The Early History of Nanocarriers as Drug Delivery Systems

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Abstract: Nano-scale drug delivery systems (NDDS) are very special. NDDS are special because they are usually targeted to specific cells in specific tissues or organs. They are also special because most of them are focused on cancer chemotherapy. The size scale of these NDDS ranges from a few nm up to several hundreds of nm. In recent years, the number of FDA-approved NDDS has grown significantly.

There are many different types of NDDS, and many of them may also be PEGylated (conjugated with PEG molecules). **Table 1** lists the different nanocarriers. Most of these systems are composed of water-soluble and hydrophilic synthetic polymers, although they may be combined with hydrophobic components. The liposome is a different kind of nanocarrier; it is a small aqueous vesicle enclosed by a lipid bilayer made of phospholipids.

Table 1: Examples of Nanocarriers

Liposomes
PEGylated Liposomes
Polymer-drug Conjugates
PEGylated Drugs
Antibody-drug Conjugates
Polycation-nucleic acid Complexes (Lipoplexes and Polyplexes)
PEGylated Lipoplexes and Polyplexes
Polymeric Micelles
PEGylated Polymeric Micelles
Albumin-drug Nanoparticles
Drug NPs, Nanogels, Nanotubes
Dendrimer-drug Nanoparticles
Polymersomes

A nanocarrier may be conjugated to a drug, (eg, PEGylation) or it may be complexed ionically to a drug of the opposite charge, (eg polyplexes and lipoplexes) or it may physically entrap a drug (eg, liposomes). It may also be biologically ("actively") targeted to specific cells using monoclonal antibodies or peptide ligands, or physically ("passively") targeted to tumors via leaky blood vessels (EPR effect). The molecular weight (or size) and biodegradability of the nanocarrier are both very important to its eventual clearance from the body after delivering the drug.

There are three key discoveries / developments that have helped to bring NDDS into the clinic. They are 1) PEGylation 2) active targeting and 3) passive targetting (EPR). The history of these technologies will be described, and this talk will continue as a review of the early history of the types of nanocarriers listed in Table 1.

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Nanomedicine for molecular imaging: interest of bimodality in preclinical studies

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Abstract: Nanoparticles have potentials in imaging in particular as enhanced contrast agent for techniques with low sensitivity, such as MRI or Ultrasound imaging. They also have a role to play in bimodal imaging. Indeed, thanks to their possible functionalization, various chromophores or contrastophor can be linked to the surface or within the core to provide new properties or to allow obtaining a high number of valuable informations in preclinical studies, while highly reducing the number of animals. Few examples of the interest of bimodal agents in preclinical evaluation will be shown. First, the conception of a protein scaffold targeting the Asialoglycoprotein receptor (Chaumet-Riffaud et al. 2010). A radioactive label provide quantitative informations on the liver function while an optical label will provide evidence on the specificity of the targeting. A second example will concern tumor imaging thanks to integrin targeting. A bimodal silica based scaffold allowed to approach within the same experiments the kinetic by optical imaging and a relative quantitative estimation using MRI (Ramniceanu et al. 2015). Another example from gaz microbubbles. These agents enhanced the ultrasound imaging signal however to follow the excipients and approach their elimination profile, optical imaging can provide some additional informations (Manta et al. 2015).

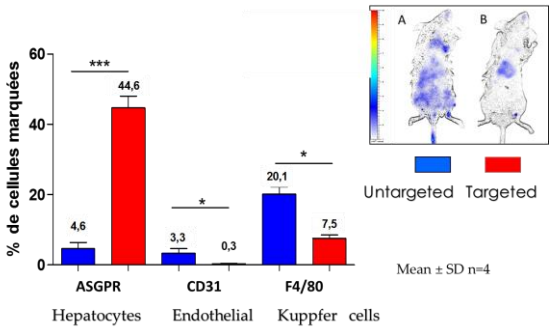

Figure 1: Figure illustrating the distribution of targeted and untargeted protein. The radioactive labeling gives the quantitative informations on the amount of the tracer in the various organs. The addition of an optical tracer will allow demonstrating the specificity of the signal at the cellular level, using immunohistology or flow cytometry on preleved organs and dissected cells.

Keywords: Targeted nanoparticles, Integrin targeting, Asialoglycoprotein receptor, Silica nanoparticles (AGUIX), protein scaffold, molecular imaging, MRI, optical imaging.

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Nanotechnologies for the treatment of severe diseases

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Even if new molecules are discovered to treat severe diseases, the clinical use and efficacy of conventional chemotherapeutics is hampered by the following limitations: (i) drug resistance at the tissue level due to physiological barriers (non cellular based mechanisms), (ii) drug resistance at the cellular level (cellular mechanisms), and (iii) non specific distribution, biotransformation and rapid clearance of the drugs in the body. It is therefore of importance to develop nanodevices able to overcome these limitations.

This will be illustrated by two nanomedicine platform developed in the laboratory: the design of biodegradable nanoparticles loaded with doxorubicin for the treatment of the resistant hepatocarcinoma (a nanomedicine currently in phase III clinical trials) (1) and the "squalenoylation" (2), a technology that takes advantage of squalene's dynamically folded conformation to link this natural and biocompatible lipid to anticancer (3), antimicrobial (4) or neuroprotective compounds (5) in order to achieve the spontaneous formation of nanoassemblies (100–300 nm) in water, without the aid of surfactants. The design of "multidrug" nanoparticles combining in the same nanodevice chemotherapy and imaging (ie., "nanotheranostics") or various drugs with complementary biological targets will be also discussed (6). Finally, it will be shown that the construction of nanodevices sensitive to endogenous (ie. pH, ionic strenght, enzymes etc.) or exogenous (ie., magnetic or electric field, light, ultrasounds etc.) stimuli may allow the spatio-temporal controlled delivery of drugs and overcome resistance to current treatments (7).

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Emergence of Ag_2S Qunatum Dots and Their Magnetic Hybrid Structures as New Promising Bionanomaterials

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Abstract: Nanobiomaterials offer nano-solutions for bioapplications. Nanoparticles are very important class of nanomaterials that are utilized biotechnology and medicine. Best example that found commercialization is probably superpramagnetic iron oxide used as contrast agent. A unique class of nanoparticles is semiconductor quantum dots that provide size tunable emission behavior in addition to broad absorabce and narrow emission window. This offers a trenedous advantage as a fluorophore, yet, QDs can be taioled as drug/gene delivery vehicles with suitable coatings and receptor specific tags. Yet, most of the studied QDs are cadmium based and pose significant toxicity. There are many attempts such as core/shell structures, PEGylation etc to decrease the toxicity yet, excitation in the UV and emission in the visible region pose significant limitation to the relatively unsafe exciatation wavelengths, limited penetration depth and autofluoresence of the tissue in the visible region. Therefore, QDs that can be excited in the visible and emitting in the NIR region is highly desirable. Ag_2S with bandgap energy of 0.9 eV and very high cytocompatability offer a great potential in realization of practical QDs for medicine and biotechnology(Hocaoglu *et al, 2012;* Hocaoglu *et al, 2014).*

Aqueous synthesis of Ag_2S QDs that emit in the medical window (700-900nm) with different surface coatings and the highest quantum yields reported in the literature (6 to 150 % with respect to NIR Dye) will be discussed. Cytototoxicity and hemocompatability of such particles will also be discussed showing their great potential as biomaterials. These NIR QDs will be demonstrated as strong optical imaging agents in the in vitro cell studies (Figure 1).

Another group of desired and popular nanomaterials are multifunctional hybrid nanoparticles. We will discuss the synthesis of magnetic and luminescent hybrid nanoparticles (Figure 2) composed of SPIONs and Ag_2S NIRQDs, discuss their hemo and cyctocompatibility as well as their optical and magnetic properties.

Overall, we will demonstrate Ag2S with variety of surface coatings, with good optical properties and very good cyto/hemocompatability as a new QD platform for medicine and bioapplications.

Keywords: Ag_2S, Near-infrared, magnetic, luminescent, hybrid, hemocompatibility, cytocompatibility, optical imaging

Figure 1: Cellular uptake and localization of Ag_2S QDs by NIH/3T3 mouse fibroblast cells (200 µg/mL QD, 24h incubation). (A) Fluorescence, (B) Transmission and (C) Overlay channels of confocal micrograph. The scale bar represents 20 µm. (excitation at 532nm)

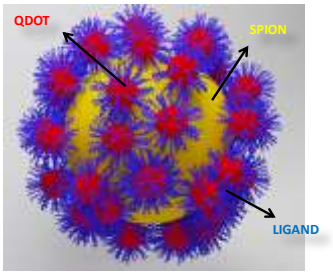

Figure 2: Schematic representation of a hybrid nanoparticle.

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Stimuli-sensitive combination nanopreparations of siRNA and chemotherapeutic drugs to treat multidrug resistant cancer

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Abstract: Tumor therapy, especially in the case of multidrug resistant cancers, could be significantly enhanced by using siRNA down-regulating the production of proteins, which are involved in cancer cell resistance, such as Pgp or survivin. Even better response could be achieved is such siRNA could be delivered to tumors together with chemotherapeutic agent. This task is complicated by low stability of siRNA in biological surrounding. Thus, the delivery system should simultaneously protect siRNA from degradation. We have developed several types of lipid-core polymeric micelles based on PEG-phospholipid or PEI-phospholipid conjugates, which are biologically inert, demonstrate prolonged circulation in the blood and can firmly bind non-modified or reversibly-modified siRNA. Additionally, these nanopreparations can be loaded into their lipidic core with poorly water soluble chemotherapeutic agents, such as paclitaxel or camptothecin. In experiments with cancer cell monolayers, cancer cell 3D spheroids, and in animals with implanted tumors, it was shown that such co-loaded preparations can significantly down-regulate target proteins in cancer cells, enhance drug activity, and reverse multidrug resistance.

In order to specifically unload such nanopreparations inside tumors, we made them sensitive to local tumor-specific stimuli, such as lowered pH, hypoxia, or overexpressed certain enzymes, such as matrix metalloproteases. Using pH-, hypoxia-, or MMP2-sensitive bonds between different components of nanopreparations co-loaded with siRNA and drugs, we were able to make the systems specifically delivering biologically active agents in tumors, which resulted in significantly improved therapeutic response.

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Nanocapsules of perfluorooctyl bromide as theranostic agents: formulation and in vivo evaluation

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Abstract: The need to detect cancer at its early stages, as well as, to deliver chemotherapy to targeted site motivates many researchers to build theranostic platforms which combine diagnostic and therapy. Among imaging modalities, ultrasonography and MRI are widely available, non invasive and complement each other. Both techniques often require the use of contrast agents.
We have developed nanocapsules of perfluorooctyl bromide as dual contrast agent for both imaging modalities. The soft, amorphous polymer shell provides echogenicity, while the high-density perfluorinated liquid core allows detection by ^{19}F MRI. We have used a shell of poly(lactide-co-glycolide) (PLGA) since this polymer is biodegradable, biocompatible and can be loaded with drugs. These capsules were shown to be efficient *in vitro* as contrast agents for both ^{19} F MRI and ultrasonography. In addition, for *in vivo* applications a poly(ethyleneglycol) (PEG) coating promotes stability and prolonged circulation. Being stealth, nanocapsule can accumulate passively into implanted tumors by the EPR effect.A anticancer drug paclitaxel was encapsulated within the PEGylated capsules with the goal of triggering its release upon the application of ultrasound. Capsule surface can also be decorated with RGD moieties to actively target integrins overexpressed on tumor neovessels. We will present nanocapsule formulation and characterization, and will show promising in vivo results obtained for both ultrasonography and ^{19} F MRI, as well as the efficacy of the formulation on xenograft tumors in mice.

Keywords: Nanocapsules, Theranostic, ^{19}F MRI, ultrasonography.

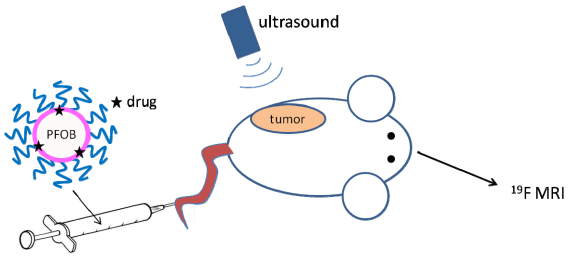

Figure 1: Schematic representation of our objectives

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Formulation and evaluation of Rosuvastatin Calcium-loaded solid lipid nanoparticles

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Abstract: Rosuvastatin Calcium is an anti-hyperlipidemic drug which is sparingly soluble in water and possesses low oral bioavailability (20%). The aim of this investigation was to design and evaluate solid lipid nanoparticles (SLNs) of Rosuvastatin Calcium. The SLNs were prepared by high pressure homogenization using glyceryl monostearate as lipid carrier and poloxamer-188 as surfactant. A two factor, three level (3^2) full factorial design was applied to study the effect of independent variables i.e. amount of glyceryl monostearate (Y1) and % concentration of poloxamer-188 (Y2) on dependent variables i.e. Particle size and % entrapment efficiency (%EE). Particles size, %Entrapment efficiency, zeta potential, drug content, *in vitro* drug release and particles morphology are evaluated for SLNs. The experimental results of optimized batch (batch A10) exhibited particle size 529.6nm with PDI of 0.506. %EE was found to be 48.90 ± 1.85% and drug release was found to be 97.41 ± 1.48 % in 36 hours. Accelerated stability studies showed no significant change in the mean particle size and %EE after storage at 40°C/75% RH for the period of three months. The developed formulation may be absorbed via the lymphatic route thereby avoiding hepatic first pass metabolism. This may lead to improvement in bioavailability, reduction dose and dose related side effects etc.

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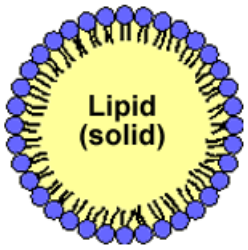
Figure 1: Figure illustrating solid lipid nanoparticle



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A blueprint for modified siRNA-cationic peptide dendrimer-based therapy of Type 2 diabetes through *'PTPN1'* gene silencing

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Abstract: In 2014, the WHO reported that over 347 million individuals worldwide were affected by diabetes mellitus (DM), of which T2DM accounted for ~90% of all cases. The T2DM-affected population mainly comprises individuals where insulin resistance (IR) and pancreatic β-cell dysfunction are the key drivers of the disease, with a complex interrelationship responsible for initiating its pathogenesis. Genome-wide association studies have identified >40 susceptible genes closely associated with T2DM. One of those genes, *PTPN1* encodes for tyrosine phosphatase-1B (PTP-1B) protein. Documented evidence firmly supports the role of PTP-1B in T2DM and obesity through negative regulation of insulin as well as leptin signalling (Koren *et al.*; 2007). At the genetic, molecular, biochemical and physiological levels, PTP-1B has proven beyond reasonable doubt to be a valid target for correcting the key underlying cause of T2DM i.e. insulin resistance. To-date the pursuit of traditional drug-based therapies against PTP-1B have been plagued with issues of "drugability"; this has meant that siRNA-based approaches (through PTP-1B silencing) are a highly attractive therapeutic proposition towards combating insulin resistance in T2DM patients.

