Abstract Book

NANOMEDICINE

VITERBO 2014

Viterbo

University of Tuscia

September, 17-19, 2014

CONFERENCE PROGRAM

Wednesday September 17

9.00 Registration

10.00 Opening Ceremony

Yechezkel Barenholz,
The Hebrew University, Jerusalem, Israel

Giovanna Mancini,
Institute of Chemical Methodologies, CNR, Rome

Agnese Molinari,
Department of Technology and Health, ISS, Rome

Invited Authorities

Alessandro Ruggieri,
Rector of Tuscia University

Luigi Nicolais,
President of CNR

Gualtiero Ricciardi,
Commissioner of Istituto Superiore di Sanità

*Chairpersons****: Yechezkel Barenholz, Dick Hoekstra***

Invited lectures

10.20 ***Salvatore Cannistraro***
Atomic force nanoscopy and spectroscopy applied to nanomedicine

10.50 ***Stefaan De Smedt***
Advanced fluorescence microscopy to study nanomedicines

11.20 *Coffee break*

Selected lectures

11.40 ***Clizia Guccione***
Poli(ethyl cyanoacrylate) nanoparticles for brain delivery of andrographolide

12.00 ***Riccardo Marega***
Selective targeting, sorting and killing of cancer-like cells in-vitro with magnetic carbon nanotubes

12.20 ***Greta Varchi***
In vivo sonodynamic activity of TPPS loaded polymeric nanoparticles

Industry Report

12.40 ***Nicolás Cassianelli*** **(nB nanoScale Biomagnetics SL)**
Monitoring the stability of a colloid for magnetic hyperthermia by specific power absorption measurements

13.00 *Lunch and poster session*

Chairpersons: ***Smadar Cohen, Stefaan De Smedt***

Invited lecture

14.30 ***Roberto Pini***
Novel strategies to actively target gold nanoparticles to malignant cells for cancer diagnostics and treatment

Selected lectures

15.00 ***Irene Xochilt Cantarelli***
Lanthanide doped alkaline earth fluoride nanoparticles as biocompatible, multifunctional materials for biomedical imaging

15.20 ***Francesco Porcaro***
New radiosensitizers agents from nanotechnology: a novel approach for the synthesis of gold nanoparticles

15.40 ***Laura Polito***
Digestive ripening of pegylated Au nanoparticles in aqueous solution: their application as X-ray contrast agents

16.00 ***Elisa Lubian***
Controlled Photo-Release Drug from Nanoparticles for PDT Applications

16.20 *Coffee break and poster session*

Thursday September 18

Chairpersons: Thomas Lars Andresen, Salvatore Cannistraro

Invited lecture

9.00 ***Luisa De Cola***
Hybrid nanomaterials for imaging and therapy

Selected lectures

9.30 ***Fabrizio Mancin***
NMR chemosensing: detection and identification of organic molecules and metabolites using nanoparticles as receptors

9.50 ***Barbara Adinolfi***
Complex nanostructures based on a specific molecular beacon and PMMA nanoparticles for the detection and silencing of survivin mRNA in human cancer cells

10.10 ***Giancarlo Morelli***
Diolein based nanostructures for theranostic applications

10.30 *Coffee break*

Invited lecture

10.50 ***Smadar Cohen***
Bio-inspired nanocarriers for siRNA delivery

Selected lectures

11.20 ***Roberto Fiammengo***
Sensitive microRNA quantification using DNA-gold nanoparticles probes

11.40 ***Nemany A. Hanafy***
Curcumin bio\nano-template for building layer by layer capsules

12.00 ***Stefania Porto***
Liposomes encapsulating doxorubicin and conjugated with cell penetrating peptides overcome drug resistance in human cancer

12.20 ***Ilaria Armentano***
Multifunctional biomaterials based on nanostructured poly(lactic acid): a way to drive stem cell responses

Industry Report

12.40 ***Elodie Ly-Morin (HORIBA)***
Nanoparticle-based approaches to push the limits of Surface Plasmon Resonance imaging for the detection of diagnostic biomarkers

13.00 *Lunch and poster session*

Chairpersons: Nadia Benkirane-Jessel, Luisa De Cola

Invited lectures

14.30 ***Salvatore Sortino***
Photoresponsive nanocarriers with a multifunctional cargo

15.00 ***Gerardo Goya***
Magnetic hyperthermia and cell death: the impact of physiological media on the heating efficiency and cell uptake

Industry Report

15.30 ***Marziale Milani, Francesco Tatti (FEI)***
Electron microscopy bradens the horizons of toxicology: the role of nanoparticles vehiculated by bacteria

Selected lectures

15.50 ***Sergio Madrigal-Carballo***
Chitosan-tannin hybrid nanoparticles as potential nanomedicine to prevent urinary tract infections

