role in the development of the tumors. This new generation of imaging tracer with a constraint nanometric size, as obtained here, leads the way to new promising therapeutic approaches to target glioma tumoral cells, and with a great potential to deeply enhance the detection threshold of small tumoral masses.

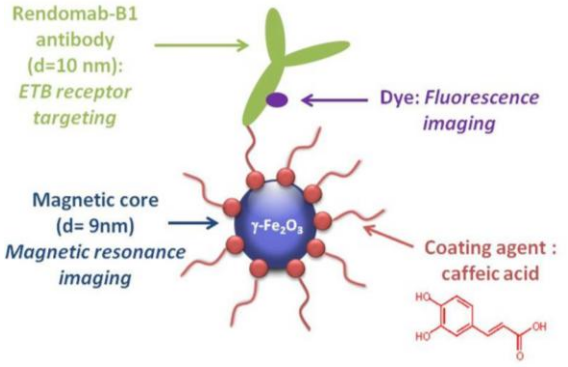

Figure 1: Multimodal Superparamagnetic nanoplatform functionnalized with antibody for dual imaging of brain tumors

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Specificity and Sensitivity comparative study between phage PVP-S1 and monoclonal antibody as receptor in polydiacetylene vesicles for *Salmonella* colorimetric detection

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Abstract: Polydiacetylene polymer (PDA) has been intensively studied because of its properties as colour change from blue to red and change from non-fluorescent to fluorescent form due to an external stimulus that lead to a reorientation of the PDA within the organized structure. External stimulus could be temperature, pH, solvent influence, bacteria presence, mechanical stresses and others (Oliveira *et al.*, 2012). Pires *et al.* (2010) support the hypothesis that such phenomena occurred due to conformational changes associated with the functional group rotation around the simple carbon-carbon bond present in PDA chains. When the backbones of PDA conjugated polymer chains are perturbed, the delocalized π-network induces changes in electronic absorption and emission properties (Huo *et al.*, 1999). For a particular colour change, it is possible to incorporate a compound in the polydiacetylene carboxyl groups that will work as a specific receptor for the bacteria detection. This technology can be used for the detection of pathogens and thus is important to avoid food contamination once the standard technology demands long time and people trained.

The selection of the receptor used in the PDA is the first critical step to develop a biosensor with improved selectivity, selectivity and stability. For this reason, the aim of this study was to make a comparative study between two recognition molecules: phage PVP-S1 (Santos et al., 2011) and a monoclonal antibody in the PDA sensor for the detection *of Salmonella*. Antibodies lack specificity, poor separation efficiency and sensitivity. Phages are extremely specific, withstand harsh environments, are economically and easily produced, show high stability during storage and thus present potential for bacterial detection. Overall the selection of the recognition molecule that show the best features is important to develop a simple and rapid sensor for the industry and consumer's life. The specificity of the sensor was proven by using *Staphylococus aures* and *Escherichia coli* as gram-positive and gram-negative controls, respectively.

Other controls as LB medium and with PDA were performed to ensure that the colour change did not occurred due to another external stimulus. Controls maintained the colour around blue (peaks in 640 nm) and upon *Salmonella* Enteretidis presence the colour changed to red (peaks in 540 nm) as verified by spectroscopic analysis. Colorimetric response was calculated as Charych *et al.* (1993) to quantify colour transition (Figure 1).

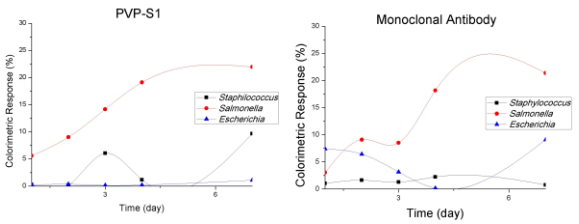

Figure 1: Colorimetric response obtained after the interaction of the different bacteria with with phage PVP-S1 (a) and antibody (b) as specific receptors in the PDA sensor

Monoclonal Antibody showed the highest colorimetric response and the PVP-S1 was more stable. Therefore both receptors improved the PDA sensor sensitivity and specificity and thus can be used as biorecognition molecules to the development of the PDA sensor.

Keywords: polydiacetylene vesicles, monoclonal antibody, *Salmonella* detection, phages, specificity, sensitivity.

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Nanoarchitectonics with Lipid and DNA Building Blocks: *In situ* Millisecond Time-Resolved SAXS Investigation

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Abstract: Cubic lipid membranes are highly efficient for uptake of DNA building blocks (Angelov *et al*., 2013, Angelova *et al*., 2015). The kinetic pathway of self-association of neurotrophic plasmid DNA (pDNA) to cationic cubosomes was investigated by millisecond time-resolved small-angle X-ray scattering (SAXS) coupled to a rapid-mixing stopped flow device. Binding of pDNA to the nanochannel-type cationic lipid nanocarriers considerably modified the curvature of the lipid/water interfaces and led to the formation of onion-type lipoplex complexes. The ultrafast structural dynamics of the complexation and assembly of the lipid particles with pDNA building blocks was revealed thanks to the high brightness of available synchrotron X-ray source. Rapid mixing stopped-flow experiments, coupled to synchrotron SAXS on the subsecond timescale, produced a vast amount of experimental structural data. The purpose of this work is to present the key steps of the SAXS data treatment for the case of self-assembly and complexation of cationic lipid particles (vesicles or cubosomes) with plasmid DNA in the course of a rapid mixing stopped-flow process. This approach is significant for the structural analysis of rapidly forming soft-matter biomacromolecular delivery systems.

Keywords: soft nanoarchitectures, lipid bilayer building blocks, plasmid DNA, bicontinuous cubic mesoporous materials, self-assembled nanochannel networks, membrane curvature, time-resolved SAXS.

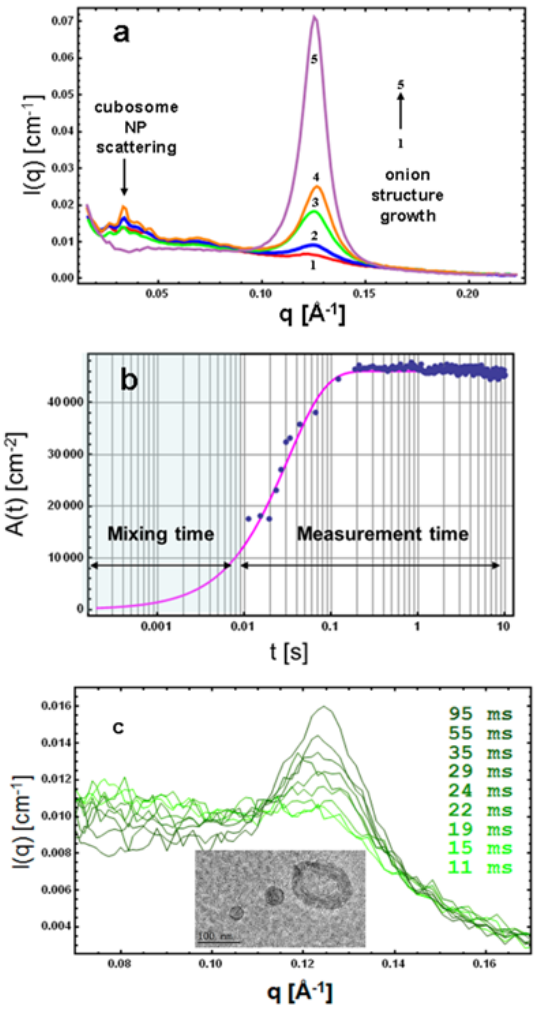

Figure 1: Plasmid DNA-driven transformation of nanochannel-type lipid carriers into tightly packed layered architectures involving compacted DNA macromolecules. (a) SAXS patterns showing that the curved lipid membranes in the cubosome nanoparticles are transformed, with the increase in the pDNA upload, into lower-curvature onion-membrane complexes encapsulating pDNA. (b) Kinetic dependence of the Bragg peaks intensity characterizing the pathway of the lipoplex formation. (c) Millisecond time-resolved SAXS patterns upon *in situ* monitoring of the lipoplex formation with a rapid-mixing stopped-flow device.

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A New Generation of Flower-Like Nanobiocatalyst for Superior Enzymatic Activity

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Abstract: Although several types of enzyme immobilization methods have been developed and used, enzyme activities were decreased or were not reached to satisfactory level yet compared to free enzymes. The activity enhancement has been remained unsolved problem till an encouraging discovery in synthesis of organic-inorganic flower shaped hybrid nanomaterial. Herein we report for the first time to the use of Fe^{2+} to form enzyme based hybrid flower shaped nano particles (HFNP) with superior catalytic activity and stability. In the project, the synthesis of HFNP was carried out by using horseradish peroxidase (HRP) as a model enzyme and Fe^{2+} metal ions as model ions. HRP, as an important enzyme, was selected for its unique properties such as wide catalytic activity, high sensitivity and common substrate specificity. HRP enzyme has been used in many areas as the removal of phenols from polluted waters, organic synthesis, biosensor design, and clinic and micro-analytic. Fe^{2+} metal ions was utilized for both reasons 1) they act as corner stones and main driving forces for the formation of HFNP and 2)it is known that even very low magnetic field has a positive effect on activity of HRP. Thus, we proved that the HFNP exhibited superior enzyme activity and stability due to several reasons such as high surface area of HFNP which does not result in significant mass-transfer limitations, cooperative effects due to nanoscale-entrapped HRP molecules, effective localized HRP concentration and unique shape effect. We demonstrated that the HFNP formed at +4°C in 10 mM PBS (pH 7.4) exhibited dramatically high catalytic activity towards guaiacol used as a model substrate. The HFNP only lost %2 of their initial activity within 30 days when the mixture of HFNP and substrate was stored at +4°C in 10 mM PBS (pH 6.8). In addition to that, we benefited from excellent activity performance of the HFNP, they were rationally and successfully used to oxidize dopamine molecule to colored quinone-type product

Keywords: Enzyme, hybrid nanoflower, activity measurement, metal ion and dopamine detectio

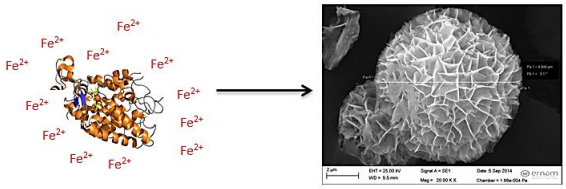

Figure 1: Figure illustrating the formation of organic-inorganic hybrid flower shape nano materials: Fe^{2+} ions incorporated horseradish peroxidase enzyme for the formation of nanoflower.

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Porous nanoparticles entrapped pipette tips for sensitive detection of biomolecules

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Abstract: In recent years, nanomaterials with ordered pores have been widely used for biomolecules detection due to the properties of tuneable pore size, high surface area and easily surface modification. Particularly, the combinations of nanomaterial with some advanced techniques, such as mass spectrometer, ensure the accurate analysis of biomolecules.(Lei, Qian et al. 2013) However, the analysis of some low abundance biomolecules (e.g. insulin) in complex biological samples is still difficult. To solve the problem, a combo-pore approach utilizes materials with different pore sizes for sample pre-treatment has been developed for the sensitive detection of insulin. This advanced approach has been applied prior to mass spectrometer or enzyme linked immunosorbent assay (ELISA) to achieve better detection sensitivity in urine/serum.(Lei, Noonan et al. 2014, Qian, Zhou et al. 2014) Furthermore, a novel combo-pipette-tips (Figure) with different porous materials entrapped are developed as "smart" device for convenient pre-treatment. The application of nanoparticle entrapped pipette tips for sample pre-treatment has the advantages of simple operation and less sample loss. As both combo-pore approach and nanoparticle entrapped tips can be special designed for different targets, they are expected to hold great potential for application in the sensitive detection of various other biomolecules of commercial and clinical significance.

Keywords: nanomaterial, mesoporous, mass spectrometer, biomolecules, pipette tips, insulin, sensitive detection.

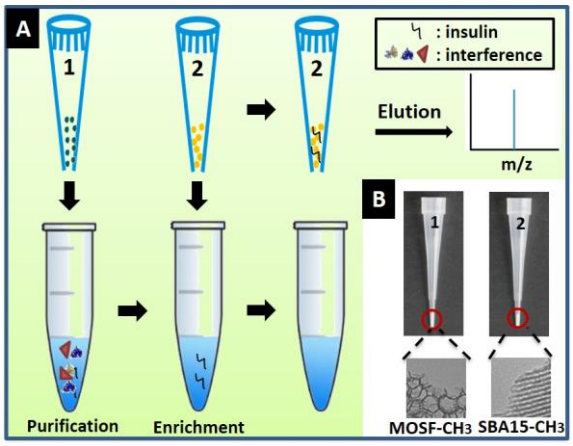

Figure Schematic illustration showing (A) the process of using combo-pipette-tips for insulin detection; (B) TEM images of fabricated tips with macroporous material MOSF-CH_3 (1) and mesoporous material SBA15-CH_3 (2).