Of the various nucleic acid modifications reported, sugar modifications resulting in locked nucleic acids (LNA) and unlocked nucleic acids (UNA) (Figure 1) have gained considerable interest (Veedu *et al.*; 2010). Our current research demonstrates superior stability (**Figure 2**) and gene silencing ability of LNA/UNA-modified siRNA towards the PTP-1B gene, over their unmodified counterparts (**Figure 3b**). Furthermore, effective delivery of these modified siRNAs using hybrid chemical carrier systems (vesicular and non-vesicular) is also under investigation (Shah *et al.*; 2014). Unmodified siRNA specific to rat PTP-1B (commercially sourced) and LNA/UNA-modified siRNA's were synthesized and annealed. Studies were performed on a rat hepatoma cell line (H4IIE) with PTP-1B expression and insulin resistance induced using tumor necrosis factor-α (TNF-α), palmitic acid and tunicamycin. We used RT-PCR and western blot to assess PTP-1B mRNA and protein levels, and a significant (30%) increase in PTP-1B mRNA expression was observed at 180 min post-incubation with TNF-α (**Figure 3a**). Our finding indicate that insulin resistance is being invoked, and forms the basis of evaluating the effects of our modified siRNA delivered using chemical carrier systems. It is expected that modified siRNA-based therapeutics when combined with appropriately engineered carrier systems will create the next generation of gene-based treatment strategies for T2DM.

Keywords: T2DM, PTP1B, siRNA, LNA, dendrimer.

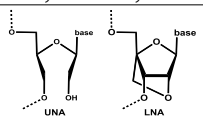

Figure 1: Chemical structure of LNA and UNA.

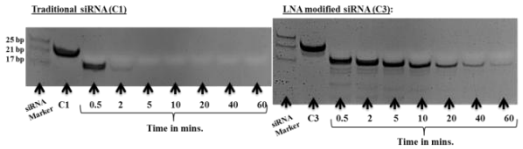

Figure 2: Stability of LNA modified siRNA against its traditional counterpart incubated in 1 mg/mL RNase A @ 37 °C.

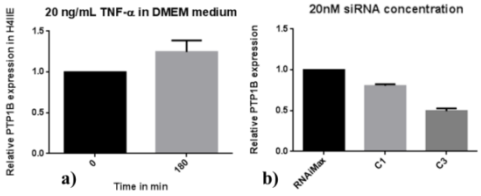

Figure 3: a) Relative expression of PTP1B mRNA in H4IIE cell line incubated with 20 ng/mL of TNF-α for 3 hr. **b)** Comparative gene silencing efficiency between C1 and C3.

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Instability of Carbon Nanoparticles Interacting with Lipid Bilayers

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Abstract: Due to the large number of possible applications of nanoparticles in cosmetic and medical products, the possible hazards of nanoparticles in the human body are a major concern. A worst-case scenario is that nanoparticles might cause health issues such as skin damage or even induce diseases such as cancer. As a first step in the study of the toxicity of nanoparticles, we investigate here the energy behaviour of three distinct carbon nanoparticles interacting with a lipid bilayer; namely nanotubes, nanocones and fullerenes. We determine the energy behaviour of these nanoparticles interacting with a lipid bilayer using the Lennard-Jones potential together with the continuous approximation, which assumes that a discrete atomic structure can be replaced by a surface of uniform atomic density. First, the equilibrium spacing between the two layers of a bilayer is predicted to be 3.36Å. For an assumed circular hole in the lipid bilayer, a relation for the molecular interaction energy is determined, involving the circular hole radius, and the perpendicular distance of the nanoparticle from the hole (Fiugre 1). For each nanoparticle, the relation between the minimum energy location and the hole radius b is found, and for example, for the fullerene, for $b > 6.81$Å, the nanoparticle relocates from the surface of the bilayer to the interior, and as the hole radius increases further moves to the centre of the bilayer, remaining there for increasing hole radii. When the system has no external forces, the nanoparticle will not penetrate through the lipid bilayer but rather remains encased between the two layers.

Keywords: mathematical model, Lennard-Jones potential function, continuous approximation, lipid bilayer, carbon nanoparticles.

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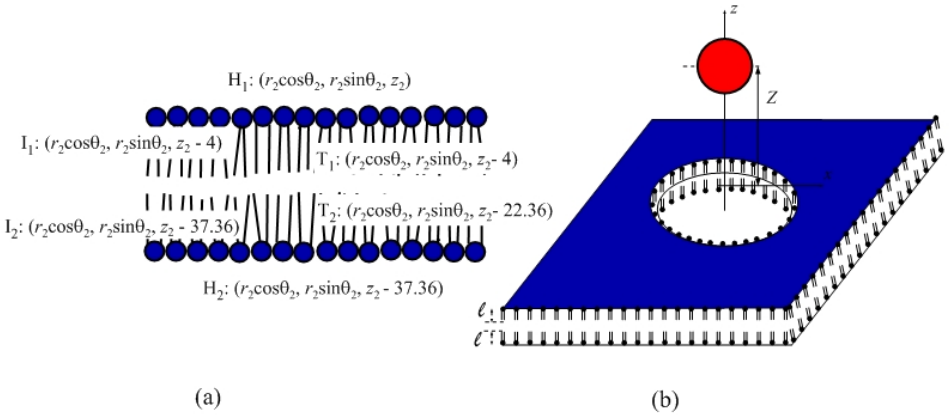

Figure 1: Model for (a) structural dimensions of lipid bilayer where H_1 and H_2 represent upper and lower head groups, I_1 and I_2 denote upper and lower intermediate layers and T_1 and T_2 are upper and lower tail groups and (b) a hole in bilayer.

Targeting of asymmetric amino acid-based cationic dendrimers to caveolae

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Abstract: Caveolae are plasma membrane subdomains ranging from 50-100 nm in diameter. They are abundant in many mammalian cells and are rich in lipids such as glycosphingolipids and cholesterol. They participate in endocytosis, transcytosis, and numerous signal transduction events (Parat; 2009). Membrane proteins of the caveolin family, and cytoplasmic proteins of the cavin family are essential for caveola formation. Gene disruption of either caveolin-1 or cavin-1 (also known as PTRF i.e. polymerase I and transcript release factor) results in caveola deficiency.

Cationic, amino acid-based dendrimers (Parekh *et al.;* 2011) are highly branched chemically-derived gene-vectors developed to transport cargo with therapeutic potential such as pharmacological agents or genetic material, across the plasma membrane. We prepared a panel of cationic dendrimers and investigated whether they use caveola as a route of internalization. We further evaluated the role of cationic charge density and head group chemistry in promoting dendrimer endocytosis via caveolae. Cell-based studies were performed using wild type (WT) or caveolin-1 gene-disrupted i.e. caveola-deficient (KO) mouse embryo fibroblasts. Cells were exposed to biotinylated peptide dendrimers for 12h and internalization was detected after fixation, using Cy3-streptavadin and fluorescence imaging. Nuclei were stained using 4',6-diamidino-2-phenylindole (DAPI). The number of dendrimer-containing red cells per field was counted and expressed as % of the total number of cells per field. Furthermore, to confirm internalization of dendrimers, time dependent kinetics of internalization was performed.

There was a statistically significant difference in entry of cationic dendrimers between WT and KO cells. Internalization was not abolished in KO cells, suggesting that other routes of entry also participate in dendrimer endocytosis **(Figure 1)**. We further unveiled differences between dendrimers with varying charge density and head group chemistry **(Figure 2)**. In addition, significantly increased uptake for WT cells was observed in a time course study at the earliest time point (1 min) for a 16+ charged Arginine dendrimer.

Our results show, using a molecular approach, that (i) caveolae mediate at least in part the entry of cationic dendrimers in cells and (ii) dendrimer structure can be modified to promote caveolar endocytosis.

Keywords: Caveolae, endocytosis, drug delivery, dendrimer

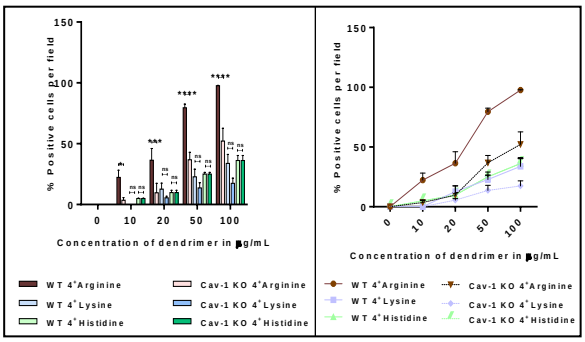

Figure 1: Caveolae participate in binding and internalization of tested dendrimers
Fibroblasts isolated from wild type or caveolin-1 knockout mice were tested for biotinylated 4 charged cationic dendrimer binding and internalization. The cells were treated with dendrimers for 12 h. Results are reported as % positive cells per field. N=3 separate experiments. **p*<0.05, ***p*<0.01, ****p*<0.001, *****p*<0.0001.

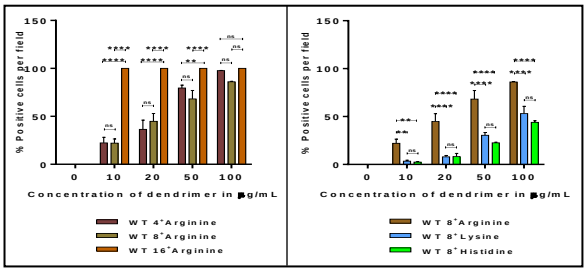

Figure 2: Dendrimer head group, charge density and generation modulate cellular entry
Fibroblasts isolated from wild type mice were tested for biotinylated 4, 8 and 16 charged cationic dendrimer surface binding and internalization. The cells were treated with dendrimers for 12 h. Results are reported as % positive cells per field. N=3 separate experiments. ***p*<0.01, *****p*<0.0001, ns, not significant.

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Polymeric self-assemblies for photodynamic therapy: a critical approach

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Abstract: The work presented here suggests a new approach in the critical development of polymeric nanovectors for photodynamic therapy (PDT) against cancer. Whereas hundreds of studies quickly jump forward from formation of self-assemblies to biological application without having a thorough examination of the vector solution, we suggest having a parallel assessment of formation/characterization of the nanovectors and biological activity. This is possible by first using a careful physical chemistry characterization of the vectors by both batch techniques (light and neutron scattering, electron microscopy, atomic force microscopy) and Asymmetrical Flow Field-Flow Fractionation (AsFlFFF) coupled to adequate detectors (refractometry, light scattering). This enables us to have a deep knowledge of the solution of the vectors regarding purity, size and zeta potential. The case of both polymeric micelles and polymersomes will be presented, using poly(ethyleneoxide-b-ε-caprolactone), poly(ethyleneoxide-b-D,L-lactide) and poly(ethyleneoxide-b-styrene). Self-assemblies exhibiting size range of 20-200 nm will be shown.
The work clearly shows the possible presence of different populations of nanovectors in some cases. For each new vector, its ability to carry a photosensitizer (Pheophorbide a) for PDT is examined. The activity in PDT either in 2D and 3D cell culture will be presented and compared on different batches, in link with the purity analysis. Here again, it becomes highly recommended to develop a critical approach considering in vitro analyses, since differences of efficiencies are clearly observed depending on the vectors and the 2D or 3D culture type.

Keywords: Polymeric micelles, self-assemblies, Asymmetrical Flow Field-Flow Fractionation, light scattering, photodynamic therapy, spheroids

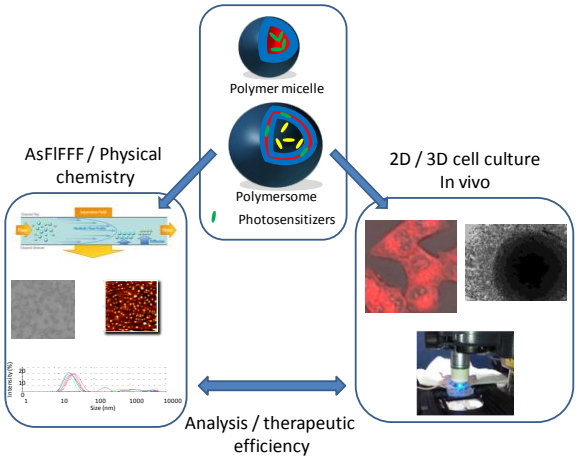

Figure 1: Process of nanovectors development for PDT. The therapeutic efficiency is systematically linked to a thorough characterization of the vector solution, including its purity.

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Biodegradable Nanoconstructs for Pharmacology: Development of Biomimetic Systems for Drug Delivery and Pathogen Blockage

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Abstract: The urgent task of modern care of public health is developing completely brand-new, "smart" drug formulations that can provide a superior therapeutic efficacy with a minimum of side effects. Moreover, the research in this direction might able to achieve the unexpected decisions when choosing therapy for various, including widespread and extremely dangerous, diseases. In particular, biodegradable nanocontainers with stipulated physical and chemical (particle size, size distribution, degradation rate of chemical bonds defined by a structure of polymer) characteristics of nanoparticles, as well as biological (biomimetic) properties of their surface, which smoothly invaded in the natural processes of human body, enable to provide such results.
The aim of presented research is design and synthesis of special biologically functionalized nanoconstructs based on nanoparticles of various chemical nature, including those modified with biofunctional gold nanoclusters, which have been recently developed in our team. The methods of biological functionalization of nanoparticle surface for addressed drug delivery to specified biological targets with properties of blocking the pathogens of various origins to facilitate their rapid clearance from the body by phagocytosis are developed.
The proposed nanoconstructs and means of their medical application certainly can be transferred to the pharmaceutical industry.
Reducing the risk of side effects due to the accurately designated molecular targets and reduction of time for therapy determine the social significance of the results and may have significant economic benefits.
Application of the unique information of the multifunctional Biobank SPbSU and bioinformatics tools for searching of specific sequences of natural biopolymers in order to select the optimal complementary pairs and *in silico* modeling of highly specific intermolecular interactions allowed functionalization of nanoconstructs to develop the new personalized approaches to diagnosis and treatment of various diseases (hepatitis C, diabetes of 1 and LADA types, AIDS, different types of cancer, etc.).

Keywords: biodegradable polymer nanoparticles, surface biofunctionalization, biomodels, ligand-research interactions, biomimetics, drug delivery, pathogen blockage, socially significant deseases

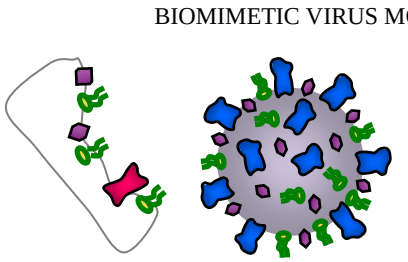

Figure 1: Figure illustrating the algorithm of nanoconstructs based on biodegradable drug-containing (inside) nanoparticles provided by multifunctional ligands both to address the carriers to corresponding natural receptors and to bind the pathogens in a bloodstream.

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Evaluation of Gelled Oil Nanoparticles as New Vehicles for Drug Delivery

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Abstract: In recent years, a growing interest has emerged in the development of semi-solid colloidal careers for the delivery of water-insoluble drugs. Solid lipid Nanoparticles (SLN) and Nanostructured Lipid Careers (NLC) are example of such systems. Their preparation consists of a hot emulsification over the melting temperature of the solid lipids and freezing at room temperature to form an aqueous dispersion of solid particles. However, crystallization occurring in the lipid matrix leads to significant drug expulsion during storage, and the formed objects also present a low biodisponibility because of a solid in solid encapsulation.

As an alternative to SLN, we propose the use of an original family of organogel nanoparticles, obtained through a smilar process. Organogels are semi-solid materials in which an organic solvent (e.g., vegetable oil) is entrapped in the three-dimensional fibrous network formed by self-aggregation of a low molecular mass organic gelator (12-Hydroxystearic acid). The preparation process of the gelled oil nanoparticles is based on the sol-gel phase transition of the organogel obtained by hot emulsification (T°>T°gel) in presence of an aqueuous solution of stabilizing agent (Polyvinylalcohol 80), leading to a stable semi-solid dispersion after cooling (T°<T°gel), (Figure 1).