16.10 ***Maria Condello***
Liposome-encapsulated plant alkaloid voacamine improves the efficacy of chemotherapy on osteosarcoma resistant cells

16.30 *Coffee break and poster session*

Social Dinner (poster prize)

Friday September 19

Chairpersons: Gerardo Goya, Salvatore Sortino

Invited lecture

9.00 ***Nadia Benkirane-Jessel***
Smart implants equipped with active nanoreservoirs for regenerative nanomedicine

Selected lectures

9.30 ***Lucia Giampetruzzi***
Collagen-based scaffolds loaded with nanoparticles

9.50 ***Federica Valentini***
New nano-carriers based on graphene oxide (GO) derivatives

Industry Report

10.10 ***Roberto Santoliquido (ALFATEST)***
Assessment of the efficiency of encapsulation of a fluorescent drug using nanoparticle tracking analysis

10.30 *Coffee break*

Invited lectures

11.00 ***Thomas Lars Andresen***
Enzyme sensitive nanomedicines for delivery of platinum based therapeutics

11.30 ***Gerben Koning***
Thermoresponsive liposomal nanomedicine and hyperthermia for the treatment of tumors

Selected lectures

12.00 ***Emanuele Papini***
Functional dissection of the role of *hard* and *soft* corona proteins in the interaction of SiO_2-NPs with cells in human plasma

12.20 ***Silvia Zappavigna***
Transferrin-conjugated self-assembled nanoparticles incorporating ZOL as a tool for the targeting of glioblastoma

12.40 ***Amalia Luce***
Zoledronic acid encapsulated in self-assembly pegylated nanoparticles in combination with radiotherapy on glioblastoma cell lines

13.00 *Closing remarks and lunch*

Invited Lectures

Atomic force nanoscopy and spectroscopy applied to nanomedicine

Salvatore Cannistraro

Biophysics & Nanoscience Centre, Dipartimento DEB, Università della Tuscia, Viterbo, Italy

Email of presenting author: cannistr@unitus.it.

Atomic Force Microscopy (AFM) is a rewarding nanotechnological tool which has boosted research in the field of nanomedicine. It is able to measure interaction forces in biomolecular systems in the order of picoNewton, without labelling, in physiological media, at the level of single molecular structures, and even when molecules and cells are at work. It is therefore possible: i) to image structures at the nanoscale; ii) to measure single ligand-receptor couple interaction strength; iii) to determine the viscosity/elasticity of cells or of their substructure; iv) to capture the molecular or cellular motions; v) to study the unfolding of biomolecules; and so on. The most important applications of AFM of particular relevance to nanomedicine will be reviewed.

See refs at the website: www.unitus.it/biophysics

Advanced Fluorescence Microscopy to Study Nanomedicines

Stefaan C. De Smedt, Kevin Braeckmans, Katrien Remaut, Koen Raemdonck, Jo Demeester

Laboratory of General Biochemistry and Physical Pharmacy, Ghent University, Ottergemsesteenweg 460, B-9000 Ghent, Belgium, 0032-9-2648098, Ghent Research Group on Nanomedicines Ghent University, B-9000 Gent, Centre for Nano- and Biophotonics, Ghent University, B-9000 Gent, Belgium

Email of presenting author: Stefaan.DeSmedt@UGent.be

In the drug delivery field, interest goes to developing 'intelligent' nanoscopic particles that are capable of efficiently delivering biopharmaceuticals to target cells. These nanoparticle formulations should fulfill several requirements. Besides efficiently encapsulating the biopharmaceuticals, they also have to provide protection against degradation during the entire delivery process. Furthermore, the nanoparticles should not aggregate e.g. after intravenous injection. Nor should they release the therapeutic cargo while being suspended in the blood circulation or when traversing the extracellular space. Release of the biopharmaceuticals in many cases should only occur after being internalized in the target cells. Obtaining a better insight into the physicochemical and biophysical behaviour of the nanoparticles during the various phases of the delivery process is required to achieve efficient optimization of their structure and composition.

For more than 10 years, our group has been exploring the use of advanced fluorescence microscopy methods for this purpose. Detailed information on the mobility and potential binding of nanoparticles in extracellular tissues can be obtained using fluorescence recovery after photobleaching (FRAP) or fluorescence Single Particle Tracking (fSPT). These techniques were for instance used to examine the mobility of nanoparticles in lung sputum of cystic fibrosis patients and vitreous humour. We have used fluorescence correlation spectroscopy (FCS) to study the association and dissociation of nucleic acids from nanomedicine formulations in biological media, such as sera, cell lysates and cells. Complementary to that, fSPT has proven to be a unique tool to accurately measure nanoparticle aggregation and concentration in biological fluids. Finally, using fSPT and live cell microscopy we succeeded in unraveling the intracellular trafficking of nanoparticles in great detail.