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Ultrasensitive magnetic particles/DNAzymes based biosensors for clinical applications

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Abstract: Traditional *in vitro* diagnostic is based on assays and procedures optimized for modern clinical laboratories, equipped with all the necessary instrumentation. However, these high-tech and costly tests are not suitable to address the current diagnostic challenges of preventive medicine, ultra-early diagnosis, and large-scale population screenings. To address these issues, innovative diagnostic tests need more simplified procedures, resulting in cheap, rapid, instrument-free analyses that can be performed also in non-specialized settings, at the point-of-care (POC). In this framework, we show here two hybrid strategies for the sensitive detection of Human Papilloma Virus (HPV) and RNases activity. HPV is a very important analytical target, due to its broad diffusion and clinically relevant association with cervical cancer. Generally, the main obstacle for developing simplified testing for nucleic acid targets is their rather low concentration in clinical samples that does not match the sensitivity of most common assays; usually, the target needs to be amplified by PCR (Polymerase Chain Reaction), which requires dedicated instrumentation. In this context, we developed a polymerase reaction free, low-cost and sensitive assay for the colorimetric detection of HPV, based on the use of a smart design exploiting magnetic microparticles, chimeric RNA/DNAzyme oligonucleotides, and double signal amplification (Fig. 1). This method allows obtaining a fast response with a detection limit of 10 pM, avoiding the amplification of the target via traditional PCR. On the other side, the detection of RNases activity has also great importance in a wide range of biomedical applications, including assessment of RNase contamination in molecular biology and screening of new antiviral drugs and RNase inhibitors. In this regard, we proposed a new versatile strategy that allows the detection of several classes of RNases with better sensitivity then existing assays. Our two-step approach consists of a DNA-RNA-DNA chimeric Hairpin Probe (cHP) conjugated to magnetic microparticles (MMP), a DNAzyme sequence and molecular beacons (see Fig. 2).
Our assays permit the detection of low amount of analyte by the use of an amplification step exploiting the catalytic activity of DNAzyme molecules. These characteristics, coupled with the small volume of samples needed by our approaches, make our assays suitable for future implementations, such as low cost Lab-On-a-Chip and high-throughput screening applications.

Keywords: HPV, DNAzymes, Magnetic microparticles, RNase, chimeric DNA-RNA.

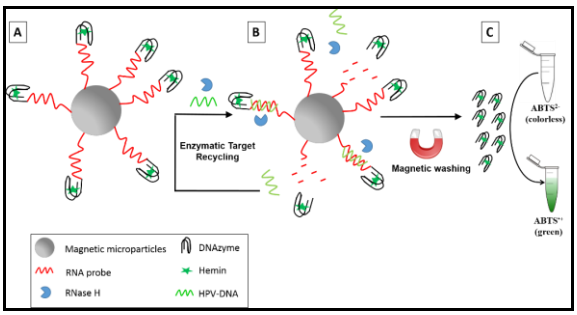

Figure 1: Schematic representation of the colorimetric detection platform based on RNase H-assisted target DNA recycling and generation of the colorimetric signal catalyzed by the peroxidase-mimicking DNAzyme. (A) DNAzyme-hemin complexes are immobilized onto magnetic microparticles (MMPs) via streptavidin-biotin interactions. (B) HPV DNA target recycling in the presence of RNase H. (C) DNAzyme-catalyzed oxidation of the ABTS substrate and generation of the colorimetric signal.

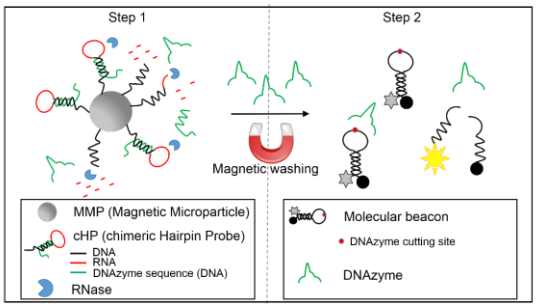

Figure 2. Schematic illustration of the two-step RNase assay. The DNA-RNA-DNA chimeric Hairpin Probe (cHP) is immobilized by biotin-streptavidin interaction onto Magnetic Microparticles (MMP) in order to obtain the MMP-cHPs complex. In the first step, the digestion of the RNA portion of cHPs by RNase allows the release of DNAzyme. After magnetic washing, the released DNAzyme is added to FAM/Dabcyl molecular beacon. In the second step, the catalytic activity of the DNAzyme on the molecular beacons generates fluorescence signal.

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Immobilization of pyranose 2-oxidase onto functionalized electrospun regenerated cellulose ultrafine fibers: a novel heterogeneous catalyst

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Abstract: Pyranose 2-oxidase (P2O), catalyzing the oxidation of aldopyranoses and useful for sugar synthesis, was successfully immobilized onto electrospun regenerated cellulose (RC) ultrafine fibers with diamaters of 229 ± 42 nm. First, the electrospun cellulose acetate fibers were fabricated from 17% w/w CA (MW ~ 3 x 104 g/mol) with 5% w/w Tween 80 dissolved in mixed solvent of water (25% w/w) and acetic acid (75% w/w) at an applied voltage of 25 kV and a fiber collection distance of 10 cm. Then, the fibers were immersed into 0.5 M ethanolic potassium hydroxide solution to hydrolyze the CA fibers and obtain RC fibers, treated with glutaraldehyde to generate aldehyde groups on the fiber surface, and immersed in P2O buffer solution to immobilize it onto the RC fibers. Effects of the immobilization process on thermal stability and pH of P2O activity were investigated. Results indicated that P2O immobilized onto RC fibers tolerated the temperature changes and pH 2 better than the free P2O did, and the optimum pH for P2O and P2O-RC operation was 5.5 and 5, respectively. Therefore, P2O-immobilized RC fibers are potentially useful as a heterogeneous catalyst under the conditions in which free P2O could not endure.

Keywords: electrospinning; ultrafine fiber; enzyme immobilization; cellulose; pyranose 2-oxidase.

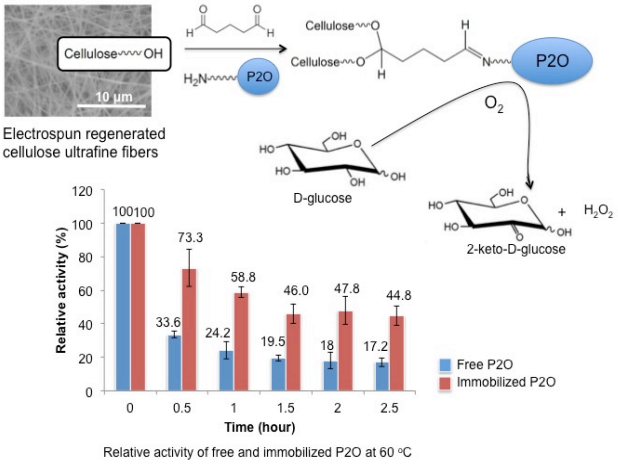

Figure 1: Pyranose 2-oxidases (P2O) were immobilized onto the electrospun regenerated cellulose ultrafine fibers (RC) and were used as catalyst in oxidation of D-glucose. P2O-RC shown better high temperature tolerance than that of P2O.

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Ultra-sensitive Silicon Nanowires for hormone detection

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Abstract: From clinical diagnostics, explosive detection to biomarker detection, POC devices have many applications. Detecting biomarkers at low levels can lead to the detection of illness and disease earlier on, resulting in better prognosis. As more and more biomarkers are being discovered the need for the development of biosensors is becoming increasingly apparent. Salivary biosensors are particularly desirable as they exhibit many advantageous traits such as decreased sample preparation time whilst also limiting the need for more invasive procedures. The ability of semiconducting Silicon nanowires (SiNWs) to convey electrical signals along with their small, portable size and relatively low costs make them a desirable platform for the development of point-of-care (POC) biosensors. Here we demonstrate the fabrication process of SiNWs using a combination of electron beam lithography (EBL), ultraviolet (UV) lithography and Substrate Conformal Imprint Lithography (SCIL). The devices were patterned onto a 100 mm diameter silicon-on-insulator (SOI) wafer. This method is highly repeatable with a high-throughput patterning high quality nanostructures on a reduced timescale when compared to more traditional techniques of just EBL and UV lithography making the SCIL method more cost effective [1]. The SiNW devices made with this method were then functionalized to have amine groups attached using the electrochemical method of diazotization. Diazotization consists of the reductive grafting of the aryl salt diazonium to the sensor surface using cyclic voltammetry followed by the reduction of a nitro group to an amine group by chronoamperometry. As a result this presents an opportunity to attach of a bio-receptor of choice [2]. The devices were then biofunctionalized with an antibody against a hormone biomarker and a calibration was carried out for different hormone concentrations. The methods used for analyte detection were electrochemical impedance spectroscopy (EIS), cyclic voltammetry and simple I(V) measurement. The devices were compared to an ELISA to compare the limits of detection, both at the lowest concentration and at the concentration of saturation

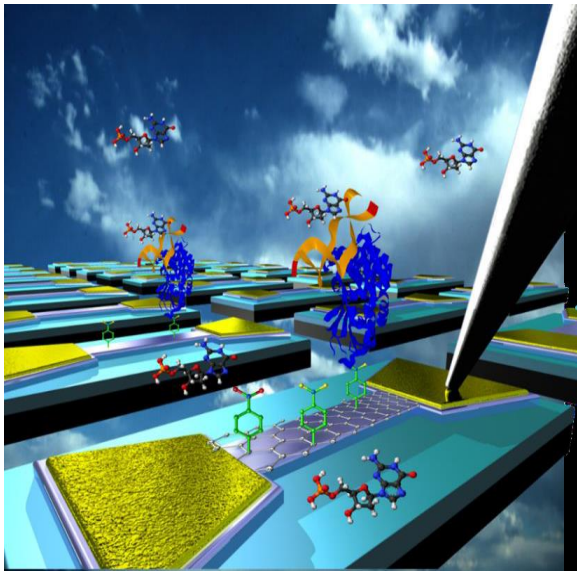

Figure 1: A schematic showing the functionalization of the SiNW surface using the diazotization method (reprinted from Mohd Azmi MA).

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Blocking Viral DNA Replication by Employing a Resonance Frequency Generated by Ag4O4 — A Real World Solution.

Presented by: Leigh J. Mack, MD, PhD
CIMTESES Foundation, Curacao

Abstract: Ag4O4 is a tetrahedral nanocrystalline silver particle that has been found to have some very specific attributes in inhibiting DNA polymerase and reverse transcriptase in several strains of viruses. It is believed that the virus to cell signalling prior to cell receptor contact is hindered. This signalling is disrupted by the outer ring electrons of Ag4O4 creating a small magnetic/charged field that disrupts the viruses from being able to make direct contact with receptor on the cell membrane or the phospholipid layer . The multiple valency property of the Ag4O4 particle is short multiple electrons in the outermost ring. This condition creates a gaining and losing of electrons at a rate that has been found to create a small magnetic field. This field can thus disrupt viral replication efficacy without damaging the surrounding cells of the body. The presentation shall include multiple studies with solid evidence of the efficacy of this particle against viruses along with information and studies of the safety level of this technology.

Keywords: Ag4O4, silver oxide, silver crystalline nano particle, frequencies for viral disruption, viruses, nanomedicine in practice.

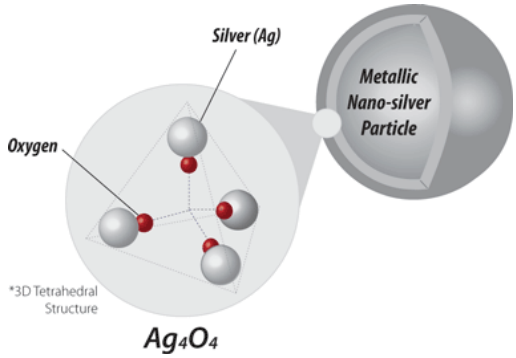

Figure A

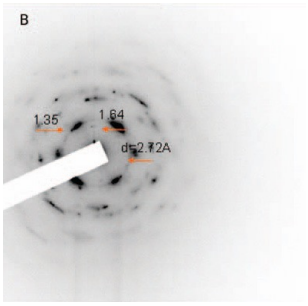

Figure B

Figure A: Figure illustrates the Ag4o4 tetrahedral structure and how it is a unique silver ion. Figure B illustrates the size and electron rings of the Ag4O4 nanoparticle. Figures are courtesy of American Biotech Labs, Inc.

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Synthsis and cytocompatability of functionalized multi-walled carbon nanotubes derivatives

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Abstract: Innovative functionalized multi-walled carbon nanotubes (MWCNTs) are emerging tool in the nanobiotechnology applications. However, thier toxic effects on environment and health have become an issue of strong concern. In the present study, we address the impact of functionalized MWCNTs on different cell lines such as: normal melanocytes (HFB4), liver carcinoma (HEPG2) and breast carcinoma (MCF7). Moreover, the prepared nanomaterials were characterized using different analytical tools; Fourier transform infrared spectroscopy (FTIR), X-ray diffraction patterns (XRD), differantial scanning calorimetry (DSC), scanning and transmission electron microscopes (SEM and TEM). The results showed that most of the functionalized MWCNTs exhibited low cytotoxic effects in comparison with the unfunctionalized ones.

Keywords: nanobiotechnology, multi-walled carbon nanotubes, cytotoxicity, cell growth, cell adhesion, grafting copolymerization.

Acknowledgment: This work was a part from the project which financially supported by National Research Center, Cairo, Egypt.

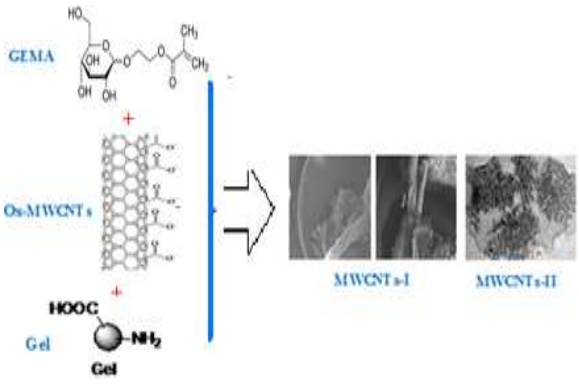

Figure 1: illustrating schematic representation of the prepared functionalized multi-walled carbon nanotubes using (Gel) gelatin and 2-glycosyloxyethyl methacrylate (GEMA).