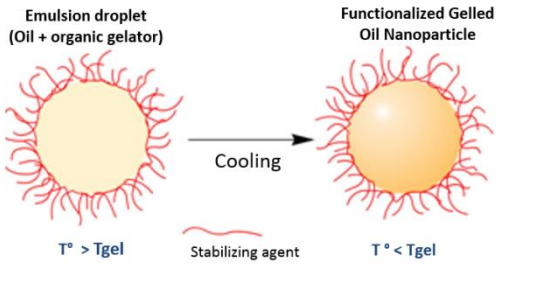

Figure 1: Concept of the elaboration of the gelled nanoparticle aqueous dispersion.

In a first part, we will present the preparation and the characterization of the aqueous dispersions of the gelled oil particles. Then we will evaluate the encapsulation properties and delivery of the prepared systems using different drugs and models, varying their hydrophobicity and pKa (indomethacin, ibuprofen, ketoconazole, efavirenz, Nile red).

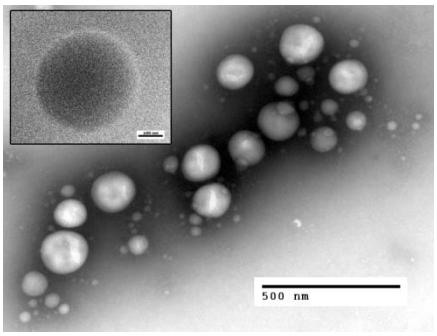

Figure 2: TEM micrograph showing organogel particles made of soybean oil and HSA as gelator

Encapsulaton efficiency studies showed an impressive drug loading of 99% and no significant drug leakage during storage.

In vitro dialysis release experiments showed different kinetic profiles in comparison with control buffer solutions, underlying the importance of the drug solubility in the gelled oil and its possible ionisation in water. The results obtained have enabled us to evaluate the drug delivery capabilities of these gelled particles and their possible use in different pharmaceutical pathways (oral or skin).

Keywords: Organogels, Gelled Nanoparticle, Aqueous dispersion, Drug delivery

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Bio-inspired catanionic vesicles as drug delivery systems: Study of the cell internalisation pathways

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Abstract: The cell membrane is a selective barrier that regulates the transfers of drugs into the cell. During the last forty years, a number of drug delivery systems have been developed to control drug release profile, absorption and distribution, with a view to improving efficacy and safety. The direct release of active molecules inside the cytoplasm is often considered as the most efficient and the safest mechanism for drug delivery. This promising strategy, which can proceed through membrane fusion between suitable carriers and the cell membrane, is not straightforward and it has raised important research and developments for the design of synthetic delivery systems.

Among drug delivery systems, catanionic vesicles, made of cationic and anionic surfactants, have appeared as powerful candidates for pharmaceutical applications because they are relatively cheap and easy to use (Soussan *et al.*, 2009). Using labelled vesicles made of a sugar-based catanionic surfactant, the work reported here aims at exploring the mechanisms of their internalisation into cells. The study was performed on different cell types using confocal laser scanning microscopy and flow cytometry, and confirmed the contribution of endocytotic and fusion processes with the plasma membranes of cells (Boudier *et al.*, 2011; Mauroy *et al.*, 2014). We confirmed the ability of sugar-derived catanionic vesicles to fuse with lipidic membranes using pure lipidic systems (Mauroy et al. 2012).

Finally, to validate the great potential of catanionic vesicles for further applications as drug delivery systems, examples of use of this catanionic system will be presented (Castagnos *et al.*, 2014).

Keywords: drug delivery, catanionic vesicles, membrane fusion, endocytosis, glycolipids, biomedical applications.

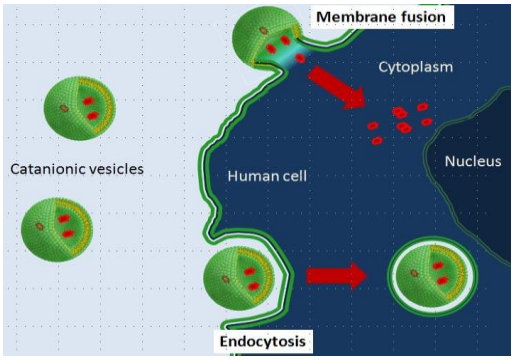

Figure 1: Figure illustrating the versatile cellular uptake mediated by catanionic vesicles

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Novel Nano-Carriers for Controlled Pulmonary Drug Delivery

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Abstract: A substantial body of research has focused recently onto pulmonary drug delivery as a well-accepted treatment for many lung diseases. This work was aiming to develop and *in-vitro* evaluate new series of carriers for controlled pulmonary drug delivery. The developed carriers systems combine the benefits of nanoparticles (NPs) and respirable/swellable microparticles while avoiding their shortcomings. The carriers are based on PEG-grafted-chitosan (PEG-g-CS) copolymer and ionotropically crosslinked with sodium tripolyphosphate (STPP) and/or Sodium hyaluronate (HAs) in form of hydrogel NPs. The drug-loaded hydrogel NPs were then used to develop respirable/swellable 2-5 microns size microparticles (MPs) through controlled spray drying of an aqueous suspension of the NPs and lactose as excipient. The particle size was determined by laser diffraction and dynamic light scattering. Surface morphology was investigated by AFM and SEM. An *in-vitro* aerosolization study was performed with the aid of a next generation impactor, NGI. Dynamic swelling, *in-vitro* biodegradation, particle's density, and moisture contents were also determined. In addition, *in-vitro* release profile of the loaded drug was investigated in simulated body fluids. The *in-vivo* investigation of the encapsulated drug was also performed using the insufflation method. The average sizes of the prepared crosslinked PEG-g-CS NPs and the MPs were found to be 83.2±2.4 nm and 4.1±0.03 µm, respectively. The NPs-MPs carriers showed high swelling within few minutes, low aerodynamic density (0.2±0.03 g/cc), moisture content of 4.1-9.0%, good *in-vitro* biodegradation, high drug loading capacity exceeding 93%, and a promising sustained drug release both *in-vitro* and *in-vivo*. In conclusion, the developed NPs-MPs systems are very promising and could be utilized as potential carriers for sustained delivery of various drugs to the lung.

Keywords: Nanoparticles, microparticles, swellable, lung, pulmonary, drug delivery

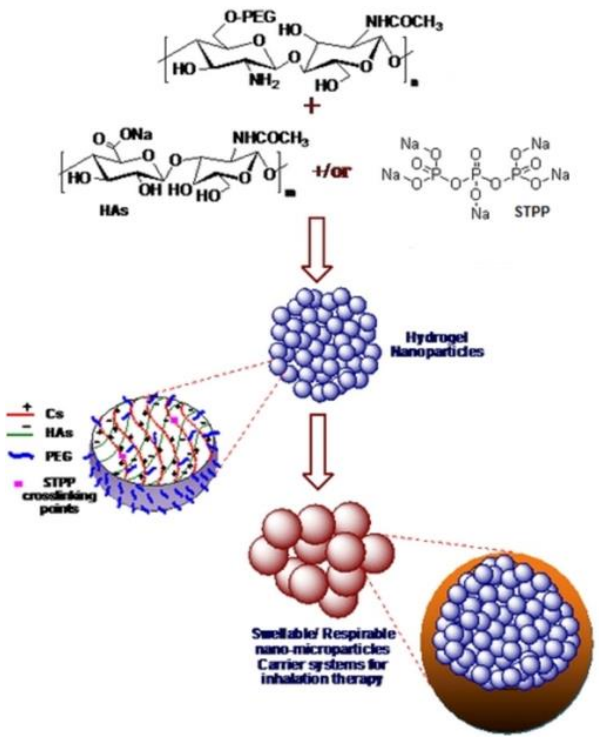

Figure 1. An illustration of the developed polymeric NPs-in-MPs as pulmonary carrier systems.

Effect of Nanotube Materials on Encapsulation of Lysozyme

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Abstract: Lysozyme is an enzyme often used in biochemical and pharmaceutical engineering and food industries as an antibacterial agent. Immobilization of lysozyme by encapsulating in a nanotube has much interest due to the enhanced property in ambient condition. Experimentally, various types of nanotubes have been proposed as a host for lysozyme. Here, we model the interaction between lysozyme and various types of nanotubes in order to compare the effectiveness of different nanotube materials. Based on the van der Waals interaction, which we model using the Lennard-Jones potential and a continuum approach, we find that different types of nanotubes have very little effect on the minimum radius (b_0) that will allow the acceptance of the lysozyme molecule and also on the critical radius (b_{cr}) that will maximise the interaction among the lysozyme molecule and the tube. However, while carbon, silicon, boron nitride and silicon carbide nanotubes possess similar energy profiles, the energy level of titania nanotube is much smaller than other tubes. This lower level of energy implies that to expel the lysozyme from the titania nanotube would require less amount of energy compared to other types of nanotubes. Based on this result, it may be said that titania nanotube has more potential for the delivery of lysozyme molecule. Since b_0 and b_{cr} for the titania nanotube are the smallest, less amount of material is needed to create the titania nanotube that allow the acceptance of the lysozyme or to generate the tube that gives optimum interaction. Following titania nanotube are carbon, boron nitride, silicon carbide and silicon nanotubes, ranging in order of the smallest to the largest b_0 and b_{cr}. For a titania nanotube, we also consider the electrostatic effect on its interaction with a lysozyme molecule by using the Coulomb potential. We find that the electrostatic energy dominates the interaction between the lysozyme and the titania nanotube. When the net charge of lysozyme is positive, we find that the smallest radius of the titania nanotube that will accept the lysozyme molecule is b_0 = 18.55 Å, noting that the radius of the lysozyme is 18.54 Å. This reduces from b_0 = 21 Å when we only consider the van der Waals energy. For the case when the lysozyme has negative net charge, the lysozyme molecule cannot enter the titania nanotube as the repulsive energy dominates. Results presented here can be extended to guide experiments to determine appropriate type of nanotube materials for effective molecular storage.

Keywords: Boron nitride nanotubes, carbon nanotubes, silicon nanotubes, silicon carbide nanotubes, titania nanotubes, lysozyme, Lennard-Jones potential, Coulomb potential, continuum approach

Structure-directing star-shaped block copolymers: Supra-molecular vesicles for the delivery of anticancer drugs

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Abstract: Amphiphilic polycarbonate/PEG copolymer with a star-like architecture was designed to facilitate a unique supramolecular transformation of micelles to vesicles in aquous solution for the efficient delivery of anticancer drugs. The star-shaped amphipilic block copolymer was synthesized by initiating the ring-opening polymerization of trimethylene carbonate (TMC) from methyl cholate through a combination of metal-free organo-catalytic living ring-opening polymerization and post-polymerization chain-end derivatization strategies. Subsequently, the self-assembly of the star-like polymer in aqueous solution into nanosized vesicles for anti-cancer drug delivery was studied. DOX was physically encapsulated into vesicles by dialysis and drug loading level was significant (22.5% in weight) for DOX. Importantly, DOX-loaded nanoparticles self-assembled from the star-like copolymer exhibited greater kinetic stability and higher DOX loading capacity than micelles prepared from cholesterol-initiated diblock analogue. The advantageous disparity is believed to be due to the transformation of micelles (diblock copolymer) to vesicles (star-like block copolymer) that possess greater core space for drug loading as well as the ability of such supramolecular structures to encapsulate DOX. DOX-loaded vesicles effectively inhibited the proliferation of 4T1, MDA-MB-231 and BT-474 cells, with IC50 of 10, 1.5 and 1.0 mg/L, respectively. DOX-loaded vesicles injected into 4T1 tumor-bearing mice exhibited enhanced accumulation in tumor tissue due to the enhanced permeation and retention (EPR) effect. Importantly, DOX-loaded vesicles demonstrated greater tumor growth inhibition than free DOX without causing significant body weight loss. The unique ability of the star-like copolymer emanating from the methyl cholate core provided the requisite modification in the curvature to generate vesicles of high loading capacity for DOX with significant kinetic stability that have potential for use as an anti-cancer drug delivery carrier for cancer therapy.

Keywords: Star-like polycarbonate; Vesicles; Doxorubicin; Biodistribution; Anti-tumor activity.

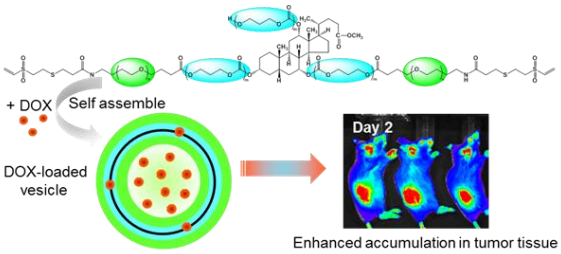

Figure 1: Star-like polycarbonate/PEG block copolymer has been synthesized and assembled into vesicles for anticancer drug delivery. When injected into 4T1 tumor-bearing mice, DOX-loaded vesicles demonstrated greater anti-tumor efficacy than free DOX.

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Tunable release of dendritic fullerene-1 modulated by an electric field across a nanochannel membrane

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Abstract: Future progress in drug delivery technologies will require precise temporal and volumetric control of therapeutic release. Chronotherapies based on aligning treatment administration with circadian rhythms have been demonstrated as more effective for several chronic pathologies (Youan 2010), such as colorectal cancer (Li et al. 2013), rheumatoid arthritis (To et al. 2011), and hypertension (Hermida et al. 2011), among others. Here we present a silicon membrane with hundreds of thousands of nanochannels fabricated with sub-nanometer precision using sacrificial etching techniques. While no electric field was applied (configuration A in Fig. 1) zero order transport was observed across 5.7 nm nanochannel membranes. The temporal, reproducible control of the dendritic fullerene-1 (DF-1) was established by application of a low potential (1.5 V) across two platinum electrodes positioned on either side of the membranes (configuration B, Fig. 1). Conductance measurements were performed in order to demonstrate that the interruption was attributable to Ionic Concentration Polarization (ICP) at the interface between the membrane's micro- and nanochannels (Kim et al. 2009), even in concentrated solutions (≤ 1 M NaCl). Due to its low power consumption (100 nW), one envisioned goal of this technology is creation of an implantable and remotely controllable system for personalized, telemedical therapeutic administration.

Keywords: Nanochannel membrane, Controlled drug delivery, Chronotherapy, Active release, Ionic concentration polarization, Electrokinetic transport, Telemedicine, Personalized medicine.

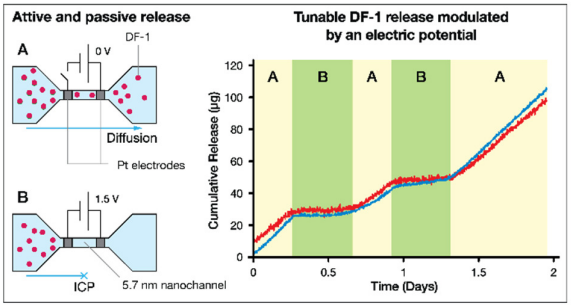

Figure 1: Figure illustrating two replicates of the cumulative release of dendritic fullerene-1 (DF-1) tuned by a passive release (configuration A) and an active release (configuration B) phase across a membrane containing hundreds of thousands 5.7 nm nanochannels.

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Preparation of Hydrolysable Biocompatible Polymersomes for Drug Delivery

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Abstract: The desire to understand molecular self-assembly and to explore potential applications motivates the study of self-assembly principles, theories, properties and structures of the assemblies. Block copolymer chains aggregate into various morphologies according to different contributions to the free energy of the system. The most common morphologies are spherical and cylindrical micelles, and polymeric vesicles, termed polymersomes. Polymersomes are hollow spheres with sizes ranging from tens of nanometres to tens of micrometres, typically having a hydrophobic wall and hydrophilic internal and external coronas. Polymersomes have been studied vastly in the last decade and new types of drug delivery systems based on their unique properties have been reported. Yet, only few studies dealt with biocompatible and biodegradable polymersomes. A better understanding of the formation mechanisms of polymersomes and of the relation between nanostructure and properties are still required. Therefore, in this work will investigate the self-assembly of block copolymer aggregates with emphasis on polymersomes. This research will deal with the formation of polymersomes from two types of biocompatible and biodegradable block copolymers, poly(ethylene oxide)-b-poly(ε-caprolactone) (PEO-b-PCL) and poly(ethylene oxide)-b-poly(lactic acid) (PEO-b-PLA). The study of the relation between aggregates morphology and preparation techniques include variation of experimental parameters and evaluating their effect on the formed structures. The main tools that used in the research are size and structure characterization using light and X-ray scattering techniques and electron microscopy. Further experiments would be conducted in order to explore the usage of the polymersomes carriers as drug delivery vehicles for three model drugs of different hydrophilicity. Three types of block copolymer aggregates have been produced and and characterization of structure has been conducted. In order to allow a rational design of polymersomes, insights into the formation and properties of biocompatible polymersome systems are needed. Therefore, the overall goal of this research is to establish a better understanding of biocompatible block copolymer systems with an emphasis on systems that lead to formation of polymersomes

Keywords: polymersomes, PEO-PCL, hydrolysis, drug delivery.

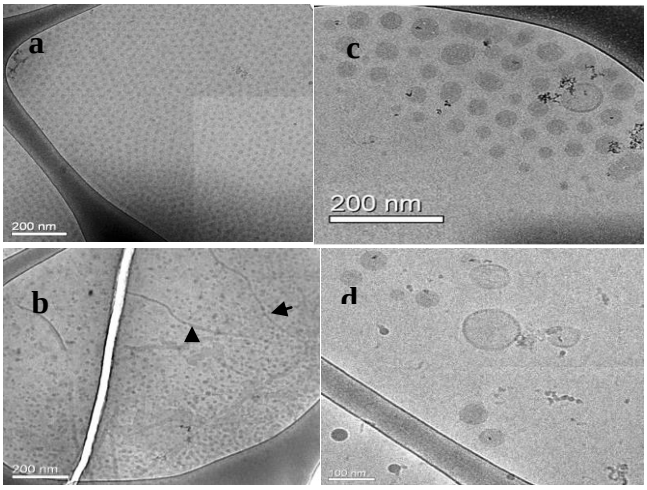

Figure 1: cryo-TEM micrographs of OLA2 (a) , OCL1(b) and OCL2 (c,d). The copolymers differ by their hydrophilic fraction,f

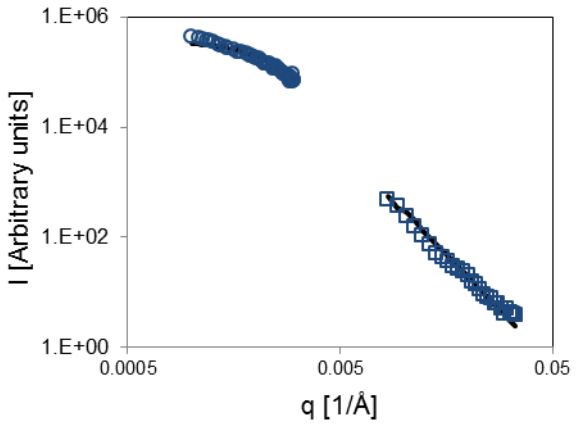

Figure 2: SLS (O) and SAXS (□) data for PEO-PCL 0.1%wt. The line represents the best fit model

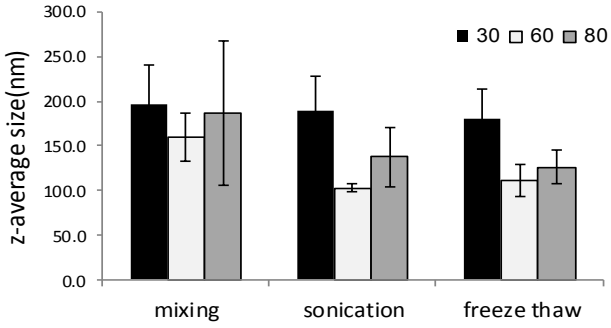

Figure 3:-average size of aggregate dispersions throughout the preparation process at different temperatures

Enhanced permeability througth the blood-brain barrier using targeted solid lipid nanoparticles for brain drug delivery

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Abstract: The challenging of cross the blood-brain barrier (BBB) and reach the brain in a therapeutic concentration is the Holy Grail for effectively treat and cure brain diseases. In fact, more that 98% of all the potential new drug for the treatment of brain diseases are unable to cross the BBB. Here, nanotechnology can be an important tool to improve the specificity and permeability of drugs in the BBB. In this context the aim of this work was the development of a new delivery system to direct drugs to the brain, by functionalizing solid lipid nanoparticles (SLNs) with apolipoprotein E (ApoE) molecules, aiming to enhance their binding to low-density lipoprotein (LDL) receptors overexpressed on the BBB endothelial cells.

SLNs were successfully functionalized with Apo E, by two distinct strategies which took advantage of the strongest known non-covalent interaction between biotin and avidin. The functionalization of SLNs with ApoE was demonstrated by infrared spectra and fluorimetric assays. Transmission electron microscopy (TEM) images revealed spherical nanoparticles, dynamic light scattering (DLS) gave a Z-average under 200 nm, polydispersity index below 0.2 and zeta potential between -10 mV and -15 mV. A stability study revealed that these characteristics remained unchanged for at least 6 months. In vitro cytotoxic effects were evaluated by MTT and LDH assays in the hCMEC/D3 cell line, a human BBB model, and revealed no toxicity up to 1.5 mg/ml of formulation solid amount for 4 hour of incubation. The enhanced permeability of functionalized SLNs was evaluated in transwell devices cultured with hCMEC/D3 monolayers and it was found a 1.5-fold increase in the permeability of functionalized SLNs when compared with non-functionalized ones.

The different molecular mechanisms of endocytosis and transcytosis processes were also studied in order to clarify the transport pathways of the nanoparticles through the BBB. It was used flow cytometry system (FCS), confocal laser scanning microscopy (CLSM) and fluorimetric assays with tracers and different pathway inhibitors. The transport of SLNs across the hCMEC/D3 monolayer was found through a transcellular but not a paracellular route. Functionalized SLNs exhibited higher intracellular uptake compared with non-functionalized ones and were found to enter cells through a specific clathrin-mediated mechanism, related to the expression of LDL receptors on BBB.

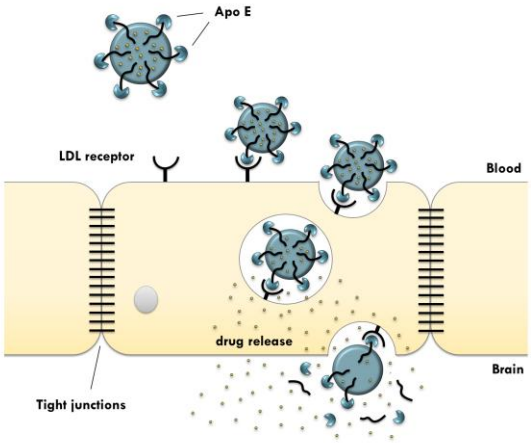

Figure 1: Schematic representation of the proposed mechanism of Apo E - functionalized SLNsuptake in brain (not to scale).

These novel ApoE-functionalized SLNs resulted in dynamic stable systems capable of being used for an improved and specific brain delivery of drugs through the BBB.

Keywords: Blood-brain barrier, drug-delivery, nanotechnology, solid lipid nanoparticles, functionalization, apolipoprotein E.

Acknowledgments: This work received financial support from the European Union (FEDER funds through COMPETE) and National Funds (FCT, Fundação para a Ciência e Tecnologia) through project Pest-C/EQB/LA0006/2013. The work also received financial support from the European Union (FEDER funds) under the framework of QREN through Project NORTE-07-0124-FEDER-000067. To all financing sources the authors are greatly indebted.

Design of Magnetic Molecularly Imprinted Polymer for Controlled Release of Doxorubicin under Alternative Magnetic Field.

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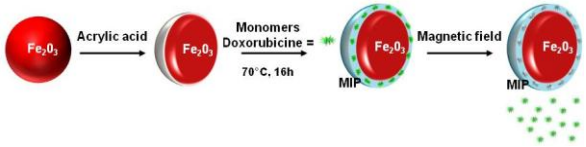
Abstract: Magnetic nanoparticles (MNPs) have attracted considerable attention for magnetic targeting and hyperthermia applications owing to their ability to generate heat when exposed to an alternative magnetic field (AMF) without penetration depth limit. Novel magnetic materials for therapeutic agents release based on thermosensitive polymers or vesicles have been developed. In this case, the approach was to induce an increase of permeability of the vector by heat dissipation under AMF excitation. Another way to release drug is to use hyperthermia to break bond between superparamagnetic iron oxide nanoparticles and the target in presence of oscillating magnetic fields. Recently, multifunctional ligands linked to iron oxide nanoparticles took benefits from local heating of nanoparticle's surface to release a fluorophore on demand. Our approach is motivated by these last developments, i.e. to use local heating as the key parameter to trigger drug release.
We synthesised an innovative magnetic delivery nanodevice for targeted cancer therapy showing active control over drug release by using hyperthermia effects. Our material, wich combines the drug controlled release ability of non thermosensitive Molecularly Imprinted Polymers (MIP) with magnetic properties of iron oxide nanoparticles, allows the control release of doxorubicine.



Scheme 1. Multistep synthesis of Fe_2O_3@DOX-MIP via a subsequent grafting of an acrylic acid compound and the growth of the polymer at 60°C. By applying an AMF, the doxorubicin is released.

Upon AMF exposure, the bonds between the MIP and the doxorubicin are broken and the molecule is released without any significant heating of the medium. These materials offer great promise for the doxorubicin release under alternating magnetic field but moreover we think that this approach can be expanded to other polymers or molecules. Using Magnetic molecularly imprinted polymers for drug delivery under AMF is a major advance in the development of multifunctional targeted drug delivery technologies and may become important theranostic tools in nanomedicines for in vitro and in vivo applications.

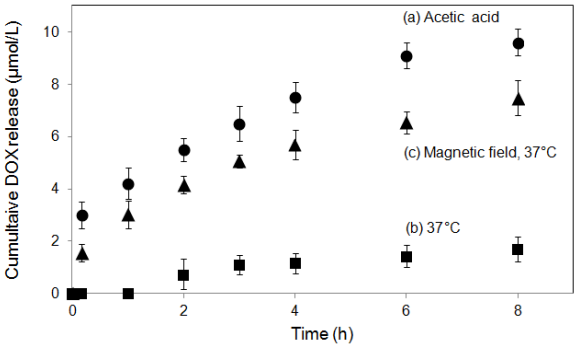


Figure 1: Influence of an AMF on the release kinetics of Fe_2O_3@DOX-MIP nanoparticles at (a) after acidic treatment, (b) 37°C without magnetic field and; (c) under alternative magnetic field (335 kHz, 5A), 37°C in µmol/L.

Keywords: hybrid nanomaterials, hyperthermia, alternative magnetic field, molecularly imprinted polymer, controlled release, biomedical applications.

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BioConjugated Gold Nanoparticles for Enhaced Delivery and Cellular Uptakes

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Abstract: Gold nanoparticles (Au NPs) have demonstrated promising properties for enhanced cellular interaction including; size and shape dependent optical properties originated from the surface plasmon resonance band, a low cytotoxicity and an ease of bioconjuguation (Khlebtsov et al; 2010). Moreover, the nanoscale nature of Au NPs provides opportunities to interact with biomolecules (i.e. antibodies, nucleotides, peptides, proteins) and living cells. Therefore, Au NPs have received considerable attention for a wide range of applications in optoelectronics, diagnostics, thermal therapy, drug and gene delivery and have contributed to the advancement of bionanotechnology (Tiwari et al; 2011; Alkilany et al; 2011). A key challenge to applications of Au NPs in drug delivery is *in vivo* instability as unmodified Au NPs are subject to aggregation in the physiological environments prior to arrival at the site of action. Attachment of neutral polymers such as polyethylene glycol (PEG) has been shown to increase the biological stability of NPs. However, attachment of PEG can also dramatically decease the cellular uptake of NPs (Pozzi et al; 2014). To overcome this issue, PEGylated Au NPs can be conjugated with bioactive targeting ligands to facilitate site-specific delivery via ligand-receptor mediated endocytosis. In this study proteins (ApoE, BSA and Transferrin) were grafted onto Au NPs through an ethylene glycol linker. Transferrin (Tf) was shown to enhance cellular uptake of Au NPs relative to untargeted Au NPs (Figure 1). In addition, in this presentation we will also show that cationic AuNPs were synthesised by conjugation of L-cysteine methyl ester hydrochloride and large branched polyethylenimine. These NPs also have potential for enhanced cellular uptake and siRNA delivery.

Keywords: gold nanoparticles, polyethylene glycol, protein, drug delivery, cellular uptake, biomedical applications.

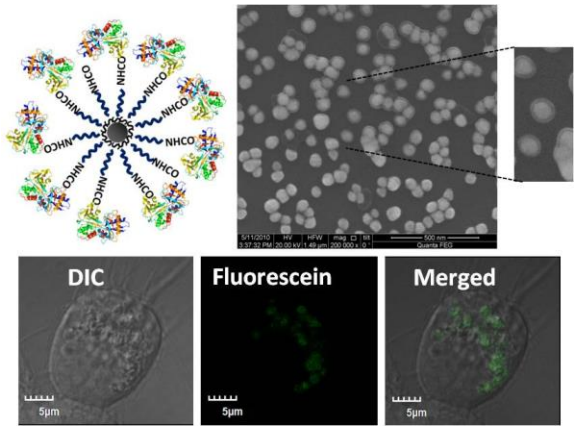

Figure 1: Schematic illustrating the final nanparticle structure with an ethylene glycol and protein coating (up left), SEM image showing the nanopartilces surrounded with an organic layer (up right). Intracellular distribution of fluorescein-labelled AuNPs-Tf in prostate cancer (PC3) cells following 24h post-transfection (lower images). These TEM images, left to right represented Differential interference contrast (DIC), fluorescein, and merged images of DIC and fluorescein.

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Prednisolone-loaded pH-sensitive liposomes as an active targeting strategy for rheumatoid arthritis

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Abstract: Rheumatoid arthritis (RA) is a chronic systemic inflammatory and autoimmune disease mainly characterized by the progressive inflammation of the synovial tissue of the body joints, destruction of cartilage and further bone erosion. Currently available treatment options include non-steroidal anti-inflammatory drugs, glucocorticoids and disease modifying anti-rheumatic drugs, either used as monotherapy or in combination therapy.

In this project we focus on the use of glucocorticoids, namely prednisolone, which is one of the most used by the physicians once RA is diagnosed, due to its rapid effect in suppressing the characteristics inflammation of RA. However, these therapeutic agents are associated with severe side effects resultant from limited selectivity and widespread biodistribution of drug molecules into non-target tissues. In order to overcome the drawbacks of conventional therapy, the aim of the following project was to design pH-sensitive liposomes as suitable drug delivery nanosystems for the treatment of RA. Although these liposomes are stable at physiological pH, they undergo rapid liposomal destabilization under acidic conditions as those presented in endosomes of target cells.