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Development of high sensitive devices using optical tweezers and diamond nanocrystals

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Abstract: The development of high sensitive detectors at the nanoscale is of crucial importance for understanding the dynamics of individual biological specimens such as proteins or analytes and the subsequent implementation of protocols in molecular diagnoses and early disease detection. Here we report recent progress in studying the mechanical properties of DNA aptamers related with the red tide using optical tweezers. We have constructed a macromolecule consisting of a lambda DNA sleeve – DNA aptamer – lambda DNA sleeve. By attaching functionalized dielectric microspheres to both ends of this macromolecule we study its mechanical properties using a multiple-trap home-built optical tweezers. For generating multiple traps we used a two-acousto optical deflector in x-y configuration able to switch from trap to trap in less than a microsecond. Each dielectric particle is trapped by intermittently by switching the trap on each particle creating a trapping potential on average. The force applied to the macromolecule is measured by a quadrant photodiode. In addition, we report the development of a device based on diamond nanocrystals able to read the concentration of proteins/analytes or reading the properties of a fluid. We use the optical response to external perturbations, such as magnetic and electric fields, of nitrogen-vacancy color centers in diamond nanocrystals (Maze *et al.*, 2011) to monitor in real time the concentration of analytes and fluid properties. We functionalized the surface of nanocrystals with ad-hoc ligands such as peptide CLPFFD to enhance the affinity to specific analytes such as toxic aggregates of A-beta protein. The effective change of surface charge at the surface of nanocrystals changes the properties of color centers such as their fluorescent spectra and spin coherence that we monitor using a home-built confocal microscope and microwave spectroscopic techniques such as Ramsey and echo spectrocopy.

Acknowledgements: we acknowledge support from CONICYT Associative Investigation Program (PIA) grant No ACT1108 – Nanobiotec.

Keywords: aptamers, optical tweezers, diamond nanocrystals, color centers, ligand/analyte interaction.

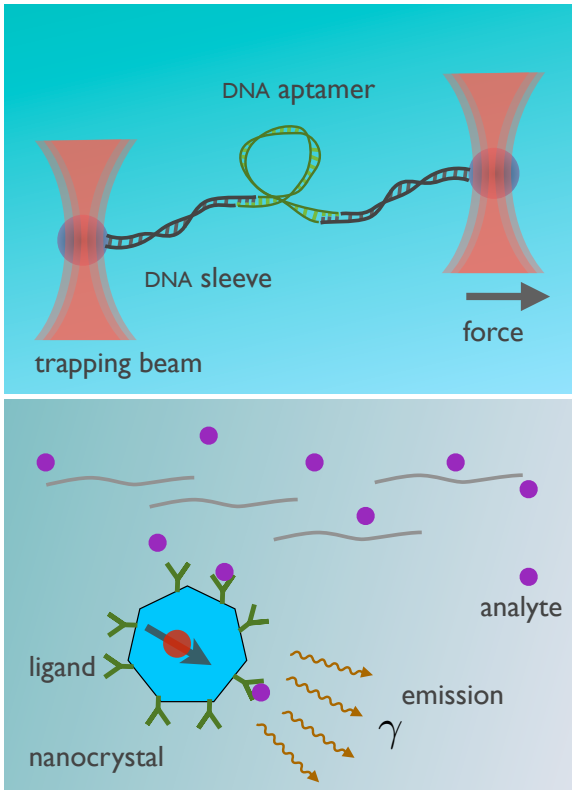

Figure 1. Top: illustration of the optical tweezers configuration to study DNA aptamers. A particular termination of DNA sleeve has been used to attach a DNA-aptamer at both ends. Each DNA sleeves is attached to dielectric microspheres trapped by a multiple trap optical tweezers. Bottom: illustration of the device using surface functionalized diamond nanocrystals. By observing the emission of color centers in the nanocrystal we infer the concentration of analyte in the fluid.

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Versatile and easy to fabricate advanced surfaces to enhance the performance of DNA microarray detection

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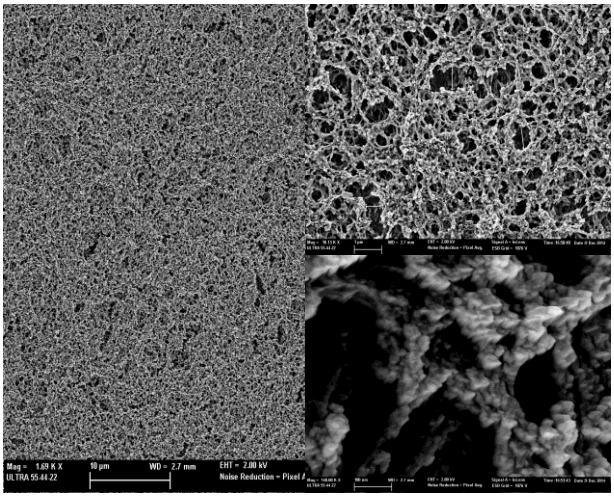
Abstract: Nature shows us the capability of micro/nanostructured surfaces to become into water-repellant surfaces, a phenomena known as *lotus effect*, because it is observed in lotus leaves (Dawood et al. 2011). Advances in nanotechnology, including micro/nanoelectromechanical systems (MEMS/NEMS) have stimulated the development of new materials directed at artificially replicating such biomimetic surfaces for applications such as self cleaning, antifogging surfaces, humidity control for electronics devices, etc (Celia et al. 2013). But, most of the described methods are costly, and require specific equipments, or harsh conditions, being difficult to use them on many fields, specially those related to the biological field.

Regarding DNA detection, the microarraying of nucleic acids (NA) on solid supports has become an area of fundamental interest (Sassolas, Leca-Bouvier, and Blum 2008). Microarrays are an alternative to homogeneous assays because they allow easy continuous monitoring and miniaturization. Thus, they have been used in clinical diagnosis and environmental monitoring, among many other applications.

However, enhance the performance of microarray technologies in issues such as sensitivity, and specificity, remains still a challenge that can be hardly solved using conventional surface chemistries.

In this work, we hypothesised that to consider the material, the surface, the probe attachment and target recognition in an holistic manner; and combine it with superhydrophobicity/superhydrophilicity surface modulation, can drive to the design of advanced microarrays with improved performance. For that, we functionalised glass slides with a micro/nanoporous polymer employing a modification of the procedure described recently by Levkin (Feng et al. 2014). Then, the polymer was modified by organosilane chemistry, and thiolated NA probes were covalently attached to the surface by thiol-ene click chemistry, to create superhydrophilic domains. The rest of the surface was derivatized to reach superhydrophobicity. The microarray created in that way was evaluated by hybridization assays with the fluorescence labelled anti-probe, and compared to other conventional microarray surfaces. Our results show that the holistic consideration increases up to 5fold the signal observed after the hybridisation, compared to standard microarrays. These high performance microarrays could have potential application in fields where high sensititvity is required, such as microRNA detection.



Keywords: micro/nanoporous surfaces, nucleic acid detection, microarray, thiol-ene click chemistry, holistic surfaces.

Figure 1: FESEM images at different magnifications of the micro/nanoporous polymer employed to develop the high performance microarray.

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DNA detection using Si-nanosandwich

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Real-time amplification and detection of nucleic acid has given rise to the development of life science research and molecular diagnostics. These methods became a basis of the techniques that are applied for express detection and quantification of small amounts of nucleic acid and have a wide array of applications. However these techniques for real-time detection of nucleic acid require precision optics as well as fluorescently labelled sequence-specific probes or fluorescent dyes for DNA labelling. Therefore this is a huge disadvantage of such techniques due to the indirect oligonucleotides signal collection. Several attempts have been made to resolve this problem.

Recently, a semiconductor-based nucleic acid sequencer that uses the pH-sensing capability of ion sensitive field effect transistors (ISFET) has been demonstrated[1]. Another device that is able to amplify and simultaneously detect DNA using embedded heaters, temperature sensors and ISFET sensor arrays appears to be also very effective[2]. The most important result of the works mentioned was to provide the amplification and the detection simultaneously. Nevertheless, despite the development of an ISFET technology[3], there are still challenges that cannot be met with it. Since the principle of an ISFET-based sensor is the pH sensing mechanism that is not target-specific, this leads to the most crucial disadvantage of it.

Here we present a new method of oligonucleotides detection by the excitation of their self-resonant modes that correspond to the unique combination of the nucleotide sequence and the whole molecular shape.

Therefore the method suggested to detect the oligonucleotides is based on the interaction of Silicon nanosandwich with nucleic acids deposited on its surface. This Silicon nanosandwich represents the ultra-narrow p-type Silicon quantum well, confined by the delta barriers heavily doped with Boron on the n-Si (100) wafer. The edge channels of this QW have been shown to be effective source of THz emission[4], [5]. In order to enhance the selective THz line emission, the corresponding system micro cavities is inserted in the plane of QW. Such devices allow the creation of the THz spectra that are close to oligonucleotides self-resonant modes. Thus, the self-resonant modes excitation of oligonucleotides deposited in the QW plane becomes possible, with provided by feedback giving rise to the changes in the conductance of edge channels.

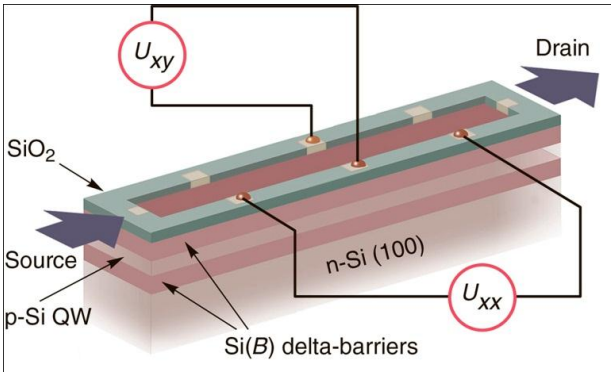

Figure 1: Silicon nanosandwich that represents the ultra-narrow silicon quantum well of the p-type Si confined by the delta-barriers heavily doped with boron on the n-type Si (100) wafer.

Keywords: DNA detection with semiconductor device, THz pumping, silicon nanosandwich, short oligonucleotides detection.

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DNA Sensing at femtomolar level using microfluidic electro-chemical cell: advantages of carbon-based transducers

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Abstract: The development of extremely sensitive, highly selective, simple, robust and yet inexpensive biosensing platforms is essential for a wide range of applications, including clinical diagnostics, environmental monitoring and food safety testing. Since accurate diagnostics need specific detection of very small amounts of DNA, there is a need to develop simple label-free DNA hybridization platforms without extensive sample pretreatments able to detect very low concentration below the femtomolar.
Microfluidics for the manipulation of small volumes of biological fluid is a tool of choice that allows multiplex analyses in independent micro-channels. Electrochemical detection appears as a gold start method for biosensors development since it gives direct sensitive measurement in real sample and is fully compatible with handheld systems (Miodek *et al*, 2013). Pairs of electrodes can be simply integrated into microchannels using conventional lithography processes (Faure *et al*, 2014; Méance *et al*, 2014). These electrochemical fluidic devices can thus be connected to handheld electronic readers giving access to portability and facility for their application in point of care system in diagnostic.
We report a microfluidic-multiplexed platform that integrates several electrochemical cells with a reduced volume at one hundred nanoliter scale capable of achieving ultra-sensitive direct electrochemical detection of DNA. Our approach is based on the localized immobilization of DNA on carbon nanotubes in each microfluidic chamber by the electrochemical patterning method. Introduction of redox marker such as ferrocene between the biological receptor and the MWCNTs transducer allows to measure the charge transfer through current of redox signal. The hybridization reaction of immobilized single strand sequence from Hepatitis C virus and their complementary target (HCV virus) is used as biological model. We demonstrate that this hybridization reaction in the confined space of the fluidic microchamber improves the sensitivity of the signal. Electrochemical DNA sensing in such microfluidic device allows direct detection at 0.1 femtomolar level. Compare to a bulk electrochemical cell featuring an identical biosensor and displaying a limit of detection of 1 picomolar, it corresponds to a decrease with four orders of magnitude. Comparison of NTCs with other promising carbon-based transducer materials, such as epitaxial graphene multilayers and reported CVD graphene bilayers will be reported. We envision that this microfluidic approach will find useful future applications in the field of biosensing for practical devices for biology, medicine and environment.

Keywords: DNA, direct electrochemical sensing, microfluidic plateform, detection limit, carbon nanotubes, ferrocene, biomedical applications.

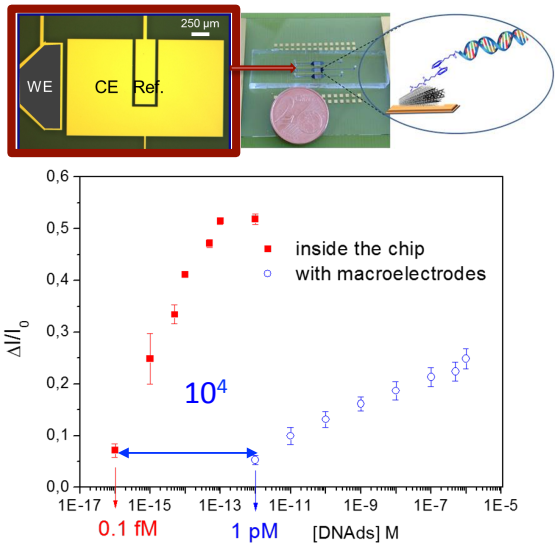

Figure 1: Top view of the fluidic chip with 3 independent microchannels and the electrochemical response as function of DNA concentration.

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Selection of peptide motifs for the detection of small molecules in biotechnological applications

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Abstract: Mycotoxins are fluorescent low-molecular-weight natural products produced as secondary metabolites by fungi in milk and dried fruits.These metabolites are chemical stable molecules that cause disease and death in human beings (Bennet *et al*.,2003). Control strategies are therefore necessary for any food where such toxins could be presented . Current analytical determination includes immuno- and bio-luminescent assays, HPLC (fluorescence detection), TLC, gas and liquid chromatography coupled to mass spectroscopy (Rai *et al*., 2012). However all of them present high costs and skilled quality control operators.

Our idea is to develop a peptide-based biosensor for fluorescence detection in a sensitive, specific and unsophisticated manner. To this aim a integrated approach has been developed to select specific peptide motif to capture aflatoxins. On one side it has been conducted through computation modeling using a Cdocker alghoritm to determine the Binding energy of all possible peptide combinations against Aflatoxin, using as building blocks eight different amino-acids chosen by considering their different chemical properties. On the other, from combinatorial peptide libraries obtained with the same building blocks best Aflatoxin binders were selected by SPR (Surface Plasmon Resonance). In figure 1A in according to docking and SPR analysis, an example of best binder sequence of Aflatoxin M1 is reported.

Peptides sequences selected by proposed approach can be easly integrated in microparticles or covalently attached on microfabricated surfaces, opening the rout towards a direct detection of aflatoxins in small volume both in liquid and solid environments.

An example of selected peptide conjugated on Polystirene-PEG beads for the fluorescence detection of Aflatoxin M1is reported in figure 1C.

Proposed approach is also applicable to other small molecules or contaminat whose sensitive detection is indispensable for food, biochemical or medical applications.

Keywords: Mycotoxins, Peptide Library, Molecular Docking, Polymeric microparticles, Material Functionalization.

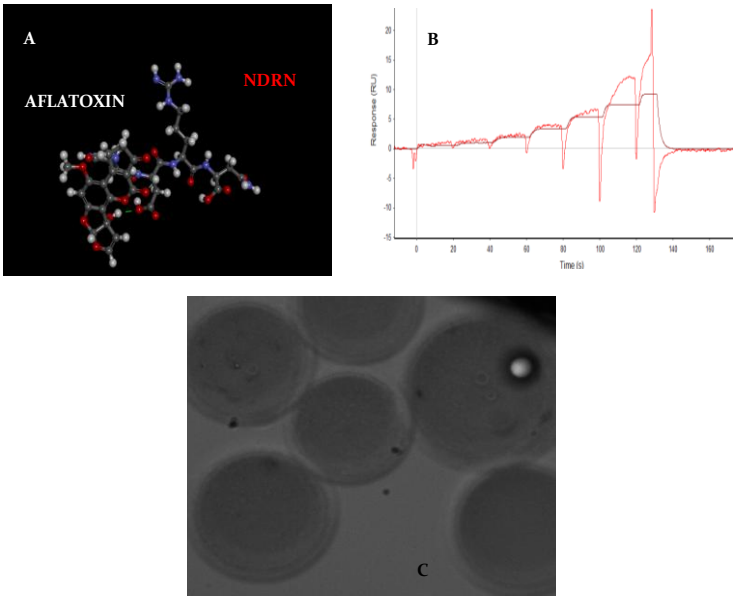

Figure.1:Docking (A) and SPR (B) results of the best Aflatoxin M1 binding peptide sequence. (C) Example of selected peptide conjugated on Polystirene-PEG beads for the fluorescence detection of Aflatoxin M1.

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Covalent functionalization of SWNT with Ciprofloxacin for enhancing its antibacterial activity

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Abstract: Despite the modern medicinal chemistry in designing new therapeutic agents by using different new innovative techniques in molecular modeling and combinatorial chemistry, beside to their expensive cost, infectious diseases continue to be one of the greatest health challenges worldwide (Cohen, M. L., 2000). The main disadvantages for conventional antibiotics are the development of multiple drug resistance and adverse side effects. Recent advancement in nanoscience and nanotechnology has expanded our ability to design and construct nanomaterials with targeting, therapeutic, and diagnostic functions (Cheon, J., et al. 2008; Huang, W. C., et al. 2008). Among nanotechnology-derived materials, carbon nanotubes (CNTs) have stimulated a great interest for biomedical applications because of their unique mechanical, electrical, thermal and spectroscopic properties (Liu, Z.; et al. 2009). Nevertheless, advances in these directions have been hampered by the insolubility of CNTs in most solvents, and most importantly in water where they exist as ropes and large bundles. To overcome these problems we have recently development various approximations for the water solubilzation of SWCNTs (Assali, M. et al.; 2009, 2010).

So, here we aim to develop a new nano-antibiotic based on carbon nanotubes by functionalizing them covalently with Ciprofloxacin antibiotic and proposing that the large surface area of CNT and/or this new nan-prodrug will prevent the bacteria to throw them out once they penetrate the membrane, figure 1.

In the present communication, the following points will be discussed: (i) the modular synthetic strategies developed for the synthesis of the nanoconjugate, (ii) the characterization of the formed nanoantibiotic by various analytical techniques like AFM, UV-Vis, FTIR, TGA, and (iii) the ability of the prepared nanosystems to specifically attack different types of bacteria.

Keywords: nanoantibiotics, carbon nanotubes, ciprofloxacin, bacterial infection.

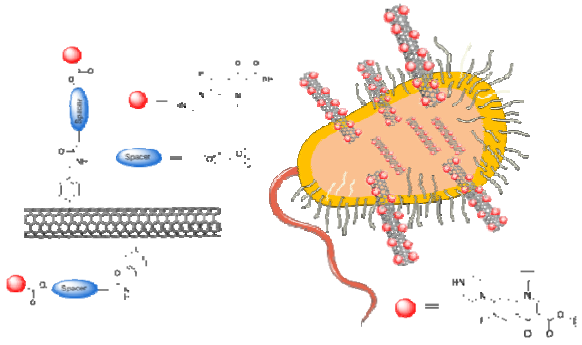

Figure 1: Figure illustrating the covalent functionalization of SWCNT with ciprofloxacin antibiotic (left), the right figure demonstrates the entrance of SWNT-cipro inside the bacteria.

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High-aspect ratio nanostructures for cellular applications

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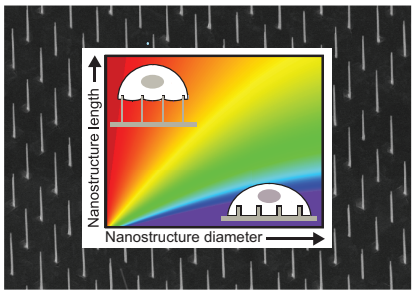
Abstract: The endeavour of exploiting arrays of vertical one-dimensional nanostructures for cellular applications is experiencing a pronounced surge of activity (Bonde *et al.* 2014). The interest is rooted in the intrinsic properties of high-aspect-ratio nanostructures.
With a height comparable to a mammalian cell, and a diameter 100-1000 times smaller, arrays of nanostructures can be interfaced in various ways with cells and are thereby suitable for various applications spanning from transfection of cells to monitoring of intracellular signals. Each of these applications requires a particular interface of nanostructures with living cells, which can be achieved by tuning the nanotopography of the surface (diameter, length and density).
In this communication, we will present a theoretical model of cell settling of arrays of nanostructures allowing the rational design of a suitable nanotopography for the application foreseen (Buch Månson *et al.* submitted). After validating the model experimentally, we will present a study behaviour on arrays of nanostructures and evaluate the effect of the nanotopography on their adhesion, migration and proliferation (Berthing *et al.* 2011, Berthing *et al.* 2012, Bonde *et al.* 2013). The study was performed using ordered arrays of vertical InAs nanowires and the throughput of the systematic cellular studies was facilitated by the design of multidensity nanowire arrays exhibiting 6 different types of nanotopography on a singe chip. We demonstrated that arrays of InAs nanowires provide a cell-promoting surface, which affects both cell division and focal adhesion up-regulation. Furthermore, a systematic variation in NW spacing affects both the detailed cell morphology and adhesion properties.

Keywords: nanowire, nanostructure, nanopillars, nanotopography, live cell biosensing.

A



B

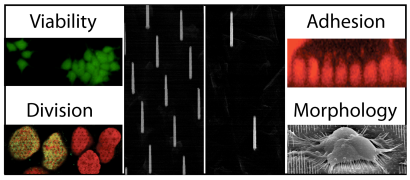


Figure illustrating the influence of nanostructure topography on cell settling (A), and cell behavior on various nanotopgraphies (B).

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A Development of Microstrip Patch Antenna with Graphene and Titanium Dioxide For Orthopaedic Implants

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Abstract: An implantable antenna in biomedical devices is used as a wireless communication device to send and receive data signal between an implantable circuit inside the human body and an electronic receiver outside, as shown in Figure 1. However, implantable antenna design still has many problems including a large size of antenna, biotoxicity of antenna materials to the human tissue caused by metallic material, and the low efficiency of radiation of implantable antennas (Soontornpipit *et al.*, 2004, 2011). This work is a mathematical method to solve these problems. We developed a new design of antenna using mathematical modeling with MATLAB programming. Specifically, in this study, graphene is used as a patch antenna since it has an excellent biocompatibility and flexibility to modify its electrical properties (Fatikow *et al.*, 2012). Titanium, widely used for orthopaedic implants, is used as a dielectric substrate to reduce the antenna size. An EMCoS Antenna VLab software is used to simulate our new design antenna in two environments: inside and outside the human body in order to test the efficiency of antenna radiation. The results of this study suggest that our designed antenna has small size and light weight which can reduce the burden of patient when wearing. The effect of graphene on implantable antenna includes good electromagnetical properties such as return loss or power radiation, moreover, its notching can also improve radiation efficiency of the overall implantable antenna.

Keywords: Biomedical devices, biomedical application, orthopaedic implant, graphene, antenna.

Figure 1: A diagram of implantable antenna used in orthopaedic implant. The antenna is a receiving and transmitting wireless device communicating with the antenna outside the humam body. The efficiency of antenna is varied with its material, size, and shape.

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Nanoscale Modification of Natural Cell-derived Matrices for Tissue Engineering Applications

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Abstract: Interest in the interaction between cells and their surrounding environment continues to grow, underscored by a wide spectrum of studies demonstrating substrate-dependent cellular behavior. Given that the extracellular matrix (ECM) plays a structural role in addition to one of biophysical transduction, understanding its effect on cells is essential. In particular, many groups have shed light on the effect of substrate stiffness on cell differentiation. However, non-biological materials such as polyacrylamide or PDMS are often used in such studies, and as a result, examining the effect of topography and chemistry of natural ECM on cellular behavior is difficult. From this perspective, natural cell-derived matrices – ECM obtained via removal of overlying cells – is an extremely attractive option recently emerging. Herein, we synthesize natural preosteoblast-derived matrices (PDM) and crosslink them with genipin to varying degrees to induce structural changes of the ECM fibers at the nano- and micro-level. An increase in crosslinking density results in a transition of secondary structure from α-helix to β-sheet, and then to random coil structure, as determined via circular dichorism spectroscopy (Figure 1A). Qualitative analysis of TEM images corroborate these observations (Figure 1B). Given the increase in crosslinking density, changes to the PDM stiffness accompany the structural modifications, as determined via AFM. Finally, we examine the effect of using PDM on preosteoblast behavior and report that increasing crosslinking of PDM results in smaller cells with larger aspect ratios (Figure 2A). Furthermore, preosteoblasts were shown to undergo osteogenic differentiation more effectively with increasing crosslinking density of PDM (Figure 2B). This study highlights the advantages of using natural cell-derived matrices over conventional substrates, and we propose that secondary structural changes to an underlying substrate in conjunction with changes in stiffness affect cellular behavior. We envision the expansion of such information for more efficient applications in tissue engineering.

Keywords: natural cell-derived matrices, ECM, osteogenesis, decellularization, tissue engineering

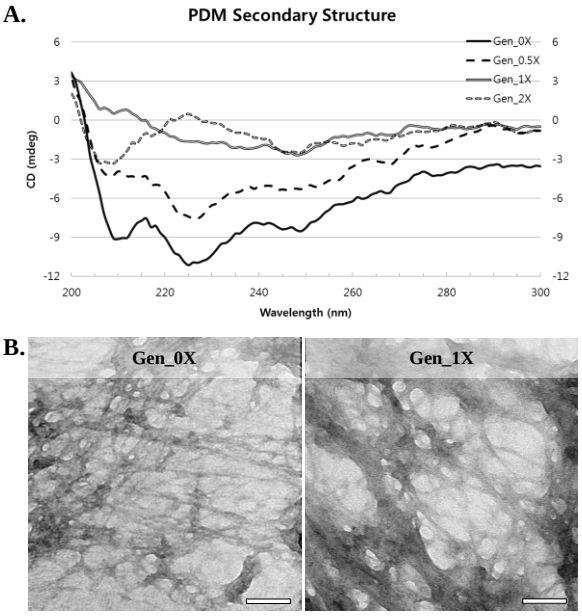

Figure 1. A. CD readings indicate changes to secondary structure of PDM. B. TEM (scale: 100nm)

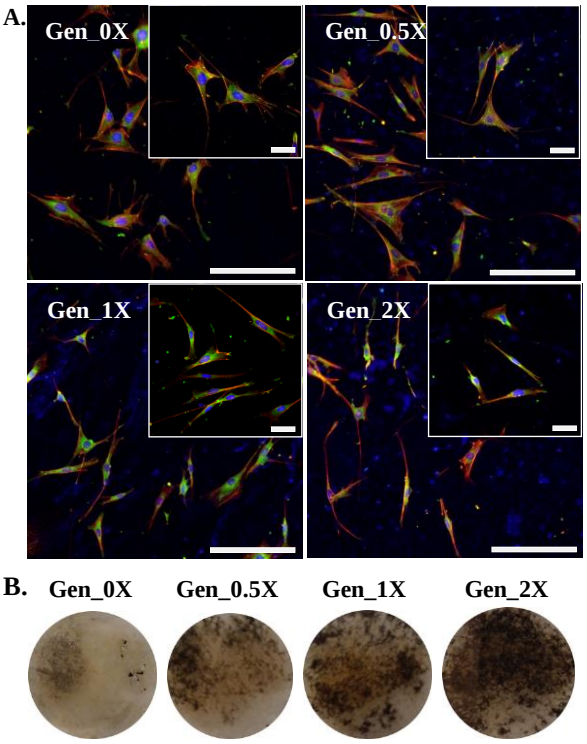

Figure 2. A) Immunofluorescence (R: f-actin; G: vinculin, B: dapi) (scale: 200μm; inset: 50μm). B) Ca^{2+} deposition is examined via Von Kossa staining.