In addition to the pH sensitivity, the extension of liposomes binding and internalization were enhanced either with polyethylene glycol-folic acid (PEG-FA) or hyaluronic acid (HA). By this means that designed pH-sensitive liposomes will, firstly by the PEGylation of liposomes, improve long-circulating times; secondly by the coupling with specific targeting conjugates, enable high binding to over-expressed target cell receptors, hence ensuring cell uptake; and thirdly by using decreased endosomal pH values, trigger and control the release of drug. Thus, the design of targeted pH-sensitive liposomes for the treatment of RA, aims to improve the therapeutic efficiency and efficacy of prednisolone, due to liposomes ability to mediate a specific and controlled release of the drug molecules into target cells, while limiting adverse off-target unwanted effects.

The *in vitro* performance of the designed pH-sensitive liposomes was evaluated through the physicochemical characterization, in terms of encapsulation efficiency, drug loading capacity, size, size distribution, zeta potential and TEM analyses underline a difference in the targeted pH-sensitive liposomes from the non targeted ones. Addicionally, drug release studies were performed mimicking both physiologic (pH 7.4) and acidic (pH 5.0) conditions. Finally, *in vitro* cellular studies were carried out to evaluate both cell viability and cytotoxicity character of designed liposomal formulations, using MTT and LDH assays. And, cell uptake kinetics and mechanism were assessed by flow cytometry, showing that synthethised targeting conjugates specifically improved the uptake by target cells.

This project proves that the selectivity and stability of the designed pH-sensitive liposomes increases the bioavailability of the drug molecules at the site of inflammation, once the liposomes specifically internalize into the target cells, where is trigger controllably the release of drug. Therefore, pH-sensitive liposomes have a huge potential as drug delivery systems to enhance the therapeutic efficiency of prednisolone, minimizing the well-know deleterious side effects in the treatment of RA.

Keywords: rheumatoid arthtitis, pH-sensitive liposomes, prednisolone, folic acid, hyaloronic acid.

Knowledgements: This work received financial support from the European Union (FEDER funds through COMPETE) and National Funds (FCT, Fundação para a Ciência e Tecnologia) through project Pest-C/EQB/LA0006/2013. The work also received financial support from the European Union (FEDER funds) under the framework of QREN through Project NORTE-07-0124-FEDER-000067. To all financing sources the authors are greatly indebted. CN also acknowledge the FCT for financial support through the Post-Doc Grant (SFRH/BPD/81963/2011).

Rapid nanoformulation and cGMP preparation of antiretroviral drugs for oral HIV nanomedicine and human clinical dosing studies

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Abstract: The first planned in-human clinical dosing studies for oral dosed nanomedicines for HIV are discussed. The global increase in patients surviving with HIV has given rise to greater demand for anti-retroviral drugs (ARVs). Oral dosage of ARV drugs is extremely desirable for HIV patients due to patient adherence and the need for long term daily dosing over prolonged periods. Herein, a strategy for the progression from small laboratory scale to industrial cGMP manufacturing scales of solid drug nanoparticles (SDNs) is demonstrated. A novel emulsion templated, freeze drying method was used to prepare more than of 4500 ARV SDNs for material evaluation.[1] Subsequently, pharmacological testing of more than 450 nanodispersions was carried out which demonstrated significant pharmacokinetic enhancement with respect to non-nanoformulated ARVs.[2] A large amount of both physical and pharmacokinetic data were collected from less than 4.5 g of ARVs.

Lead SDNs were progressed towards novel emulsion spray-drying synthesis. With the use of an industrially relevant Niro Mobile Minor spray dryer, establishment of scale was achieved leading to large scale production of viable powders for loading into capsules for oral dosage to healthy volunteers for clinical trial studies. Physical characterization *via* PXRD show the dry powders obtained to be amorphous. Upon addition of water (1 mg/mL) powders easily dispersed showing SDN particle size of approximately 250 nm, determined by DLS. Stability testing of aqueous nanodispersions showed SDN particles to remain stable for several hours as well as stable to acid and basic conditions. Storage stability studies were also conducted on the produced bulk powder as well as the clinically prepared drug powder capsules under three temperature and humidity regimes and monitored by HPLC yielding long term storage stability data.
Documentation and MHRA approval has been applied for and granted and as such clinical development and human clinical trial dosage for the first oral dosed nanomedicine for ARV therapy against HIV is imminent.

Keywords: Human clinical trial, Solid Drug Nanoparticles, HIV, Antiretroviral drugs, industrial scale up.

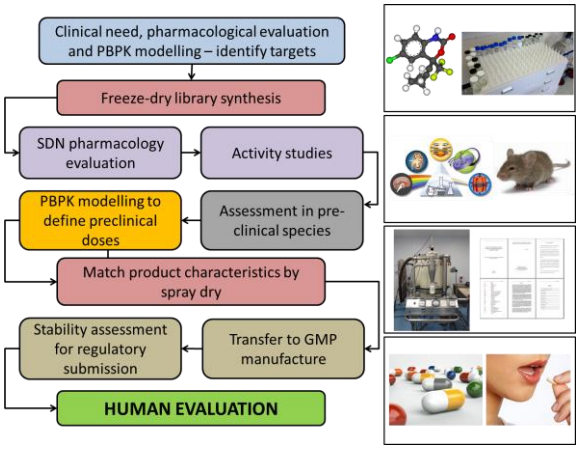

Figure 1: Strategy showing progression from small laboratory scale SDN production through to both *in vitro* and *in vivo* pharmacological analysis followed by industrial scale up and finally through to human clinical dosing studies.

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Combined PTT & PDT cancer therapies mediated by hybrid carbon nanotubes and assessment of ultrasound elastography for monitoring tumor treatment

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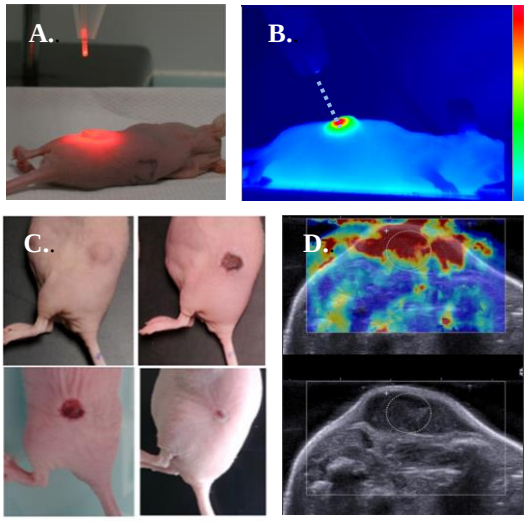
Abstract: Nano-composites combining multiple functionalities in one single nano-object hold a lot of promises for controlled drug delivery and targeted therapy [1].

In this work carbon nanotubes (CNTs) were functionalized with a therapeutic agent, namely a photosensitizer (mTHPC) already approved in clinical practice for photodynamic therapy (PDT). The aim is to couple the intrinsic properties of CNTs as efficient drug vector and potent photothermal agent with those of the photosensitizer and so benefit from the action of a double light-activated therapy i.e. photothermal therapy (PTT) and PDT.
The first part of this study consisted in quantifying the internalization of drug-loaded CNTs by tumor cells, intracellular release of mTHPC and the absence of intrinsic dark toxicity. Then, we investigated *in vitro* the biologic effects of the distinct therapies and highlighted their combined effects. For this, tumor cells were illuminated either by a 808 nm near-infrared laser radiation (to mediate PTT and local intracellular heating) or by 650 nm laser radiation in order to excite the photosensitizer and thus trigger ROS production. Through different approaches, we studied cell viability, apoptotic cascade and gene expression and demonstrated that each treatment impacts the cell by distinct mechanisms leading to synergistic effect.

The photothermal therapy was also tested *in vivo* on epidermoid carcinoma xenografts implanted in mice. This study had a dual objective: evaluate the therapeutic efficacy of the PTT mediated by CNTs by monitoring the tumor regression and assess the utility of ultrasound elastography to provide mechanical properties of tumor tissue. Indeed, on one hand, the tumor stiffness has been shown as a predictive marker of tumour malignancy. On the other hand, thermal therapy has been associated with local damage on the tumor extracellular matrix with impact on tumor solid stress. Therefore the stiffness of the tumors was monitored throughout the period of the treatment using shear wave elastography. Two distinct types of heating were experimented: moderate hyperthermia (temperature of the tumor monitored at 43°C - 45°C for 20 min, repeated twice) and thermal ablation (50°C for 3 min, repeated twice). We aimed to determine the best compromise between overheating limitation, thermal damage and outcome on tumor progression. It appeared a dramatic decrease of the tumor stiffness for tumors responding favorably to the treatment compared to the others. This non-invasive and non-ionizing imaging technique may allow correlating the effects of hyperthermia on tumor tissues with the evolution of their mechanic properties but also with pathological features. The final objective is to enlarge the use of ultrasonic elastography as a tool for non-invasive personalized monitoring of therapy.



Keywords: cancer treatment, carbon nanotubes, photodynamic and photothermal therapies, elastography

Figure 1: (A) Tumor treatment by laser illumination (B) Thermographic infrared camera photograph of a mouse under laser illumination (laser beam in dotted line): right scale represents the color code for surface temperature (C) Tumor regression (before treatment, and at +1, 8, 12 days) (D) Tumor imaging (top) shear wave elastography : monitoring of the tumor stiffness (bottom) conventional ultra-sonography.

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Penetration of mucoadhesive chitosan-dextran sulfate nanoparticles into the cornea

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Abstract: Nanoparticles-based drug/gene delivery has been reported for potential therapeutic management of various ocular surface and corneal disorders. In this study, we have examined the efficiency of mucoadhesive chitosan-dextran sulfate nanoparticles (CDNs) penetration across the cornea at the microscopic level by using porcine eyeball as a model. CDNs were produced by polyelectrolyte complexation of the positively charged chitosan and negatively charged dextran sulfate. CDNs were labeled with fluorescein isothiocyanate by reaction of the amino groups of chitosan and the isothiocyanate groups of FITC. Specifically, we have employed a custom-built confocal scanning microfluorometer (CSMF) for record the depth of fluorescence across the cornea. CSMF, equipped with a water-immersion objective (Zeiss 40x; 0.75 NA and wd = 1.2 mm), was employed to quantify the penetration dynamics of SRB. The output of a white LED, which was modulated at 10 kHz, was filtered through an interference filter (565 + 10 nm) and led to the excitation port of the CSMF. The FITC fluorescence (475 nm) and scattered light passing through a parfocal exit slit positioned in the eyepiece were detected by two photomultiplier tubes (R928 Hamamatsu) coupled to two lock-in amplifiers. All measurements were performed with eyeballs held underneath the objective on a precision and motorized XYZ linear translation stage (Newport). FITC-labeled CDNs (FCDNs) was applied onto the porcine cornea for 1.5 and 6 hours with and without intact epithelium. After the experiments, each cornea sample was separated and observed under fluorescence microscopy to confirm the removal of the corneal epithelial layer. Moreover, FCDNs were exposed to primary porcine corneal epithelial cells (PCE) for 30 and 120 min to investigate the cellular uptake mechanism. Meanwhile, the PCE were stained with LysoTracker Red to visualize their appearance in the late endosomes. FCDNs showed a spherical shape with a mean particle size of < 400 nm with positive surface charge. Instillation of FCDNs on the whole cornea with epithelium-intact over 1.5-6 hours led to a peak in fluorescence close to the scatter peak corresponding to the superficial epithelium, while instillation on the bare stroma showed higher fluorescence from deeper layers of the stroma (Figure 1). FCDNs could be endocytosed by PEC via clathrin-dependent pathway and also found in the late endosome/lysosome compartments. Based on the results, it can be concluded that the research into the penetration of mucoadhesive CDNs into the cornea has been very successful. This strategy can be used to deliver drugs payloads to corneal epithelium using CDNs.

Keywords: chitosan-dextran sulfate nanoparticles, mucoadhesive, cornea, custom-built confocal scanning microfluorometer.

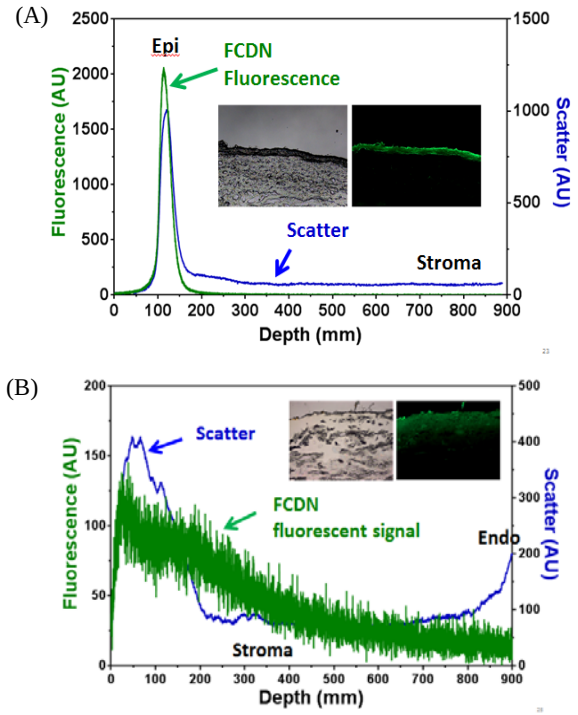

Figure 1: Penetration of FCDNs across the epithelial surface (A) and into stroma (B) after 1.5 hours using CSMF to measure depth-resolved fluorescence. The insert pictures showed the histologically of cornea after experimenting.

Magnetic nanoprobes with anti-HER2 single chain antibody fragments for active targeting of breast and ovarian cancers

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Abstract: Polyethylene glycol (PEG)-coated superparamagnetic iron oxides nanoparticles (SPIONs) are known as attractive platforms for anticancer theranostic nanomedicine combining magnetic resonance imaging, drug delivery and hyperthermia functions (Gautier *et al*., 2013; Hervé *et al*., 2008; Liu *et al*., 2011). The major challenge for all the injectable nanomedicines consists in improving their ability to target efficiently the disease cells while preserving their stealthyness relatively to the immune system of the body. The conjugation of antibodies to nanoprobes is intended to insure cancer cells targetting: for instance, anti-HER2 antibody Herceptin® is known to specifically recognise breast and ovarian cancer cells (Colombo *et al*., 2012). However, the conjugation of whole antibody (MW~150 kDa) to nanomedicines induces several drawbacks that limit the efficiency of cancer targeting: poor control of functionnalisation geometry, decreased colloidal stability *in vitro* and stealthyness *in vivo*. To overcome these drawbacks, it is possible to decorate nanomedicines with a scFv fragment (MW ~27 kDa), i.e. the smallest functional antigen-binding domain of the antibody (Vigor *et al*, 2010). In the present study, we describe synthesis and characterization of new generation of magnetic nanoprobes: SPION-PEG functionalised with recombinant scFv of Herceptin®. The physico-chemical properties of our SPION-PEG-scFv were assessed by atomic absorption, fluorescence and photon correlation spectroscopies. The presence of scFv fragments was analyzed both qualitatively and quantitatively, by means of optical spectroscopy. According to the analysis data, our SPION-PEG-scFv nanoprobes combined several advantages such as: (i) regio-specific covalent binding of the scFv to the PEG external surface of nanoprobes; (ii) small and regular size (hydrodynamic diameter of ca. 100 nm); (iii) nearly neutral surface insuring good colloidal stability and stealthyness. From the biological point of view, the scFv-nanoprobes were immunoreactive as it was shown by ELISA and immunofluorescence methods. In addition, absence of the Fc constant domain should reduce the nanoprobes immunogenicity – this will be studied in the very close future.