Modular Assembly Gadolinium-Coated Nanoliposomes Enabling Detection of Ischemic Vasculature

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Abstract: Nanoparticle formulations incorporate multiple components, including both diagnostic and therapeutic functionalities, within a single construct. Direct encapsulation of such units, however, can lead to unfavorable interactions between encapsulated materials, as well as reduced performance of the nanoparticle. While surface functionalization serves as a method of spatial organization, subsequent reaction and purification steps can be detrimental to the formulation. To this end, we synthesized a chitosan modified with hydrophobic, octadecyl chains and gadolinium-chelating diethylenetriaminepentaacetic acid, named as a polymeric fastener. In this way, the chitosan fastener was able to link gadolinium, an MRI contrast agent, to the surface of liposomes by self-assembly. Due to the localization of gadolinium on the liposome surface, we were able to achieve enhanced relaxivity compared to gadolinium loaded within the liposome. The resulting gadolinium-coated liposome enabled us to successfully detect and image vascular defects in an ischemic hindlimb and kidney using MRI.

In addition, we improved the stability of the liposome-fastener complex in physiological fluid by cross-linking lipids of the liposome using $DC_{8,9}PC$ lipids, which contained the photo cross-linkable diyne moiety. This approach not only stabilized the liposome, but also enhanced its association with the functional fastener. Interestingly, cross-linking the lipids after attachment of the fastener, rather than prior, was critical in enhancing the initial association with the fastener, as well as stabilizing it in serum. Ultimately, the strategy proved effective in enhancing MRI contrast nearly two-fold per liposome dose after one hour of serum exposure. Taken together, we believe that both polymeric fastener and lipid cross-linking will be broadly useful in spatially organizing functional cues in nanoparticles and further extending their lifetimes.

Keywords: polymer fastener, MRI, vasculature, gadolinium, liposome, self-assembly

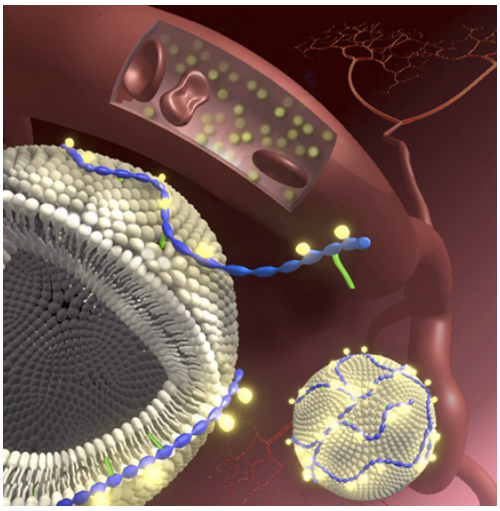

Figure 1: Figure illustrating the gadolinium-coated liposome that accumulate in ischemic vasculature and highlight the area in an MR image

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Pluronic F127 coated superparamagnetic nanoparticles for Human Umbilical Vein Endothelial Cell tracking via magnetic resonance imaging

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Abstract: Cell based therapies have emerged as a alternative approaches to conventional pharmacological treatments due to their multipotential action mechanisms. Integration in the host, immunomodulation processes or growth factors secretion are the possible ways of action, however fundamental questions related to cell type, characterization and dosage, therapeutic timing versus toxicity or the relationship between biodistribution, fate and outcome must be elucidated.[1-4]

In order to monitor *in vivo* administered cells and its relationship with the underlying mechanisms of stem cell therapy, superparamagnetic nanoparticles (MNPs) as magnetic resonance imaging (MRI) contrast agent are the perfect candidate for it. Thus, MNPs labeled cells can be tracked *in vivo* via MRI without harmful effects, providing real-time information about their biodistribution but the pathologic condition of the organ as well.[5,6]

In this study, we have synthesized Pluronic F127-coated superparamagnetic nanoparticles (P-MNPs) and we have validated their use for cell tracking. For such purpose, we have studied the influence of different concentrations of P-MNPs (35µg/mL and 15µg/mL) and incubation times (24h and 6h) in vitro in Human Umbilical Vein Endothelial Cells (HUVECs) culture, by studying cellular proliferation after labeling, the viability of labeled and non-labeled cells, Prussian Blue assessment of labeling (Fig.1A,B), transmission electron microscopy (TEM) of labeled cells to demonstrate the full internalization of the MNPs (Fig.1C), the quantification of internalized iron depending on the concentration and incubation time, and MRI signal of HUVEC cells after P-MNPs labeling. This deep *in vitro* characterization will elucidate the best conditions for HUVEC tagging with P-MNPs for further *in vivo* experiments.

Keywords: Stem cell therapy, superparamagnetic nanoparticles, Human Umbilical Vein Endothelial Cells, cell labeling, cell tracking, magnetic resonance imaging, contrast agents.

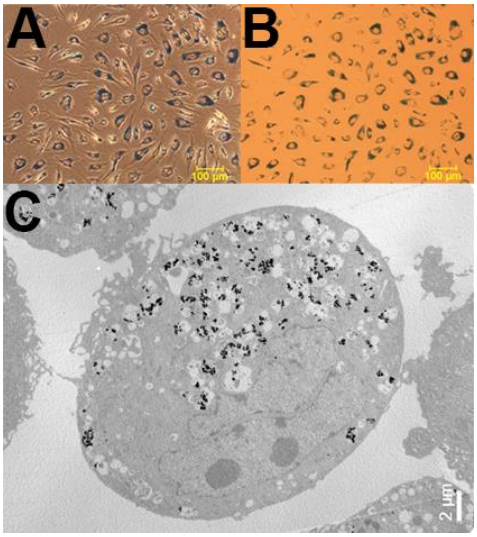

Figure 1: (A) P-MNPs labeled HUVEC cells in phase constrast optical microscopy after Prussian Blue Staining, (B) in bright field optical microscopy after Prussian Blue Staining, (C) TEM micrograph of labeled cells.

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Preparation, Biological Activity and Mechanism of Action of Ag and AgBr Nanoparticles

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[3]Department of Microbiology, Faculty of Medicine and Dentistry, Palacky University, Olomouc, Czech Republic

Abstract: Silver nanoparticles (Ag NPs) and nanoparticles of insoluble silver compounds such as Ag_2O, AgOH or AgX (X = Cl, Br or I) became one of the most discussed branches of nanoscience in the several last decades. Ag NPs attract due to their unique physical, chemical, optical and biological properties great attention in the scientific research. Because of expected inability of bacteria to develop resistance against antibacterial action of silver based nanomaterials, nowadays, there are intensively studied their biological properties, namely antifungal, antiviral and particularly antibacterial activities together with their cytotoxicity. According to this, silver based nanomaterials find their use in medicine (catheters, implants, prostheses) (Rupp *et al.*; 2004, Stevens *et al.*; 2011), and are used to improve commercial products (textiles, deodorants) (Perelshtein *et al.*; 2013).

In this work, the preparation of silver bromide NPs, their reduction to the silver NPs and the diverse mechanism of antimicrobial activity of AgBr and Ag NPs against gram-positive and gram-negative bacteria and also against several strains of candida was explored. The AgBr nanoparticles (NPs) were prepared by simple precipitation of silver nitrate by potassium bromide in the presence of stabilizing polymers (PEG, PVP, PVA and HEC). It was found, that used polymers influence significantly the size of the prepared AgBr NPs dependently on the mode of interaction of polymer with Ag^+ ions. Small NPs (diameter of about 60-70 nm) were formed in the presence of the polymers with low interaction as are PEG and HEC, the polymers which interact with Ag^+ strongly produce nearly two times bigger NPs (120-130 nm). The prepared AgBr NPs were reduced to Ag NPs by using of $NaBH_4$. The sizes of the produced Ag NPs followed the same trends – the smallest NPs were produced in the presence of PEG and HEC polymers. Prepared AgBr and Ag NPs dispersions were tested for their biological activity. The obtained results of antimicrobial activity of AgBr and Ag NPs are discussed in terms of possible mechanism of the action of these NPs against tested microbial strains. The AgBr NPs are more effective against gram-negative bacteria and tested yeast strains while Ag NPs show the best antibacterial action against gram-positive bacteria strains.

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Preparation of Novel Selenium Nanoparticles with Strong *In Vitro* and *In Vivo* Anti-cancer Efficacy Using Tiger Milk Mushroom

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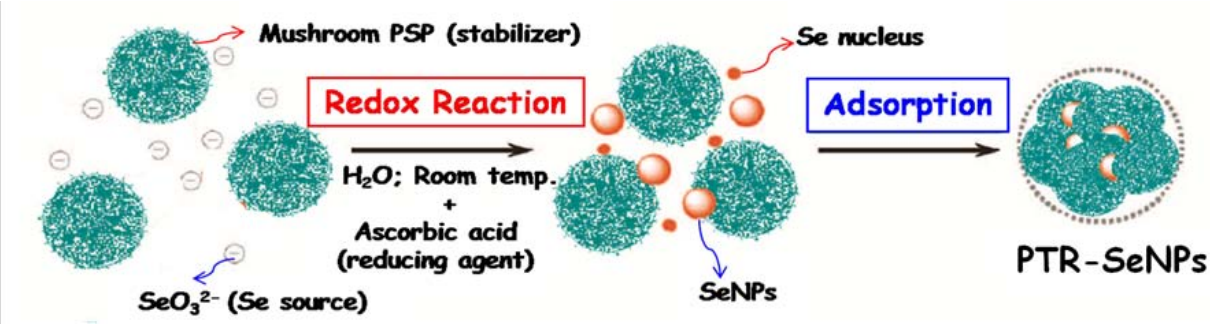

Figure 1: Schematic illustration of PTR-SeNPs preparation using PSP isolated from tiger milk mushroom

Abstract: Selenium is an essential trace minerals to human health. In the past decades, substantial amount of evidences have been acculmulated to support its role in cancer treatment. The anti-cancer efficacy and toxicity of selenium are highly dependent on its chemical form and dosage. Recently, selenium nanoparticles (SeNPs) have become the new research target, since they were found to possess remarkable anti-cancer efficacy and low toxicity.

By using a polysaccharide-protein complex (PSP) isolated from the tiger milk mushroom (*Pleurotus tuber-regium)*, our research team has successfully prepared novel SeNPs (PTR-SeNPs) under a simple redox system, in which sodium selenite and ascorbic acid were used as the Se source and reducing agent, respectively. In contrast to normal cells, the PTR-SeNPs were found to significantly inhibit the growth of human breast carcinoma MCF-7 cells (IC_{50} = 3.7μM) by apoptosis induction via activating a ROS-mediated mitochondrial pathway. Comparing with the IC_{50} value of native SeNPs (200μM) and PSP (400μg/mL), interestingly, the mushroom PSP surface decoration did not only stabilize the SeNPs, but also significantly enhance their cellular uptake and anti-proliferative effect on the MCF-7 cells. More importantly, our recent *in vivo* anti-tumor study demonstrated that PTR-SeNPs (ranged from 2.5 to 7.5 mg/kg) significantly inhibit the growth of MCF-7 xenografts (33.9-76.7%) transplanted in BALB/c nude mice in a dose-dependent manner after 16 days of intravenous injection.

It is the first study of its kind to prepare SeNPs with strong anti-cancer efficacy using mushroom PSP as the stabilizer. We anticipate that findings of this study could provide significant insights on developing the PTR-SeNPs into next generation anti-tumor agents. Our long term goal is to develop economical, safe and evidence-based anti-tumor agents for our community, hereby alleviating the now spiraling cost of cancer treatments in the public healthcare system.

Keywords: selenium nanoparticles, tiger milk mushroom, anti-cancer efficacy

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Conductive polypyrrole: a promising interface for attachment and proliferation of mammalian cells

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Abstract: Cell-based assays have emerged as low-cost, rapid and non-invasive alternatives for a great variety of biological analyses, encompassing areas from basic research to clinical and industrial applications. Development of high through-put cell-based assays depend on the availability of miniaturizable surfaces where cells could remain attached after repeated cycles of washing. At present, substrates for cellular growth in biosensors are mostly based on polydimethylsiloxane (PDMS), However, cell adhesion onto PDMS is insufficient, and high added value biomolecules, such as poly-lysine or fibronectin, must be used to increase cell attachment (Mehling and Tay; 2014). Polypyrrole, a conductive polymer, is known to be highly biocompatible, and has been evaluated for tissue regeneration purposes (Balint *et al* 2014). Due to the unique combination of properties in this material, including electric conductivity, flexibility, and low cost, its use as a biosensor support for cellular growth would be very valuable. In this work, polypyrrole was evaluated as an interface for supporting growth of various mammalian cells. Electrochemically synthesized polypyrrole films were incubated with different immortalized adherent cell lines, and analyzed by confocal microscopy, MTT cell proliferation assays and scanning electron microscopy. Our results showed that polypyrrole is a good support for cell attachment and proliferation of mammalian cells. Moreover, further assays showed that polypyrrole allowed the adherence and/or activation and proliferation of primary cultures from mouse spleen cells, thus indicating the usefulness of this material for a wide set of cell-based assays.

Keywords: semiconducting polymers, polypyrrole, cell adherence, cell proliferation, biomedical applications.

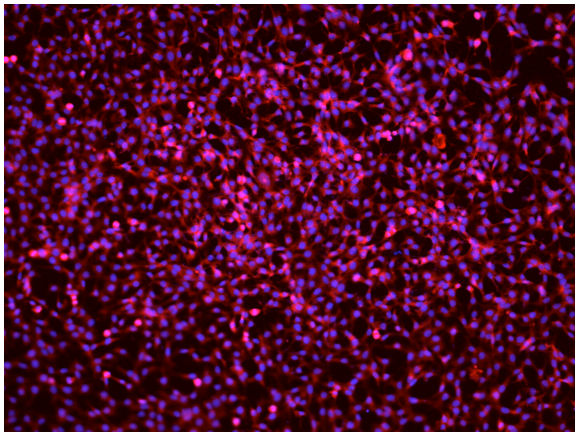

Figure 1: Fluorescence microscopy of the murine fibroblast line, NIH 3T3, after 72 hours of growth onto polypyrrole support. Cytoskeletal was stained with Cy3 coupled-anti-beta actin antibody (red). Staining with DAPI was used to visualize cell nuclei (blue).

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Acknowledgments:

This work was supported by CONACyT, Mexico, through Grant 214076. The authors thank Nydia Hernández, Martin García-Servín & Alicia del Real for excellent technical assistance.

An *In Vitro* Study of Osteoblast Behaviors on Graphene Oxide Electrodeposited on Anodized Titanium

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Abstract: Titanium is one of the most popular light-weight metals used for orthopaedic implants. Because their physical property and excellent biocompatibility (Yamaguchi *et al*., 2001). In order to increase osseointegration, surface modification of titanium mimicking a nanoscale hierarchical structure was widely studied (Zhang *et al*., 2013). Anodization is a method has been used to frabication titanium dioxide (TiO_2) nanotube arrays on titanium surface in order to increase osteointegration (Minagar *et al*., 2012). Using graphene oxide (GO) coating on biomaterial surfaces is a great potential for long-term use of orthopaedic implants due to its biocompatibility and antibacterial property (Zhu *et al*., 2010). The aim of this study is to frabicate anodized titanium coated with graphene oxide (ATiGO) using anodization and electrodeposition of graphene oxide, respectively. Scanning electron microscopy (SEM) was used to investigate surface morphology (Figure 1). Their physiochemical properties were evaluated by energy-dispersive X-ray spectroscopy (EDX), X-ray diffractrometer (XRD), thermogravimetric analysis (TGA), and X-ray photoelectron spectroscopy (XPS). Furthermore, cell proliferation of mouse osteoblastic cell line (MC3T3-E1) was investigated using MTT assay. Enzyme linked immunosorbent assay (ELISA), calcium assays and osteocalcin immunofluorescence analyses were used to evaluate osteogenic differentiation of cells. The results from ATiGO, GO coated on titanium (TiGO), anodized titanium (ATi), and pure titanium (Ti) were compared and discussed. The results in the present study suggest that graphene oxide can promote osteoblast behaviors when compared with anodized titanium and pure titanium without GO coating.

Keywords: TiO_2 nanotubes, graphene oxide, orthopaedic application, anodization, electrodeposition

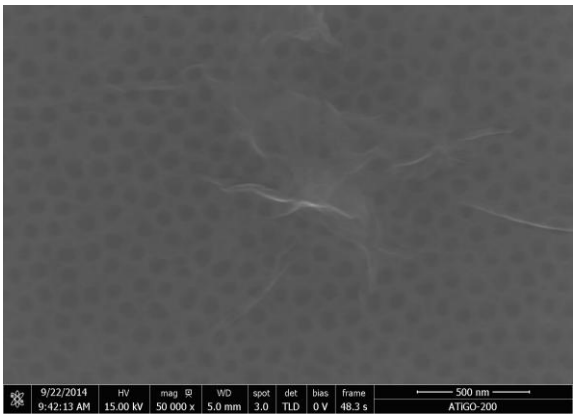

Figure 1: Top sectional scanning electron microscope image of 200 µg/ml of graphene oxide electrodeposited on anodized titanium (ATiGO200). A thin layer of reduced graphene oxide covers titanium dioxide nanotube array.

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STM Imaging of Yellow Fluorescent Protein under Ambient Condition

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Abstract: Fluorescent proteins have shown tremendous applications especially in localizing proteins of interest within cells. To our knowledge there are few scanning tunneling microscopy (STM) or scanning tunneling spectroscopy (STS) studies regarding fluorescent proteins. Several works related with fluorescence dynamics such as lifetime and anisotropy decay of fluorescent protein in solution have been reported (Suhling *et al*., 2002). Knowing the nature of adsorbed protein is interesting in the area such as biomaterials and pharmacology (Norde, 1986). In this study, a fluorescent protein (FP) has been chosen as a model to understand protein behaviour when adsorbed on a surface. Citrine from yellow fluorescent protein (YFP) variants has been chosen among other variants. Histidine tags are always used in the purification of protein and can as well be used as chemical handles on bare gold surfaces (Korpany *et al.*, 2012). STM was used to study protein's morphology on surfaces. Citrine was dried on hydrophilically and hydrophobically modified gold surfaces by drop cast protein solution on the freshly modified surfaces. The drop cast solution formed a "ring-like" pattern having a concentrated and visible rim. Citrine films were observed with an STM under ambient conditions. From STM images obtained, citrine molecules adsorbed more uniformly packed crystal-like protein layers on hydrophilic surface. While on hydrophobic surface, citrine molecules were more randomly adsorbed forming some aggregates on non homogenous layers. Furthermore, time-resolved anisotropy clearly has shown the tendency of fast and randomized layers on hydrophobic surface to compare with on hydrophilic surface.

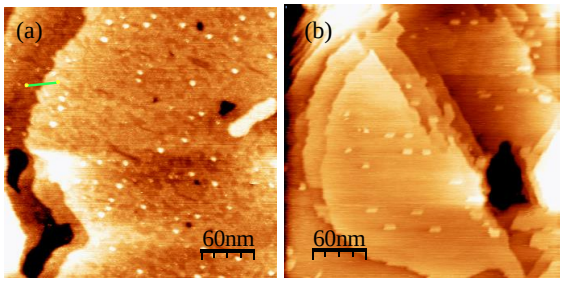

Figure 1: STM images of citrine film on hydrophilically modified surface (a) hydrophobically modified surface (b). Scanning condition I_t = 600 pA for hydrophilic surface, I_t = 800 pA for hydrophobic surface, V_{bias} = 0.2 V was used for both modified surfaces.

Keywords: fluorescent proteins, scanning tunneling microscopy, anisotropy decay

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Nano- and Microfabricated Hydrogels for Regenerative Engineering

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Abstract:
Engineered materials that integrate advances in polymer chemistry, nanotechnology, and biological sciences have the potential to create powerful medical therapies. Our group aims to engineer tissue regenerative therapies using water-containing polymer networks, called hydrogels, that can regulate cell behavior. Specifically, we have developed photocrosslinkable hybrid hydrogels that combine natural biomolecules with nanoparticles to regulate the chemical, biological, mechanical and electrical properties of gels. These functional scaffolds induce the differentiation of stem cells to desired cell types and direct the formation of vascularized heart or bone tissues. Since tissue function is highly dependent on architecture, we have also used microfabrication methods, such as microfluidics, photolithography, bioprinting, and molding, to regulate the architecture of these materials. We have employed these strategies to generate miniaturized tissues. To create tissue complexity, we have also developed directed assembly techniques to compile small tissue modules into larger constructs. It is anticipated that such approaches will lead to the development of next-generation regenerative therapeutics and biomedical devices.

SEEC Microscopy : An innovative optical technique for the live and label-free study of a enzymatic reaction in liquid

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Abstract: Nowadays, label-free In-Vitro Diagnostic (IVD) is in higher demand due to the steric, electronic influence of labeled molecules on biological samples. However, in the absence of chemical amplification, it is a challenge for label-free biosensing to achieve the same degree of sensitivity as those exhibited by standard enzyme-linked immunosorbent assay (ELISA). In this sense, significant optical label-free biosensors are reported performing well: SPR, SPRi, QCM-D, OWLS… But even if these techniques show quite good sensitivity, none of them offer high-lateral resolution imaging and direct thickness measurements capacities.
Few years ago, was developed the SEEC (Surface Enhanced Ellipsometric Contrast) Microscopy, an innovative advanced optical technique based on ellipsometric and interference principles. This technique offers live and label-free topographic imaging of organic, inorganic and biological samples with a high precision and accuracy (down to 0.1nm). This technique has been successfully applied to the study of nanometric films and structures, biological layers, nano-objects…Compared to existing techniques, the SEEC microscopy offers a unique combination of acquisition mode capabilities : non-contact, label-free, live imaging, nanometric sensitivity, topographic analysis…
Recently, a SEEC study was performed to monitor a dynamic enzymatic reaction in real-time and in liquid. Biochemical reactions taking place on surface molecular patterns were not only tracked but also fully characterized thanks to the topography imaging capability of the technique. This study was performed on 5µm x 5µm patterns to illustrate the ability of the SEEC technique to perform ultra-high density multiplexing analyses.

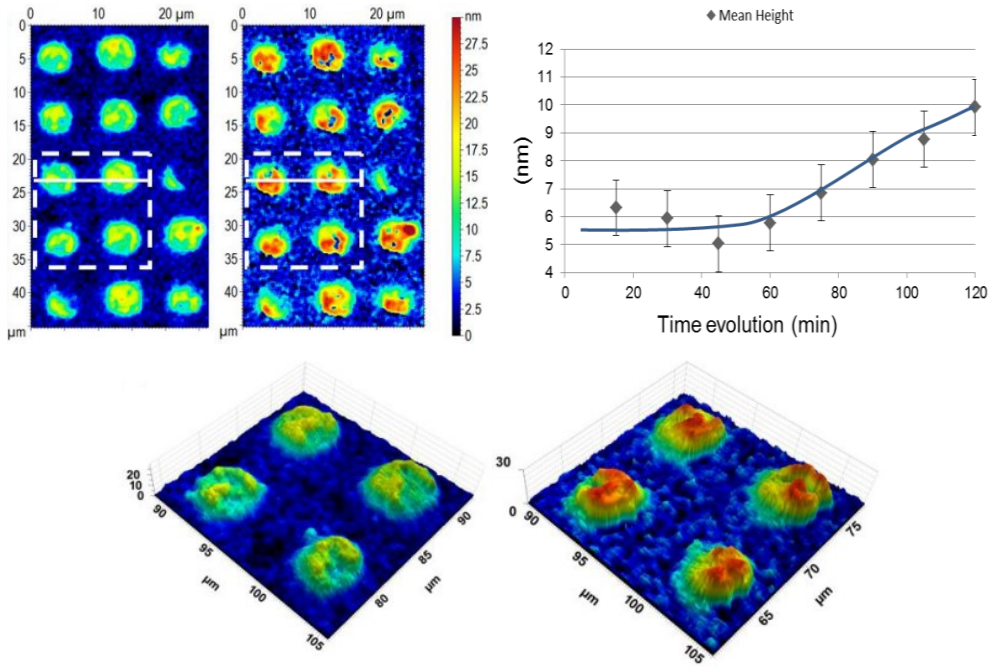

Figure 1: SEEC analysis of biochips hybridation (enzymatic reactions)
A.Topographic images (t_0 and t_0+120min) B. Biochip thickness vs. hybridation time C. 3D views

References:

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Biocompatibility of nanovesicles derived from microbial cells: an assessment towards vaccine applications

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Abstract: Due to the emergence of immunosuppressive conditions worldwide, the development of acellular vaccines constitutes a major need. Nanotechnology provides a wide set of approaches for the development of cell-free vaccines, including antigen- or/and microbial gene-functionalized nanocarriers. A recent modality of acellular vaccines is the use of cell-free nanovesicles (André et al; 2004). In this work, we explored a method for the production of *Mycobacterium*-derived nanovesicles, performed their physical and chemical characterization, and assessed their biocompatibility and genotoxicity traits.

Ultracentrifugation and dialysis were explored as purification methods. Physical characterization of nanovesicles was performed through transmission electron microscopy and dynamic light scattering analyses. Chemical identification of nanovesicles was performed through thin-layer-chromatography analysis of lipids and sodium-dodecyl-sulfate polyacrylamide gel electrophoresis of proteins. For biocompatibility tests, we focused on the study of cytotoxic, genotoxic and hemolytic effects of microbial-derived vesicles on NIH-3T3 mouse fibroblast cells. Cell viability was tested using the 3-(4, 5-dimethlthiazol-2-yl) 2,5-diphenyl tetrazolium bromide (MTT) colorimetric assay. DNA lesions were evaluated using the single-cell gel alkaline electrophoresis (comet) assay and observed by fluorescence microscopy. Hemolytic effects were tested by the Neun-Dobrovolskaia method (2011).

Physico-chemical analysis of vesicles show that a large variety of microbial molecules may compose the obtained nanovesicles, thus suggesting that both innate and adaptive immune responses could be activated by this newly developed vaccine system.

Keywords: nanovesicles, nanosafety, vaccines, comet assay, biocompatibility tests.

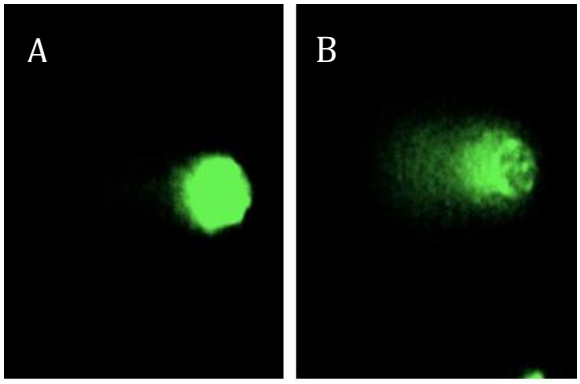

Figure 1: Single cell alkaline electrophoresis (comet assay) showing the nuclear material of a control cell (A), and the characteristic comet tail, which is attributed to alterations in the genetic material caused by exposure to various external mutagen agents (B).