Keywords: SPIONs, scFv fragment, anti-HER2, nanoprobes, diagnostic, targeting, cancer.

Acknowledgments:

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Cellulose Nanocapsules of Metoprolol and its Metabolites Produced as New Products in Pharmaceuticals Recycling Processes

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Abstract: Accert Chemistry and Biotecnology Inc. has been developing different processes for recovering expired pharmaceuticals and out-of-date medicines. Strategies include development of bioprocess using microorganisms and enzymes to promote specific reactions in active pharmaceutical ingredients (API) and then generate degradation products standards (DPS) or active metabolites (AM). Herein, cellulose nanocapsules containing metoprolol and some of its active metabolites have been prepared by emulsion–solvent evaporation method[1]. Metoprolol is a beta-blocker that affects the heart and blood flow through arteries and veins[2]. Different conditions were applied (temperature, rotation, nanoparticules concentration, API, DPS or AM concentration) and the results indicated API, DPS or AM concentration does not significantly influence the size of the nanocapsules, which are extremely affected by the employed producing method (from 250 nm to 900 nm). Nanoparticles sizes were determined by scanning electron microscopy (SEM) and encapsulation efficiency was determined by ultra-high performance liquid chromatography coupled with ultraviolet detector (UHPLC-UV). The results suggest that efficient process for recovering expired pharmaceuticals or out-of-date medicines can be achieved by generating cellulose nanocapsules. Such kinds of processes are of desired since cellulose is a widely used excipient in pharmaceutical products. Therefore, using such approach pharmaceuticals recycling can be a very good way of producing new pharmaceuticals products or formulations.

Keywords: pharmaceutical recycling, cellulose nanocapsules, metoprolol, new pharmaceutical products, metabolites.

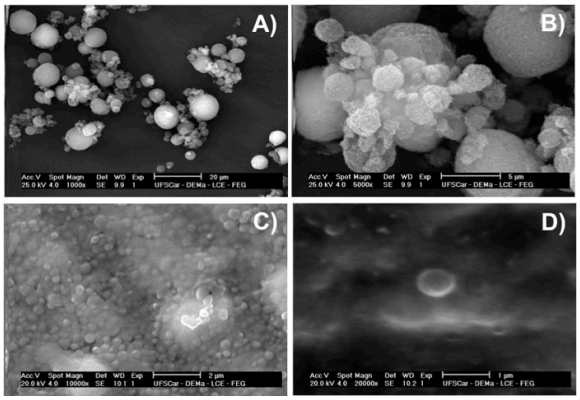

Figure 1: SEM of examples os nanocapsules obtained.

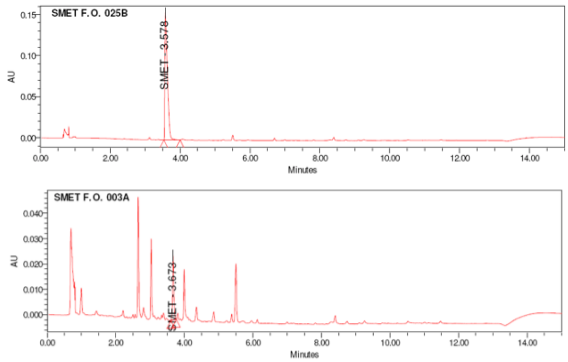

Figure 2: UHPLC-UV of metoprolol (above) and metoprolol after a biorreaction using enzymes (bellow). Such samples were encapsulated with cellulose.

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Ph/temperature and Magnetic Field Responsive Doxorubicin Loaded NIPA Coated Superparamagnetic Nanoparticles for Targeted Cancer Therapy

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Abstract:

"Intelligent" materials which are able to respond to external stimuli, represent one of the most exciting and immerging class of materials that can be applied for biomedical application. Poly(N-isopropylacrylamide) (PNIPAAm), seems as the most suitable of this group for biomedical applications (Ward et al 2011). This is because of the fact that lower critical solution temperature LCST of PNIPAM is around 32°C which is close to the body temperature (37°C) (Shimizu et al 2010). Superparamagnetic nanoparticles (SPIONs), another important group of "Intelligent" materials, have gained great attention in the fields of nanomedicine due to being biocompatible, biodegradable, facilely tunable, and superparamagnetic and thus controllable by an external magnetic field (Mok et al 2013).In this study, in order to improve the delivery and therapeutic properties, NIPAM polymer, a potent system for hyperthermia therapy was combined with superparamagnetic core which resulted in the combination of non-contact (Magnetic force) and contact (pH and Temprature) forces. We tried to design this polymeric nanocarrier more applicable as drug delivery system by increasing the LCST from 32°C to mild hyperthermia temperatures about 42°C. In order to investigate the in vitro thermo-chemosensitisation characteristics of our nanoparticles for drug delivery applications, doxorubicin, one of the most widely used anticancer drugs, was loaded on newly synthesized pH/temperature and magnetic field responsive NIPA-Fe_3O_4 nanoparticles. Thermo-chemosensitisation paved the way for increased antineoplastic drug accumulation in tumors and enhanced drug cytotoxicity. The characterization of nanoparticles was studied. Then loading efficiency was optimized and the release studies performed at different temperatures (25°C, 37°C, 42°C) and pH (7.4, 5.6) (Figure1). Then cell cytotoxicity of the complex compared with free form of doxorubicin. Finally time and temperature dependent cell internalization and drug release of the complex was studied using life time inverted florescent microscopy (Figure2). Results demonstrated that the prepared complex can be a potent delivery system especially for targeted cancer therapy.

Keywords: Cancer; PNIPAAm ; superparamagnetic; Doxorubicin; biocompatible; biodegradable; targeted cance therapy;

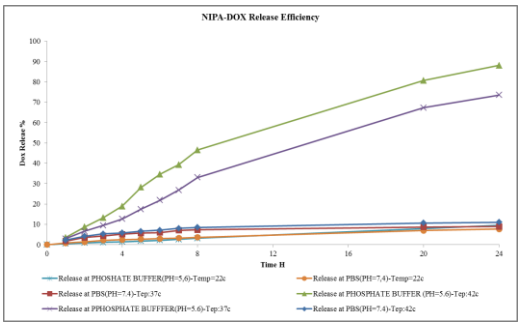

Figure 1. Release of doxorubicin from NIPA-Fe3O4 Nanoparticles at pH 5.6 and pH 7.4 with three different temperature as 22°C, 37°C and 42°C.

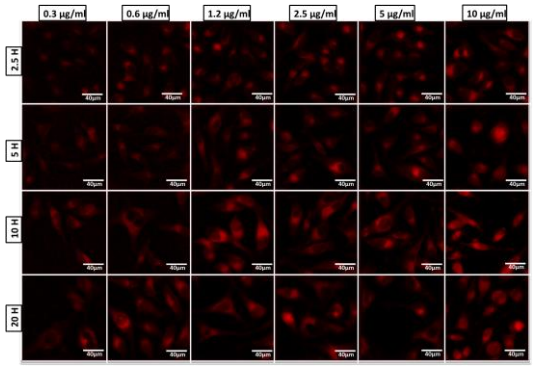

Figure 2. Uptake of doxorubicin loaded NIPA-Fe3O4 Nanoparticles by Hela Cells. Confocal laser microscopy image of Hela cells incubated together with doxorubicin loaded NIPA-Fe3O4 Nanoparticles with different concentration (0.3, 0.6, 1.2, 2.5, 5 and 10 µg/ml) of doxorubicin contents at different time points (2.5h, 5h, 10h and 20 h) at 37°C.

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Targeted polyethylene glycol gold nanoparticles for the treatment of pancreatic cancer: from synthesis to a proof-of-concept *in vitro* studies.

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Abstract: Gold nanoparticles (AuNPs) are attracting considerable interest as viable biomedical materials, and research into them is growing due to their unique physical and chemical properties (Movia et al , 2014;Cui et al 2008).
Here we report the synthesis of doxorubicin-loaded PEGylated AuNPs (DOX-PEGAuNPs) by a simple one-step method, and their further functionalization with an anti-potassium channel monoclonal antibody (mAb) by terminal carboxylic acid groups for their future application in the treatment of pancreatic cancer. The mAb efficiently recognizes a specific antigen expressed on the membrane of pancreatic cancer cells, with positive effects on the drug delivery efficiency. The relevance and major interest of the so-obtained nanoparticles in the presence of terminal carboxylic acid groups at their surface, as confirmed by infrared X-Ray Diffraction (XRD), and X-ray Photoelectron Spectroscopy (XPS), and Polarization Modulation-Reflection-Adsorption-Infrared-Spectroscopy (PM-IRRAS), in addition to the surrounding PEG chains, is essential to avoid non-specific protein adsorption (Spadavecchia et al 2014). In parallel, biocompatibility, as well as the therapeutic efficacy, were evaluated in Proof-of-Concept (PoC) *in vitro* studies by means of a high throughput technique (namely, flow cytometry analysis) (Figure 1). Data gathered from this study may have further applications for the safe design of nanostructures to be applied for therapeutic purposes in the treatment of cancer.

Keywords: PEG Gold Nanoparticles, Doxorubicin, Mab; cancer, PM-IRRAS, biomedical applications.

Acknowledgment:
This project was partially supported by EU FP7 QualityNano Infrastructure project (grant number: 262163).

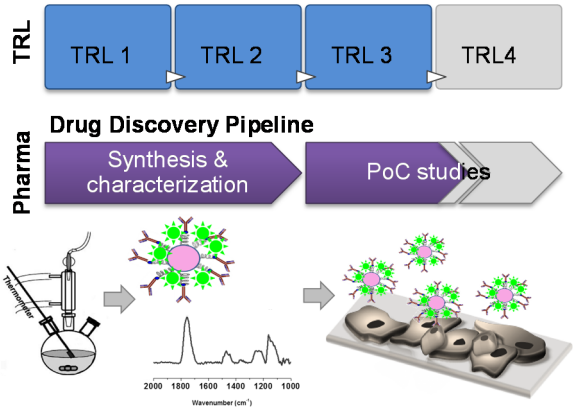

Figure 1: Technology Readiness Level (TRL) for pharmaceutical products development.

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Oral insulin delivery and biodistribution of biopolymers-based nanoparticles

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Abstract: Alginate-dextran sulfate based nanoparticles complexed with a chitosan-polyethylene glycol-albumin shell can be considered suitable carriers for oral insulin delivery. Nanoparticles were obtained by co-surfactant and ultrasonication assisted emulsification/internal gelation technology. This technology makes use of natural occurring polymers which are a suitable choice, due to their excellent biocompatibility, drug carrying ability, adjustable controlled-release property, lower cost, abundance in nature and easier application (Stops et al, 2008). Physicochemical characterization revealed them as suitable carriers for oral delivery of insulin. Nanoparticles with a mean diameter of 166±0.84nm were able to well-preserve their integrity in simulated gastric fluid. In simulated intestinal fluid, nanoparticles morphology changed and a sustained and controlled release of insulin was observed during 3h. Size variation of coated and uncoated nanoparticles across the simulated fluids were controlled over the time and pH increment, due to polymer pH dependent behavior. Insulin secondary structure during nanoparticles preparation was analysed. Biodistribution of ^{99m}Tc-albumin and ^{99m}Tc-albumin-nanoparticles was monitored during 24h after oral administration to mice. 99mTc-albumin-nanoparticles showed a different behavior compared to 99mTc-albumin, since the activity in the duodenum's and small intestine's wall was maintained for 60 min while the activity in the small intestine content was already residual. The interaction between albumin and chitosan, a positively charged biopolymer with mucoadhesive properties, allows the interaction of these nanoparticles with intestinal cells. After oral administration of insulin-loaded nanoparticles to diabetic Wistar rats, the glycemic reductions attained by both doses after 10h were -69 ($p<0.05$) and – 63% ($p<0.05$) with 50 and 100 IU/kg insulin-loaded nanoparticles, respectively. Compared with subcutaneous insulin, the oral insulin-loaded nanoparticles showed more sustained and long-term effect. After 10 h of oral administration of 50IU/Kg insulin-loaded nanoparticles, intraperitoneal glucose tolerance tests were compared between three animal models, diabetic Wistar (type 1 diabetic model), GK (type 2 diabetic model) and healthy Wistar rats. In both diabetic models, glycemia increased to a lesser extent in rats receiving the formulation and 1h30 after of the glucose challenge, a significantly faster decrease was noticed. After 3h, no statistical differences were obtained between GK groups, in contrast to the behavior of wistar diabetic rats. Although the pancreatic islets of GK rats are defective, this genetically modified model of type 2 diabetes have the islets partly damaged but could still secrete a little insulin and glucagon. (Li et al, 2013). Therefore, the exogenous insulin may cover-up the effect of insulin-loaded nanoparticles. Comparison of the response between normal Wistar and GK rats showed that GK rats have a slower metabolic decomposition rate of glucose. Our results demonstrate that these biopolymers-based nanoparticles may provide an efficient template for the oral delivery of insulin.

Keywords: biopolymers, insulin, oral delivery, nanoparticles, type 1 and type 2 diabetic models, radioactive labeling.

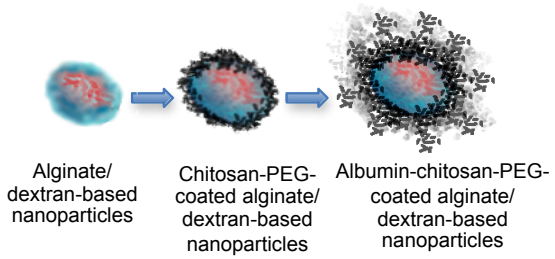

Figure 1. Nanoparticles coating process.

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IONCs: a versatile tool for hyperthermia, imaging and controlled drug delivery

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Iron Oxide nanocubes (IONCs) are among the best iron-based nanoparticles for hyperthermia treatment and magnetic resonance imaging (MRI). Properly functionalized they are also important tools for transport and controlled release of drugs. Moreover it was demonstrated that the controlled aggregation of multiple highly magnetic interacting IONCs in a polymeric shell has a significant effect on the heating performance and on the efficiency as contrast agent of these nanoparticles.

The "magnetically mediated hyperthermia" (MMH) represents a novel therapeutic concept based on the generation of heat *via* an alternating magnetic field (AMF) exploiting magnetic nanoparticles (MNPs) as heating *foci*. The heating efficiency of a heat probe is evaluated by its specific absorption rate (SAR) value, which provides the power absorbed per unit mass of magnetic material (W/g) when exposed to an alternating magnetic field (AMF).

In this work we studied the SAR values of single IONCs and clusters of the latter named magnetic nanobeads (MNBs). These samples are made water-soluble by using recently novel methods developed by us. It was observed in particular a considerable SAR decrease when the IONCs are aggregated into MNBs. This trend was also confirmed by the SAR measurements performed in solvents with different viscosity. For the same samples were also performed the relaxivity measurements which showed very high values of r_2/r_1 for MNBs respect IONCs.

To fully exploit the outstanding heating ability of IONCs, these particles were functionalized with a thermo-responsive polymer (based on *N*-isopropylacrylamide). These nanohybrid structures were used as drug carrier for chemotherapy: Doxorubicin (DOXO) was loaded at room temperature and after encapsulation in the polymer, was released under the AMF using technical conditions biomedically safe for patients ($Hf < 5 \times 10^9$ $Am^{-1}s^{-1}$). The temperature increase generated by IONCs in the AMF induces the coil-globule transition of the polymer which in turns triggers the drug expulsion from the hybrid nanostructures (Figure 1).