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Acknowledgments:

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$Fe_{3-\delta}O_4$ nanoparticles inhibit *Clostridium difficile* spore germination: an in vitro and in vivo study

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Abstract: *Clostridium difficile* infection (CDI) has emerged to be an important cause of global healthcare-associated infection (Ananthakrishnan *et al*.; 2010). Resistance of CD spores to various preventive and therapeutic measures post a significant threat in CDI (Paredes-Sabja *et al*.; 2014). Nanomaterials have been explored for potential applications in anti-microbials with intrinsic advantages of low drug-resistance issue and high efficacy. We discovered the 22 nm octahedral $Fe_{3-\delta}O_4$ single crystal nanoparticles with a strong inhibitory effect to CD spore germination *in vitro* and *in vivo*. The nanocrytal presented excellent saturation magnetization (94 emu/g) close to the bulk due to the presence of alpha iron. Such particle showed a dose dependent inhibition of CD spores germination (62% growth inhibition at 50 μg/mL) for 20 minutes of exposure. At 500 μg/mL, the inhibition rate is close to that of sodium hypochloride. CDI animal model established in NF-κB-reporter mice using oral gavage with CD spores presented significant bowel inflammation in the MOCK compared to $Fe_{3-\delta}O_4$ nanoparticle treated group as revealed by In Vivo Imaging System. S-layer protein and a conserved hypothetical protein were released from CD spores after for 20 minutes of $Fe_{3-\delta}O_4$ nanoparticle exposure. Cryo-electron tomography clearly showed binding of the nanoparticles to CD spores' surface followed by disruption of the spores. Pro-inflammatory cytokines including IL-1β, TNF-α, and INF-γ and inflammatory cell infiltrations were significantly suppressed after nanoparticle treatment. These results provide nano-material based strategy for CDI control and potential therapeutic mechanisms that encourage further clinical translational development.

Keywords: Clostridium, spore, infection, nanoparticle, Cryo-electron microscope, in vivo test

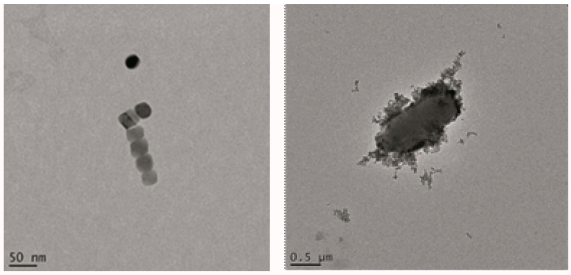

Figure 1: Transmission electron microscopy revealed the typical 22nm octahedral iron oxide nanoparticles (left). When treated with $Fe_{3-\delta}O_4$ nanoparticles, these particles attached to spore surfaces and compromise the integrity of the spaore (right).

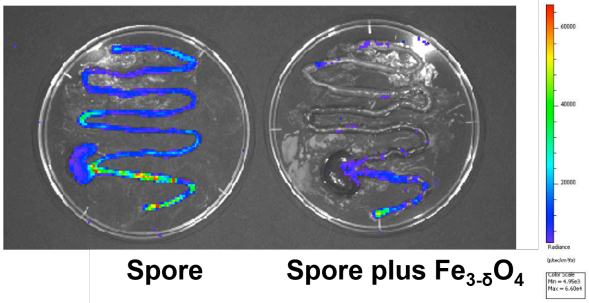

Figure 2: The spore-induced CDI presented extensive bowel inflammation in the NF-κB-reporter mice as revealed by IVIS (left). Such inflammation was significantly attenuated by oral administration of 500μg/mL $Fe_{3-\delta}O_4$ treatment (right).

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Identification of critical Monte Carlo simulation parameters in nanoparticles radiosensitization

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Abstract: The use of nano-objects as radiosensitizers is rapidly increasing and has become a major subject of interest as a new strategy to fight cancer. This concept has already been proven *in vitro* and *in vivo* by a number of authors (Butterworth *et al.*, 2012) but only a few nanoparticles (NPs) are undergoing clinical trials. Even though a lot of preclinical studies are conducted to investigate the effects of nano-objects combined with X-rays on cells/tissues/tumor, there is still a serious lack of knowledge about their physical and chemical mechanisms.

A common way to investigate their properties is to use Monte-Carlo simulation methods with particle transport codes such as Geant4 (Agostinelli *et al.*, 2003). These simulations are time consuming, strongly code or model dependent and do not compare well between each other. It has already been noticed that the lack of standardization in preclinical studies of nano-radiosensitizers could partially explain the low number of translation to clinical applications (Retif *et al.*, 2015). That seems to become problematic in the field of NPs (Kodiha *et al.*, 2015).

In this study, a sensitivity analysis is performed to estimate the impact of eight simulation parameters on the variability of two numerical responses: the dose enhancement and the dose diffusion in seven spatial regions of interest shown in Figure 1. The physical modeling and Monte Carlo simulations are supported by the Geant4-GATE environment. The simulated nano-object is a single 100 nm gold nanoparticle. A Box and Behnken design of numerical experiments was carried out to estimate the statistical relevance of the simulations factors.

Finally three simulation parameters: the Auger effect, the medium type and the fluence level, have been identified as critical factors and therefore have to be chosen carefully and to be kept constant between interlaboratory comparisons of numerical simulations. Another factor: the cutoff energy may have a significant impact on the diffusion response. However, whatever the studied response, four simulation parameters have negligible effects: the working volume, the spatial resolution and cutoff, and the computer. Those results bring new contributions to improve the robustness of numerical simulations of nanoparticles activated by X-ray and proposes directions for standardization in this scientific area.

Keywords: nanoparticle, cancer, radiotherapy, modeling, simulation, design of experiments, statistics.

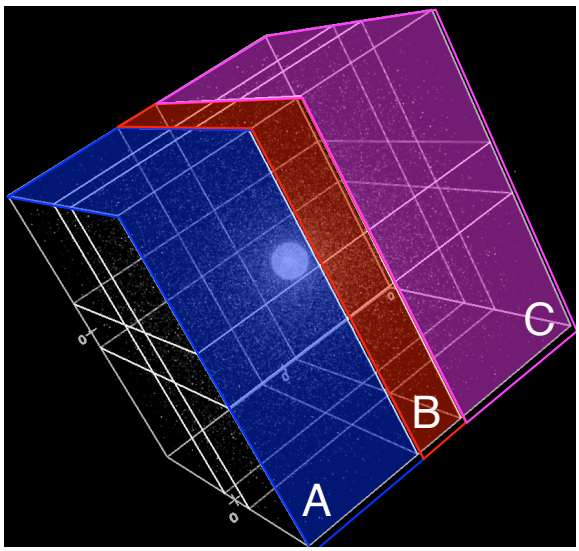

Figure 1: Cubic domain of simulation in which seven spatial regions of interest have been defined. Only the central region is composed of a single 100 nm gold nanoparticle.

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The spread of multi-wall carbon nanotubes to the room air as a result of their mixing in the fume hood

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Abstract: Processes with possible emission of multi-wall carbon nanotubes (MWCNTs) should be carried-out in fume hood (LEV) or in full containment systems, which should interact with the general ventilation system of the room. Ensure proper interaction of ventilation systems in the room is essential to provide protection from exposure to particles of both workers, who work with nanomaterials, as well as other staff in those room.
The possibility of particles emission and their spread to the room air were determined by measurements of particles concentrations (using DiscMini's) before (background) and during processes of mixing of nanomaterial (MWCNTs 10-20nm from Cheap Tubes). Location of measuring points in the room are shown in Figure 1.

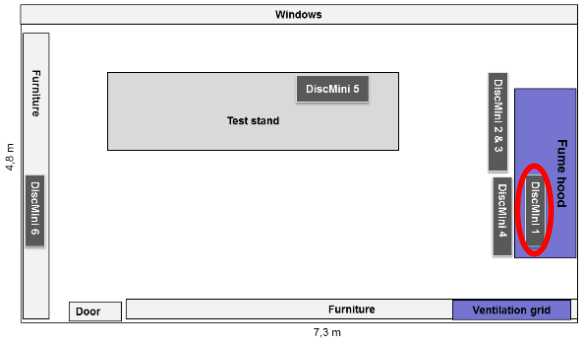

Figure 1. Location of measuring points in the room: ◯ place of MWCNTs emission during mixing.

Processes of mixing were carried-out inside LEV, in the room equiped with grid of general ventilation, for situations were LEV was switched-off (air supply to the room 137m^3/h, air exhaust 121m^3/h) and switched-on (air supply to the room 360m^3/h, air exhaust 969m^3/h).
Received results were the base for calculated ratio (P/B), showing of increase number concentrations as a result of mixing MWCNTs (P) compare to background (B). Criteria for determination of the likelihood of emission or the spread particles in the room air are following: P/B ≥ 2 - likely, P/B >1,05 to <2 - possibly/not excluded, P/B <1,05 - no likely (Brouwer *et al.;* 2013).

Keywords: ventilation, MWCNTs, emission, the spread of particles in the room air

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It was found, that multi-wall carbon nanotubes (MWCNTs) emitted during processes of mixing the spread to the room air, even in the areas located far from place of mixing (more than 6m - DiscMini6) – Table 1. Switched-on LEV results of decreasing of emission of MWCNTs (Figure 2c) compare that when LEV was switched-off (Figure 2a). However for both situations (LEV switched-off/on) to the air were emitted MWCNTs (Figure 2b and 2d).

Table 1. Likelihood emission or the spread particles in the room air.

Fume hood	Possibility of MWCNTs emission during mixing (in fume hood – DiscMini1)	Possibility of the spread of MWCNTs, emitted during mixing, to the room air (in five points in the room)
Switched - off	P/B=10,2 (likely)	DiscMini's 2: P/B=12,8 (likely) 3: P/B=10,0 (likely) 4: P/B=6,2 (likely) 5: P/B=1,8 (possibly) 6: P/B=2,4 (likely)
Switched - on	P/B=2,0 (likely)	DiscMini's 2: P/B=1,2 (possibly) 3: P/B=1,2 (possibly) 4: P/B=1,3 (possibly) 5: P/B=1,2 (possibly) 6: P/B=1,1 (possibly)

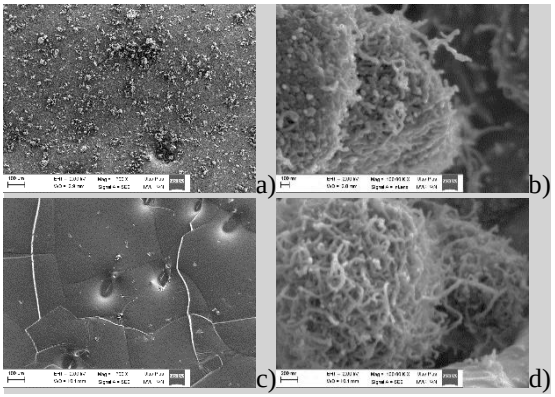

Figure 2. Electron microscopy (SEM) analysis: a), b) LEV switched-off; c), d) LEV switched-on.

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The DaNa$^{2.0}$ Knowledge Base Nanomaterials – quality-approved and easy-to-understand information on current nanosafety research

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Abstract: Nanotechnology will bring about fundamental changes of basic research as well as of many sectors of industry. It will also have a great impact on our daily life ranging from electronics to the health care system. However, consumers often miss reliable and easy-to-understand information on nanomaterials and nanotechnology and don't know where to get such information. Besides the great need to respond to basic questions such as *"Are there any risks for myself and the environment?"* there is a growing demand for tools to manage and assess the rapidly increasing number of publications related to nanosafety issues. Therefore the international DaNa2.0 expert team brings together its expertise and knowledge from different research areas dealing with all aspects of nanosafety research in order to create and provide a non-biased, quality-approved and up-to-date knowledge base for more transparency on www.nanopartikel.info. The DaNa$^{2.0}$ project publishes articles covering latest research results on nanomaterials with regard to their influence on humans and the environment in an easily comprehensible way. For this purpose, scientific publications, reports, project results and latest news on human and environmental toxicology are analysed using the *«Literature Criteria Checklist»*. This customised methodology developed by the DaNa expert team helps to discriminate between high- and low quality publications and thus facilitates the evaluation process of scientific publications. The mandatory and desirable assessment criteria were developed in accordance with common quality criteria acknowledged worldwide within the scientific community. Another unique feature of the DaNa knowledge base is the integrated application-based database that provides a unique link between nanomaterials in real applications (e.g. environmental remediation or medical products) and their potential impacts/ toxicological effect(s) that can be easily accessed by the interested visitor.

Additionally, DaNa$^{2.0}$ provides a list of FAQs, a link platform with contact data to other information portals and the opportunity to directly pose questions to our experts via E-mail. DaNa$^{2.0}$ is also present on Twitter, follow us @nano_info.

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Keywords: knowledge base nanomaterials, literature criteria checklist, quality assessment of publications

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In vitro study of lung surfactant-nanoparticle interactions for evaluating nanotoxicity

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Abstract: The nanoparticles generated from industrial processes and those designed for use in medicine has increased our levels of exposure. Exposure to nanoparticles arises from either byproducts of industry or combustion as those seen from car exhausts. Although nanoparticles are continually being developed at an accelerated pace the understanding of the health impacts these nanomaterials have not been fully evaluated. The lungs are continually exposed to the environment and have evolved efficient mechanisms to remove particulate matter. The nanosized particles are less effectively removed in comparison to their micro sized counterparts. The impact of nanoparticles on the lungs has not been fully characterized. Drug delivery aims to use inhalable nanoparticles for treatment of pulmonary disease. The lungs offer several advantages including a large surface area for deposition of molecules and a thin alveolar epithelial layer to cross before entering systemic circulation. Combining these two features allows for a large dose of drugs and materials to be delivered that can be rapidly absorbed into the body. Pulmonary drug delivery shows great potential in the treatment of lung disease however it is unclear how these may impact respiratory function. Our research focuses on understanding the interaction between nanoparticles and lung surfactant models. Through this we can understand the nanotoxicity effects and be able to create safer inhalable drug carriers.

During inhalation particles can deposit on the surface of trachea, bronchiolar and alveolar regions of the lungs. For efficient delivery of therapeutics, nanoparticles will need to deposit within the alveoli where molecules are able to cross into the blood-stream. Before reaching the alveoli epithelial cells then the vasculature, the nanoparticles must first interact with the lung surfactant. Lung surfactant is a single molecule thick layer of lipids and proteins, it is responsible for lowering the surface tension in the alveoli required to maintain breathing. Disruption of surfactant results in impaired lung function and may lead to irreversible alveolar collapse.