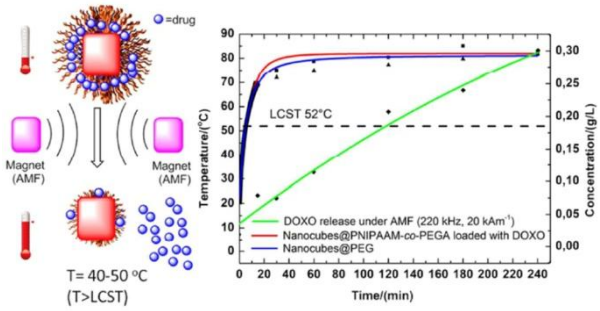

Figure 1: schematic representation of DOXO release from IONCs functionalized with a T-responsive polymer under AMF. The graph shows the amount of DOXO released over the time together with the temperature profiles for the IONCs with and without the thermo-responsive shell.

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A Novel Wound Dressing Coated with Self-Assembling Peptide Nanofibrils as a Drug Carrier

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Abstract: The delivery of therapeutic agents into wounds is a developing area. The release of a drug to wounds in a control manner is rearley reported. The uncontrolled release is a common limitation of drug releasing dressings. If the release rate is too high, the drug can be unloaded from the dressing before infection is arrested. Furthermore, a 'burst release' within the first few hours may lead to over-dose cytotoxcity, which can give rise to delayed wound healing, or to the development of antibiotic resistance [1]. Inversely, if the release rate is too low, the drug delivery may be below the effective therapeutic dose that is required to be effective. Another limitation caused by uncontrolled drug release from wound dressings is that it leads to the need for frequent changes. When the dressing is removed, the newly formed epithelium can be damaged, so limiting dressing changes is usually considered to be beneficial [1, 2].

To overcome these limitations, attention has been paid to develop materials that provide potential for controlled drug delivery. Controlled drug delivery to wounds normally means the delivery of an active agent to the wound site in a sustained manner. The ideal bioactive dressing should release the drug at the optimum therapeutic concentration followed by a sustained constant delivery [2]. These criteria can limit the frequency of dressing changes. Under these circumstances patient compliance can be improved, especially in those suffering from chronic wounds, where the patient needs to undergo extended periods of treatment [3]. In light of such considerations, there is a need for a more conservative approach for smart wound-care materials that are more effective and more functional than traditional materials. Optimistically, the addition of pH-responsive self-assembling peptide nanofibrils (Figure 1) as a biofunctional component to medical fabrics could have the potential to combine the advantages of triggered release with slow kinetic release.

This project is an attempt to contribute to the development of smart medical textiles, in particular, to development of controlled drug-release textiles for wound care that can imediatlly release the drug when triggered by the pH of bacteria. Here, for this purpose, cellulosic fabrics were coated with the fibril-encapsulated antibiotics (Figure 2) to develop a smart pH-stimulus responsivness dressing, which was then assessed for the ability to inhibit the growth of *staphylococcus epidermidis*. The results showed that the dressings were stimulated by the pH of bacteria and released the loaded drug. Hopefully, this dressing could by clinically applied on infected wounds to release the drug only when required and reduce the problem of bacterial resistance to antibiotics in wound care applications.

Keywords: self-assembling peptide, wound dressing, drug delivery.

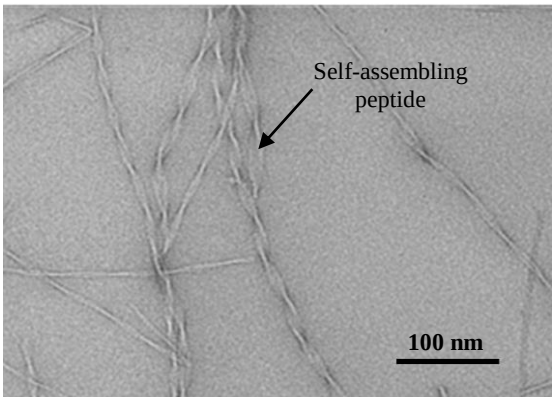

Figure 1: TEM image of self-assempling peptide fibrils.

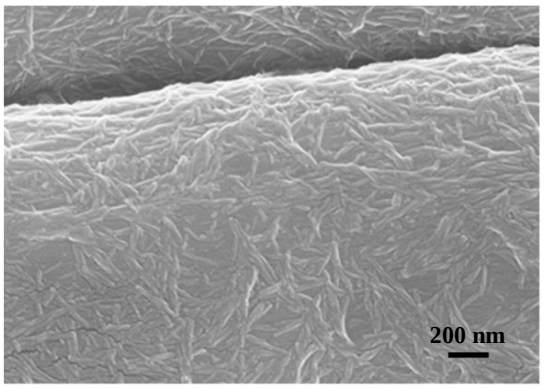

Figure 2: SEM image of a dressing coated with peptide fibrils containing vancomycin.

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Core-cone Structured Monodispersed Mesoporous Silica Manoparticles with Ultra-large Cavity for Protein Delivery

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Abstract: Mesoporous silica nanoparticles (MSNs) have attracted much attention as a new generation of drug delivery vehicles. Delivering therapeutic proteins into cells is an important challenge for many medical applications including cancer therapy, vaccination and regenerative medicine. Traditional MSNs usually have small pore sizes (<13 nm), which greatly hinders their encapsulation and intracellular delivery of therapeutic proteins. In this work a new type of monodispersed mesoporous silica nanoparticles with a core-cone structure (MSN-CC) has been synthesized. The large cone-shaped pores are formed by silica lamellae closely packed encircling a spherical core, showing a structure similar to the flower dahlia. MSN-CC has a large pore size of 45 nm and a high pore volume of 2.59 cm^3 g^{-1}, the highest pore volume of all MSN with radial structures. A supramolecular self-assembly mechanism is proposed to explain the formation of MSN-CC, and first time cone-like vesicles are reported in self-assembled objects. MSN-CC demonstrates a high loading capacity of large proteins and successfully delivers bioactive β-galactosidase (β-gal) into cells, showing their potential as efficient nanocarriers for the cellular delivery of proteins with large molecular weights. To our knowledge, our study is the first report on intracellular delivery of large functional proteins above 100 kDa using MSNs, providing a safer delivery tool for large proteins instead of previous transduction peptide based ones.

Keywords: Mesoporous silica nanoparticles, large radial pores, supramolecular selfprotein delivery

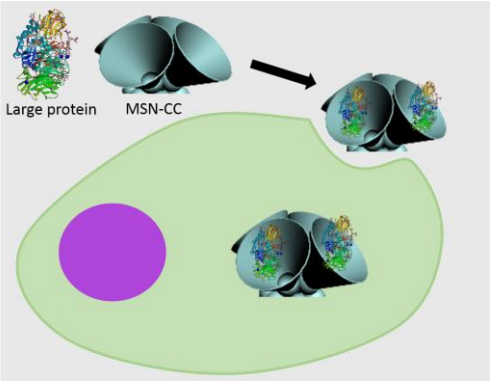

Figure 1: The process of delivery of large functional proteins into cells using MSN-CC.

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Impact of the Polyethylenimine Conjugation Mode on the Cell Transfection Efficiency of Silica Nanovectors

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Abstract: Efficient gene delivery relies largely on the design of intracellular vectors (Guo *et al.*; 2011). Among the non-viral vectors, silica nanoparticles (SiNP) have attracted increasing attention due to tunable size/ mesoporisity and versitile surface chemistry (Mamaevaa *et al.*; 2013). The main strategy is to modify SiNP with amine groups to complex the negatively charged plasmid DNA. Nowadays, it's of special interest to modify SiNP surface with a cationic polymer, polyethylenimine (PEI). PEI alone is a very efficient transgene vector and the availability of various conjugation methods to SiNP facilitates the optimization of the vector design. Nevertheless, the impact of conjugation mode on cell transfection remains unknown and so far PEI modified SiNP, especially covalent bonded ones, targets mainly at the delivery of siRNA rather than plasmid DNA (Buchman *et al.*; 2013).

In our study, we utilized three approaches to conjugate PEI (25 kDa) to SiNP surface and evaluated the systems as delivery vectors for plasmid DNA. Previously, we have systematically studied the feasibility to graft PEI through direct physical adsorption (SiNP@PEI, Figure 1a) and excellent transfection efficiency could be maintained while cytoxicity of PEI was reduced (WANG *et al.*; 2015). Meanwhile, we proposed two other methods for comparation. On the one hand, we modified SiNP first with -SO_3 and then PEI was introduced by stronger electrostatic adsorption (Figure 1b, SiNP@SO_3@PEI). On the other hand, SiNP was grafted with -Cl and -Cl was then substituted by the amine group of PEI (SiNP@Cl@PEI, Figure 1c).

Successful modifications were first confirmed with zeta potential measurement and elemental anaylsis. PEI conformation at nano-interface was investigated further with XPS and solid-state NMR. The highest rigidity of PEI over particles was observed with SiNP@PEI, followed by SiNP@SO3@PEI and finally SiNP@Cl@PEI. In principle, lowest rigidity allowed the flexibility and therefore the highest DNA compaction capability. Surprisingly, the trend SiNP@PEI> SiNP@SO3@PEI>> SiNP@Cl@PEI was obtained in terms of transfection efficiency. It should be noted that the interactions between PEI and SiNP evolved in the opposite order, correlated with the difficulty of PEI release from the interface. Hence, this sharp contrast suggested the release of PEI from the particle plays a more important role in the intracellular gene delivery. Another important outcome of our studies was concerned with safety when applied as nanomedicine. It was found that at comparable doses, PEI alone showed detrimental effect while all the SiNP modified with PEI exhibited no cytotoxicity.

Keywords: silica nanoparticles, polyethylenimine, solid-state NMR, surface modification, gene delivery.

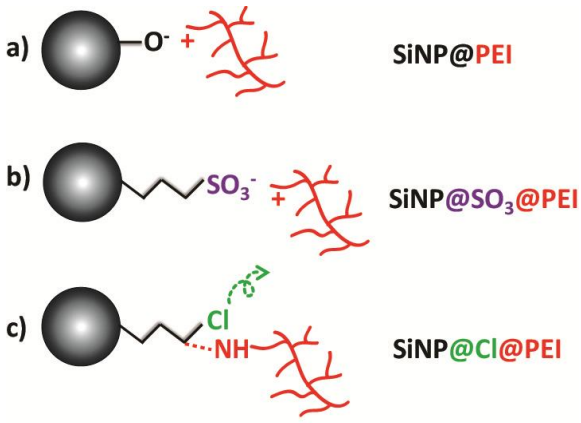

Figure 1: illustration of PEI conjugation mode to SiNP surface. a) adsorption with silanols; b) adsorption with sulfonate; c) covalent bonding by substitution of -Cl with amine of PEI.

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PEI – Starch Nanoparticles for siRNA based Gene Silencing Therapy for Cancer

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Abstract: Conventional cancer treatment techniques are not sufficiently efficient and they even are harmful for healthy tissues. Recent studies suggest that siRNA based gene silencing can be used as a highly effective targeted therapy (Ozpolat *et al.*; 2013). However, siRNA delivery into tumors and target tissues have proved to be difficult (Tekedereli et al., 2012). This study aimed to develop an siRNA delivery system to cancer cells using positively charged nanospheres constructed of PEI and S.

PEI-starch nanospheres were prepared at different ratios by water-in-oil microemulsion method. Genipin was used to crosslink PEI molecules. The PEI-starch nanospheres were characterized by measuring the zeta potential and the particle size, studying the topography with SEM, and measuring release rate of their content by spectrofluorometer. We also evaluated siRNA and nanaoparticle uptake and target downmodulation by confocal laser scanning microscopy (CLSM) and western blot analysis.

The mean diameter of PEI-starch incorporating siRNA was 84.6 nm. Zeta potential of PEI-starch nanospheres increased when the PEI /starch ratios were increased and was the highest (8.7 mV) at PEI:Starch, 9:1 (w/w) (Table 1).

Table 1: Surface potential of PEI: starch nanospheres and viability of MCF 7 cells treated with PEI – starch nanospheres constructed with PEI: starch (w/w) ratios of 1:9, 1:3, 1:1, 3:1 and 9:1.

PEI:starch (w:w)	Zeta potential (mV)	Cell viability (%)
1:9	-31	90.7
1:3	-26	86.6
1:1	-19	85.6
3:1	0	81.9
9:1	9	78.0

The nanospheres released 50 % of their siRNA content in the first 3 days and all of the content in a week. CLSM micrographs show the nanospheres and siRNA were taken up by MCF 7 breast cancer cells (Figure 1). Western blot analysis demonstrated that EF2-Kinase siRNA loaded nanospheres significantly inhibited EF2K protein expression (90-100%) (Figure 2).

Keywords: siRNA, drug delivery, nanoshere, polyethylenimine, starch, cancer therapy, nanomedicine

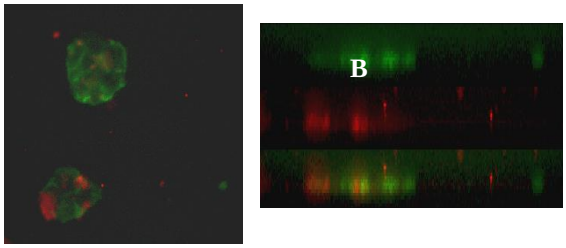

Figure 1: Confocal microscopy of MCF 7 cells treated with Alexa-555 labelled siRNA loaded PEI-starch nanospheres (A) fluorescence image of MCF 7 cells (green) treated with Alexa-555 labelled siRNA loaded PEI-starch nanospheres (red) (x 40), (B) Z-stack.

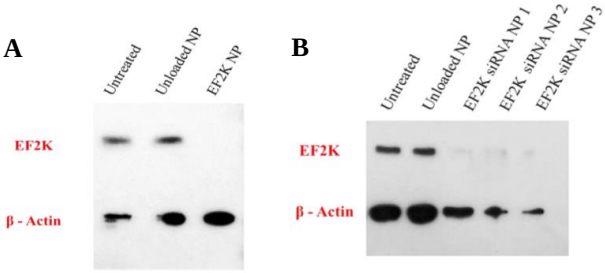

Figure 2: Western blot analysis of MCF 7 cells treated for 72 h (100 nM EF2K siRNA and 100 000 cells per well). (A) with EF2K siRNA loaded nanospheres (NP), (B) with various batches of EF2K siRNA loaded nanospheres (NP) (NP1, NP2, NP3).

In this study construction of a novel PEI-starch based siRNA carrier to initiate apoptosis in breast cancer cells was aimed. The PEI–starch nanospheres effectively penetrated into MCF 7 cells. Thus, we showed that the PEI-starch nanospheres are suitable in size, charge, release kinetics and as such they exhibit a potential to serve as an efficient vector for siRNA delivery to cancer cells.

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Synthesis of l-PEI and 2MPA Coated Biocompatible Silver Sulfide QDs as Transfection Vectors

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Abstract: Gene therapy has become very popular approach to fight cancer. In recent years, efforts of improving non-viral gene delivery techniques and transfection vectors have been increased. Due to its high transfection efficiency, cationic polyethyleneimine (PEI) polymer is one of the most popular vectors, yet molecular weight and structure dependent toxicity of PEI is an important issue. High transfection efficiency along with high biocompatibility is desired for practical purposes. Therefore, usually molecular weight is kept at and below 25kDa and sometimes lineer PEI is preferred over branched one. Besides, tracking the delivery of genes and/or the outcome of the gene therapy with diagnostic tools is highly desired. Organic fluorescent dyes conjugated to the vectors or genes can be used for optical tracking however, organic dyes have a very short luminescence lifetime which is disadvantages in long experimental procedures. Quantum dots with resistance to photobleaching and long luminescence lifetime emerged as alternative fluorescent tags in biomedical research. In literature there are few reports on cadmium chalcogenide bound PEI as a transfection vectors however, Cd-chalcogenides are usually toxic and emit in the visible region which is far from being ideal for in vivo experiments.
Here, we will describe the development of new quantum dots which emit in the medical window (Near infrared: 700-900nm) which are composed of more cytocompatable lineer PEI and highly cytocompatible Ag_2S. Effect of PEI molecular weight and reaction parameters on the properties of quantum dots and their ability in gene trasfection will be discussed.

Keywords: Quantum Dots, NIR, Nanomedicine, Transfection Agent, PEI, Ag_2S, Theranostic, P53, Gene Transfection

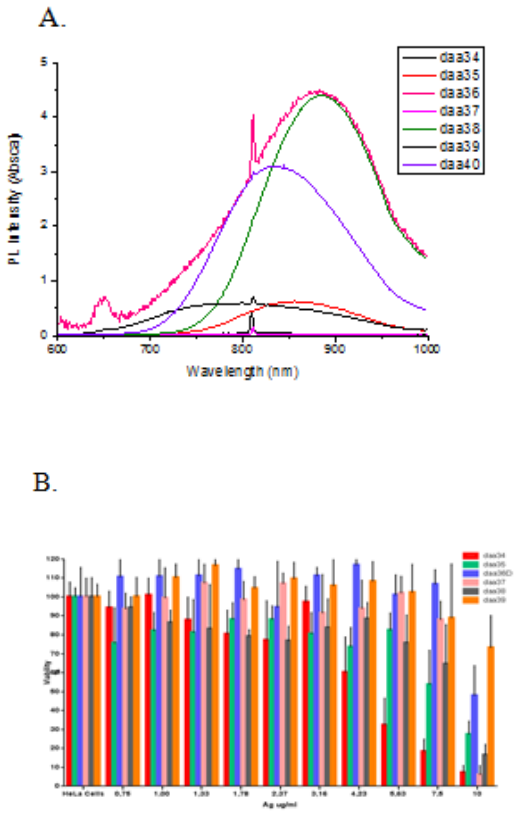

Figure 1. (A) PL intensity versus wavelength graph of quantum dots with different l-PEI and 2MPA ratios. (B) Dose dependent viability of HeLa cells determined by MTT.

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The Role of Triblock Amphiphilic Copolymers for DNA Translocation Through Lipid Bilayers

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Abstract: Gene therapy consists in delivering DNA inside a cell to obtain a therapeutic effect. In the case of intramuscular injections, the most efficient synthetic vectors are neutral amphiphilic block copolymers, compared to naked DNA and cationic polymers (Chèvre *et al.*, 2011). Block chemical nature has an influence on the transfection efficiency and toxicity (Pomel *et al.*, 2008). Neutral amphiphilic block copolymers could induce transitory pores in lipid bilayers, pores allowing plasmid translocation, which could be a good indication for gene transfer mechanism with such copolymers (Huin *et al.*, 2011).

A family of ABA triblock copolymers (TBCP), having a hydrophobic poly(tetrahydrofuran) block and two hydrophilic poly(2-methyl-2-oxazoline) blocks, was synthesized using cationic ring opening polymerization. Experimental conditions allowed a good control of the structures. Critical aggregation concentration and characteristic parameters, evidencing macromolecular self-assembly, were determined. Studies of micelle formation (using DLS, ITC, Dosy NMR, Fluorescence) led us to draw a relationship between copolymer structure and physicochemical properties in solution, showing the formation of a core-corona structure (Rasolonjatovo *et al.*, 2014).

Interactions of copolymers with model lipid bilayers were evaluated by 'Black Lipid Membrane' experiments and the ability of plasmid (9kb) translocation was studied (Figure).

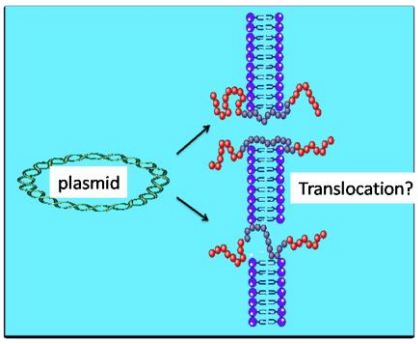

Figure : Plasmid Translocation assisted by a TBCP through lipid membrane.

TBCPs were finally used as vectors of pCMV-Luc, by using intramuscular injections in Swiss female mice.

Correlation between physicochemical properties, patch-clamp analyses and biological results was established. The TBCP structural parameters have a strong influence on the transfection efficiency, one of the most important for this kind of applications.

Keywords: amphiphilic block copolymers, gene therapy, plasmid translocation.

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Synthesis and Characterization of Folate-Targeted Poly(ethylene glycol) Coated Cationic Ag_2S QDs for Tumor Targeted Gene Delivery

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Cationic quantum dots are promising nanomaterials in biological applications, especially in drug/gene delivery. However, most of the designed cationic quantum dots are cytotoxic (Li et al., 2008) and emit in the visible range (400–700 nm) where the penetration depth of the light into the tissue is very low and autofluorescence from tissue/cells appear. Ag_2S QDs that emit in the near-infrared region (NIR) overcome these problems and present highly biocompatible structures with the negligible toxicity (Hocaoglu *et al.*, 2014).

The most prominent cationic polymer used in gene delivery is polyethylenimine with high DNA condensation capacity and transfection efficiency (Yue *et. al.*, 2011). However, the polymer shows toxicity due to highly cationic character. PEGylation is a solution to reduce the toxicity by lowering the surface charge and interactions with blood and extracellular components (Zhang *et al.*, 2010). As a result of PEGylation, the blood circulation time extends and the biocompatibility increases but uptake decreases. Targeting ligands help by receptor-mediated endocytosis to enhance the uptake. Folic acid is one of the most used ligands for tumor therapy because of the over-expression of folate receptor in cancer cells.

In this study, NIR emitting cationic Ag_2S QDs coated with poleyethleneimine (PEI) and l-cystein were PEGylated to reduce the toxicity of PEI and targeted by folic acid (FA) to cancer cells. The QDs in different coating ratio of PEI and l-cystein were studied to develop highly stable, luminescent, biocompatible non-viral gene delivery systems. Nanoparticle composition is determined by ICP-OES, NMR and elemental analysis. DLS and zeta potential measurements showed an increase in the size of the QDs and decrease in the zeta potential of the cationic QDs after FA-PEGylation. Cytotoxicity analyses demonstrated the improvement in the cell viability of the QDs thanks to PEGylation and increase in the uptake with folate targeting. These QDs successfully condensed p53-GFP plasmids at and above N/P ratio of 5. Transfection efficiency and influence of particle properties on trasfection efficiency will be discussed.

Overall, folate-targeted poly(ethylene glycol) coated cationic Ag_2S QDs present a promising gene delivery system with optical detection opportunity in the NIR, biocompatibility and high transfection efficiency.

Keywords: Near IR Ag_2S quantum dots, PEGylation, folic acid, gene delivery.

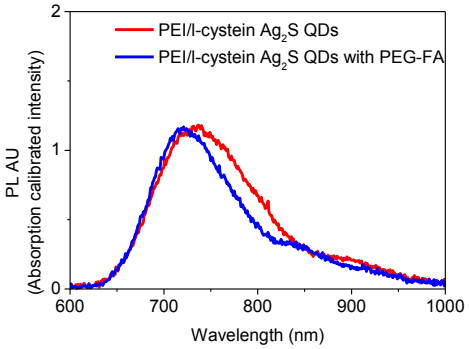

Figure 1: Photoluminescence spectra of the Ag_2S QD and FA-PEGylated Ag_2S QDs

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A Systemic Approach For Manipulating Geometries Of Nano Gold And Nano Silver in Synthesis for Controlling Vector Borne Diseases

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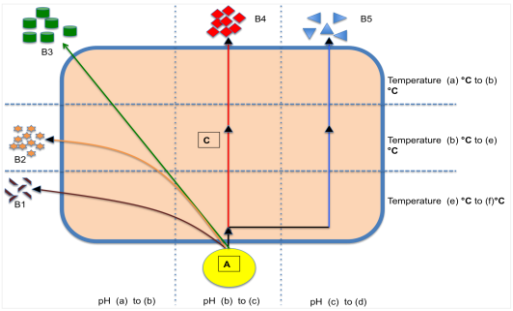
Abstract: The synthesis of metallic nanoparticles is an active area of academic and, more significantly, industrial applied research in nanotechnology. Currently nanoparticle research is an area of intense scientific interest in controlling diseases of global nature. Silver (Ag) and Gold (Au) nanoparticles (NPs) have been the focus of fungi and plant based syntheses for controlling vector o populations of mosquitoes which can be specifically of one species or more therefore could be targeted with each interfacing device explored geometrically. Silver and gold nanoparticles are nanoparticles of silver and gold. These particles are of between 1 nm and 100 nm in size. Silver and gold have been use in the wide variety of potential applications accordingly with their geometries. There is a crucial need to manipulate geometries to produce new insecticides. Synthesizing nanoparticles target specific need manipulating their geometries which we have experimented during synthesizing nanoparticles with a system science approach. The mosquitocidal activity of silver and gold nanoparticles using each fungi is a system. The system science applications in synthesis could be of great help as the precision in geometry of new particle can be predictable with dynamic model presented by us for target specific mosquitoes. (Soni and Prakash (2012a ,2012b,2014).

The holistic view precisely obtained during synthesis can be manipulated in creating equations by altering physical, chemical and biological system as shown in figures(1,2) parameters for each geometry required for the cause.

Keywords: Nano Gold, Nano Silver, Mosquito Control, System Science, Nano Geometries



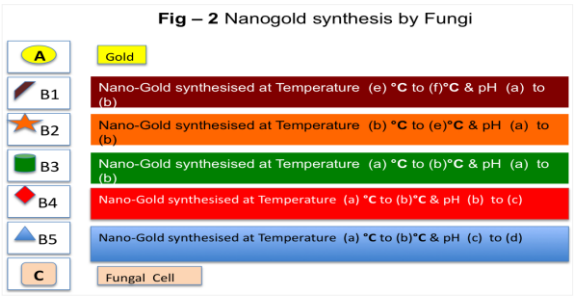


Fig1,2: illustrating diagrammatically the fundamental strategy to manipulate geometries on nanoparticles by changing their physical, chemical and biological systems

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Influence of TiO_2 and Al_2O_3 Addition on Mechanical Properties of Dental Zirconia

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Abstract: Zirconia-based ceramics have generated considerable interests in the dental community as restorative dental materials due to their high mechanical and chemical properties (Kaya *et al.*, 2012). This study was to produce dental zirconia ceramics by adding titania and alumina as a dopant. Mechanical ball milling was introduced to add the additives into zirconia practically. The shaped samples were sintered at 1350°C-1550°C for 2 hrs. Bulk density and shrinkage were calculated to investigate the effect of additives. The mechanical property was determined using the method of Vickers indentationand fracture toughness after sintering. SEM was used for analysis of grain size and surface morphology. XRD was operated to examine the crystalline phases in the titania and alumina-added zirconia ceramics during heat treatment. Table 1 shows the hardness and fracture toughness densification behaviour of various zirconia ceramics that contain different amounts of TiO_2 and Al_2O_3 at different sintering temperatures. It is noted that the addition of TiO_2 has been effective in lowering the sintering temperature, even though the density increased with increasing sintering temperature (Park *et al.*, 2008). Beside, addition of Al_2O_3 decreased the density of zirconia with increasing sintering temperature (Matsui *et al.*, 2008). However, hardness and fracture toughness were increased with adding alumina. As a result, co-doping of TiO_2 and Al_2O_3 addition decreased the sintering temperature and enhanced the mechanical properties.

Keywords: zirconia dental ceramics, TiO_2, Al_2O_3, Vicker's hardness, fracture toughness

Table 1: Hardness & fracture toughness of zirconia with different amount of alumina and titania

Sample	Hardness (H_V)	Fracture Toughness (K_{ic})
Pure zirconia	1220±4	4.55±0.40
0.5 wt%TiO_2-ZrO_2	1331±22	4.74±0.30
1 wt% TiO_2-ZrO_2	1215±3	4.63±0.06
0.5 wt%TiO_2- 1 wt%Al_2O_3-ZrO_2	1346±0.14	5.32±0.2
0.5 wt%TiO_2- 2 wt%Al_2O_3-ZrO_2	1296±6	4.61±0.05

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Piezoresistive Strain Sensing Characteristics of Nano-carbon Composites

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Abstract: In this study, the piezoresistive sensing properties of nano-carbon e.g. CNTs (Carbon Nanotubes) and xGnP (eXfoliated Graphite Nano Platelet), based composites are characterized for their applications of strain sensors. The nano-carbon composites show peculiar piezoresistive characteristics depending on their matrixes with respect to their strain variations.

In the case of hard matrix composites, they demonstrated quite linear bidirectional piezoresistive responses to strain and their piezoresistivity tend to increase to tension and to decrease to compression (Kang et al., 2006). The strain response of hard composites such as an epoxy presented fairly symmetrical and reversible behavior, and the gauge factors obtained were about ~200 within the range of 1,000 micro-strain. The xGnP composites revealed much higher strain sensitivity than CNTs composites (Kim *et al*., 2011). The static and dynamic voltage output responses of the composite sensors were also experimentally studied and were compared with those of a conventional foil strain gages. The voltage outputs by using signal processing systems were fairly stable and they showed fairy linear responses at both of loading and unloading cases with little hysteresis.

However, soft matrix based nano-carbon composites have not only non-symmetric responses but also demonstrated incrementally unidirectional piezoresistivity under both of tension and compression (Kang *et al*., 2011). The resistivity of the nano-carbon soft composites increased even compressive force condition due to the destruction of the existing conductivity of the filler (Jing *et al*., 2006). The voltage output was distorted under a quasi-dynamic test due to their unsymmetrical piezoresistive characteristics. The nano-carbon soft composite sensor showed quite tardy response to its settling time test under static deflections and that would be a hurdle for its real time applications. Furthermore, since the soft sensor did not have directional voltage output to tension and compression, it only could be utilized as a monodirectional force sensor such as a compressive touch sensor.

The strain sensor system made of nano-carbon composites can monitor structures continuously for electrical impedance and piezoresistive signals indicating structural deterioration and impact which may be sufficient to cause damage in real time. The composite sensors can be easily installed on composite structures using a spray-on technique, making the sensor low cost and practical. Strain or impact applied to the structure can be detected by the highly sensitive nano-carbon composite sensors. They induce change of piezoresistivity of the structure and that converts into voltage output consequently by means of simple signal processing system. The piezoresistive nano composite sensor is lightweight and easily applied to the structural surface, and there is no stress concentration, no piezoelectrics, no amplifier, and no storage of high frequency waveforms. Nano piezoresistive composite sensor is expected to be a cost effective and sensitive multi-functional sensor for composites and other damage monitoring applications in the field of engineering sensing..

Keywords: strain sensor, nano carbon, carbon nanotubes, exfoliated graphite nano-platelet, nano composite, smart material.

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