Here we use lung surfactant as a model to evaluate the impact of nanoparticles on respiratory function. This in vitro method uses a Langmuir trough to measure the surface pressure-area isotherms of lung surfactant (Fig. 1). A high surface pressure corresponds to the ability of surfactant to be compressed to small areas while remaining stable. The end goal is to be able to screen different nanoparticles to determine their impact on surfactant function. This will allow for a first screen of airborne nanoparticles to determine their potential impact on lung surfactant function (Al-Hallak et al 2010).

The two nanoparticles used were gelatin and polyisobutylcyanoacrylate, both are being tested for inhalable drug delivery. Gelatin is a natural polymer derived from collagen that is nontoxic and biodegradable. Polyisobutylcyanoacrylate is a synthetic polymer and derivatives are already being used in the medical field. The lung surfactant model used is made up of the two most common lipid classes found in vivo, namely phosphatidylcholines and phosphatidylglycerols. This will allow us to vary the nature of the lipids used and to have tight control over lipid ratios and concentrations to determine if there are preferential interactions of nanoparticles with specific lipid depending on the headgroup, fatty acid tail saturation or the film packing as a function of composition. This is compared against a clinical surfactant to compare results. The information gained here will be used to help create a better mod-el for lung surfactant and aid in creating safer nanoparticles.

Keywords: inhalable therapy, nanoparticles, in vitro, surface pressure-area isotherms, lung surfactant.

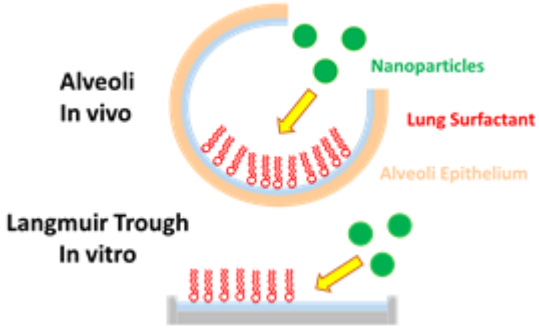

Figure 1: Schematic showing the in vitro aspect of the Langmuir trough and compared to the in vivo alveoli situation.

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Effect of Gold Nanoparticle Shape of Cellular Uptake and Toxicity

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Abstract: The ability to adapt parameters during nanoparticle synthesis has led to the creation of a vast catalogue of gold nanoparticles (AuNPs) that differ in size, shape, surface charge, surface corona and chemical composition. AuNPs are continuing to attract research interest for biomedical applications, as they appear to be well tolerated in biological systems while providing opportunities for facile surface manipulations as well as exhibiting interesting optical properties (Shukla *et al*., 2005). Spherical AuNPs possess limited potential for surface plasmon resonance tuning, however altering the shape of AuNPs gives rise to interesting optical properties which can be suited to biological sensing, cellular imaging and cancer treatment.

While developments in the synthesis of AuNPs have been rapid in recent times, our understanding of their biological impact, in particular the effects of shape, size, chemical composition and surface corona, has struggled to keep pace. It is commonly thought that changes in shape could significantly influence the way that particles are recognised, processed and excreted by cells, however this effect has not been fully explored.

All inorganic nanoparticles, including gold, invoke the formation of a protein corona upon introduction to protein rich solutions such as biological media or blood. The biological response after a nanoparticle enters the blood stream is thought to be influenced by the protein species which bind to the nanoparticle, as well as protein orientation, binding strength and conformation (Alkilany *et al*., 2009). Despite the small number of studies in this area, it is generally agreed that shape affects the manner in which a protein can bind to the surface of a nanoparticle, with the introduction of such features as variable curvature, flat planes, sharp edges, corners, and pores. Such features may favour or hinder binding of individual proteins depending on their conformation, or cause them to undergo structural changes after binding.

In this paper we synthesise AuNPs with comparable surface functionalisation in various shapes (Figure 1) and explore the biological effects in two ways: first, using human serum albumin (HSA) - the most abundant protein in human blood - we explore the role of shape in protein corona formation; and second, we report on the effects of shape on uptake and toxicity in mammalian cells.

Keywords: Gold nanoparticles, toxicity, cellular uptake, protein corona, shape.

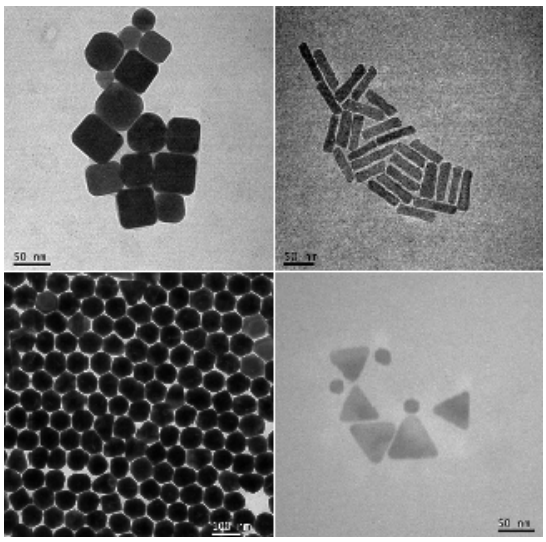

Figure 1: Scanning electron microscope image of various shaped gold nanoparticles as synthesized for biological experimentation.

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Genotoxicity and Mutagenicity Screening of Engineered Nanomaterials:

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Abstract: The rapid growth of nanotechnology and concomitantly the increased rate of synthesis of engineered nanomaterials (ENMs) has led to an increasing and ubiquitous presence of ENMs in our environment. As a consequence, it is important to be able to characterise the nano-bio interactions between living organisms and ENMs. Furthermore, despite the great scientific effort and large economic investments for research projects in the field of nanotoxicology (34 projects dedicated to nanomaterial toxicology worth over €100 million within the European Seventh Framework -FP7- Programme), nanotoxicology studies have not yet provided any clear and unequivocal answers on the toxicity of nanomaterials (McCall, et al.; 2013). In addition, recent studies have revealed genotoxic and mutagenic risks associated with ENMs exacerbating this situation (Vecchio et al.; 2012), highlighting the urgent necessity to deeply examine and define the possible toxicological effects of ENMs and their physico-chemical characteristics. It should be noted that the combinatorial diversity of nanomaterials makes their rapid toxicological classification difficult without the application of high-throughput screening (HTS) approaches. In this context, we will show the mutagenic effects induced by AuNPs in Drosophila melanogaster and a new HTS platform based on the cytokinesis-block micronucle-us (CBMN) assay, that has been successfully applied in the evaluation of the cytotoxic and genotoxic effects induced by AgNPs and SiO2NPs, and the role of their physico-chemical properties such as composition, surface coating, size and surface charge (Vecchio et al.; 2014). In particular, our results demonstrate the catastrophic effects due to the mutagenic events induced by ENMs in the Drosophila offspring and the capability of our HTS platform to assess cyto- and genotoxicity induced by different ENMs in primary human lymphocytes. Finally, we will show the future strategy to evaluate these effects and to thoroughly analyse the molecular basis of nano/bio interactions by coupling the Drosophila genetic tools and the developed HTS platform.

Keywords: genotoxicity, mutagenicity, high-throughput screening.

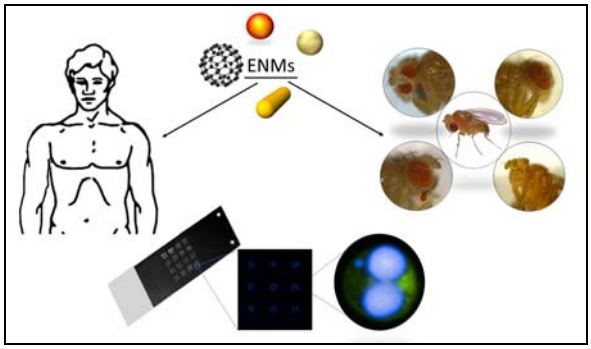

Figure 1: Schematic representation for ENMs genotoxicity and mutagenicity assessment by *in-vivo* and *in-vitro* approaches. Comparison between the ENMs induced genotoxicity evaluated in primary human lymphocytes (*in-vitro* approach) and in *Drosophila* haemocyte (*in-vivo* approach) using the new developed HTS platform. Assessment of the relationship between the mutagenic and genotoxic effects induced by nanomaterials in *Drosophila*.

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Impact of ageing and protein remediation in the life-cycle of metal oxide nanoparticles in the organism

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Abstract: The use of inorganic nanoparticles in the field of the nanomedecine is rapidly expanding allowing news treatments for various diseases. Thanks to their size, optical or magnetic properties and functionalization, metal oxide nanoparticles are widely used for their significant potential in theranostics. Although the potential toxicity of such nanomaterials are extensively studied, their long term fate, their degradation and the evolution of their properties in the organism are still poorly understood. Here we propose a multi-scale approach to study the life cycle of metal oxide nanoparticles in biological environments based on the evolution of their physical and morphological properties.

Our group have previously shown that iron oxide nanoparticles injected intravenously in mice underwent local intracellular degradation within lysosomes of macrophages in spleen and liver (L. Lartigue, ACS 2013) . The coexistence of iron rich ferritin protein in vicinity of degraded nanoparticles (Figure 1) suggests the implication of these iron storage protein in the remediation of iron released by nanoparticles. In this work, we aim to decipher the degradation mechanism of iron oxide, cobalt iron oxide and gold-iron oxide nanohybrids and their processing by endogenous proteins such as apoferritin.

We used an in vitro lysosome like medium to study the kinetic of degradation of the different nanomaterials depending on their composition and coating in presence and absence of apoferritin. The evolution of magnetic properties (currently used in medical applications like MRI or magnetically induced therapeutic hyperthermia) was studied by combining Nuclear Magnetic Resonance Dispersion (NMRD) and Electronic Paramagnetic Resonance (EPR). In parallel, the evolution of the morphology and the size of the nanoparticles were studied by using Dynamic Light Scattering (DLS) and Transmission Electronic Microscopy (TEM). Then we have investigated the transfer of iron and cobalt from nanoparticles to apoferritin by using UV-visible spectroscopy and TEM. This combination of methods highlights the mechanism of the nanoparticle degradation and the remediation and protein storage of metals from the nanoparticles.

Finally, to compare and understand the future of NPs in vivo, we have examined the evolution of the protein corona depending on the coating of the NPs, and the role of the protein corona involved in the degradation of NPs.

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Keywords: Ageing and degradation of NPs, protein remediation, magnetic nanoparticles, Electroni Paramagnetic Resonnance, Nuclear Magnetic Resonnance Dispersion, UV-visible spectroscopy.

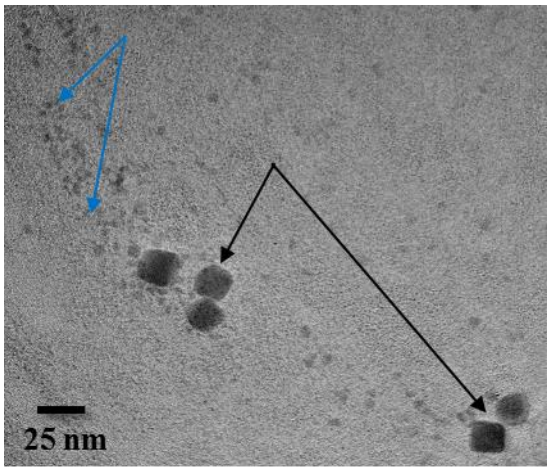

Figure 1: Figure illustrating intracellular degradation of PEG coated nanocubes (black arrows) in lysosomes after intravenous injection. Blue arrows show the coexistence of ferritin with degradated nanocubes.

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Size and morphology dependence of gold nanorods and gold nanospheres in the nanotoxicological process: *in vitro*, *in vivo* and membrane models studies

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Abstract: The need for adequate toxicology testing of nanomaterial-based products should be addressed to help clarify which classes of nanomaterials or which behaviors/characteristics of such particles may indicate potential toxicity to human health or to the environment. However, conventional toxicology protocols for pharmaceutical validation are not adequate in terms of investigating nanoparticle toxicology because of the possible interactions between the nanomaterials and some of the utilized reagents. To understanding the real interaction or mechanisms involved in the nanoparticles/cell interface as nanotoxicity aspects, several techniques have been applying . Traditional biologic protocols, physical chemical and analytical methods have been updated and combined with *in vitro* and *in vitro* analysis for this purpose (Walczyk *et al*., 2010). But there is still the need to develop new methodologies to study the interactions between cell membrane and nanoparticles at the molecular level, especially because the uptake assessment is highly dependent of the membrane characteristics and nanoparticle charges, shape and size. Here, we report the influence of morphology and size of gold nanorods and gold nanospheres coated or not with human albumin serum using *in vivo*, *in vitro* and membrane models assays (Figure 1). The increase in the nanorods size as well as the protein corona decrease the uptake process according our *in vivo* and *in vitro* results, which was also confirmed by membrane model analysis, indicating that the size and morphology have high influence in the toxicology aspect. In addition, we introduce to the nanotoxicology community an alternative technique to understand the interactions of nanoparticles and membrane models composed by phospholipids, which may contribute to understand the interaction and possible mechanisms of adsorption and uptake process (Cancino *et al.,* 2013a,b). This study will also permit us to discuss the importance of combining techniques to better understand the nanotoxicity of nanomaterials and biological systems.

Keywords: nanotoxicology, nanorods, nanospheres, phospholipids, membrane model, in vitro, in vivo.

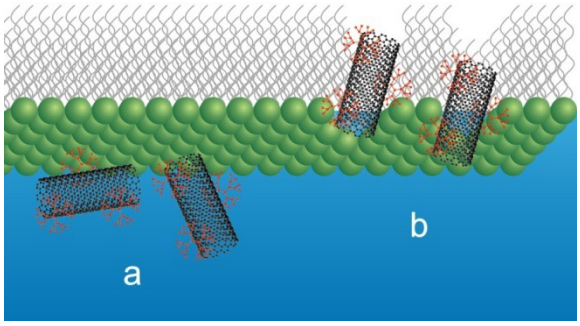

Figure 1: Illustration of a membrane composed by phospholipids which can be helpful to understand the interactions of nanomaterials and cell membranes.

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