By providing a better insight into the stability and transport of nanoparticles during the various phases of the delivery process through the use of advanced microscopy techniques, it is our aim to enable a more efficient and rational development of improved carrier materials for the delivery of nucleic acids.

Novel strategies to actively target gold nanoparticles to malignant cells for cancer diagnostics and treatment

Roberto Pini[a], Fulvio Ratto[a], Francesca Tatini[a], Sonia Centi[b], Ida Landini[c], Stefania Nobili[c], Ewa Witort[b], Franco Fusi[b], Sergio Capaccioli[b], Enrico Mini[c]

[a] *Institute of Applied Physics, National Research Council of Italy, Via Madonna del Piano 10, I-50019, Sesto Fiorentino, Italy;* [b] *Department of Experimental Biomedical and Clinical Science, University of Florence, Viale Pieraccini 6, I-50139 Firenze, Italy;* [c] *Department of Health Science, University of Florence, Viale Pieraccini 6, I-50139 Firenze, Italy.*

Email of presenting author: r.pini@ifac.cnr.it

The combination of pulsed and CW near-infrared laser light with plasmonic particles such as gold nanorods is gaining relevance for the photoacoustic imaging and photothermal ablation of cancer. Selective targeting of malignant cells with these contrast agents may rely on complementary biochemical and biological strategies, including the use of specific probes or the exploitation of cellular vehicles.

Here we move from a platform of PEGylated gold nanorods with plasmonic bands around 800 nm, good biological profiles, stability and efficiency of photoacoustic and photothermal conversion as well as potential to passively accumulate into solid tumors by their enhanced permeability and retention. In order to enhance this potential, we implemented different approaches for active delivery by functionalization with (i) antibodies against cancer antigen 125 (CA125), which is a common biomarker for ovarian lesions; (ii) inhibitors of carbonic anhydrases 9 and 12 (CAIX and CAXII), which are expressed by hypoxic cells such as those found in solid tumors; and (iii) by introducing macrophages as a versatile model of cellular vehicles that would phagocytose the particles and home to inflammatory lesions.

We challenge these alternatives in vitro under relevant conditions and discuss issues and perspectives behind their optimization and synergy.

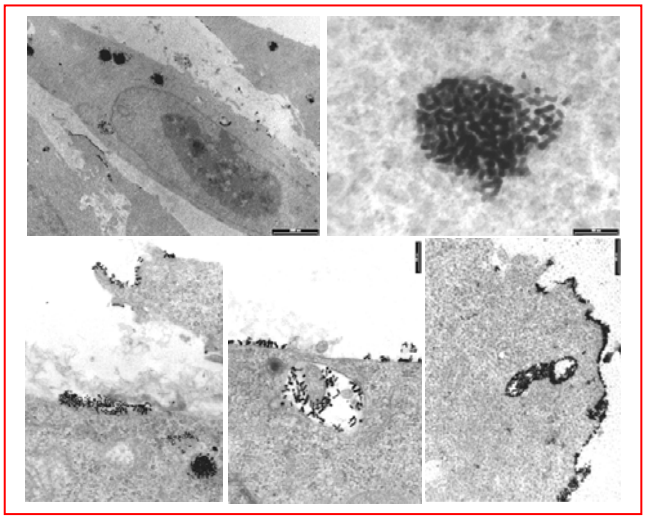

Figure 1. Cellular uptake of PEGylated gold nanorods by endocytosis in macrophages to be used as cellular vehicles.

Hybrid and breakable porous materials. Application in biomedicine

Luisa De Cola, Eko A Prasetyanto, Laura Maggini, Alessandro Bertucci, Federica Fiorini, Dedy Septiady

[a]*Department Institute de Science et d'Ingénierie Supramoléculaires (ISIS), Université de Strasbourg, 8 Rue G. Monge, 67000 Strasbourg, France and INT-KIT, Karlsruhe, Germany*

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The talk will focus on the synthesis, characterization and applications of porous organosilicates, silicates, to be used for in vitro and *in vivo* imaging. The use of ordered mesoporous silica will be illustrated as containers for molecules and for delivering PNA [1] and DNA [2] inside HeLa cells. The fate of the silica particles will be discussed and in particular their accumulation in specific location in the cells will introduce our newest finding of porous systems able to disintegrate upon an external stimulus [3]. Their delivery properties and their destruction kinetics will be shown with some examples. On the other hand such porous systems can be used as components for special type of hydrogels. This hybrid soft/hard material has very exciting properties such as a very high swelling, a high water content (>95%) and excellent mechanical stability. The hybrid scaffold has been used as matrix for 3D growth of cells. The pores of the particles can be indeed employed as food reservoir and we have demonstrated that cells in such conditions can live several days [4].

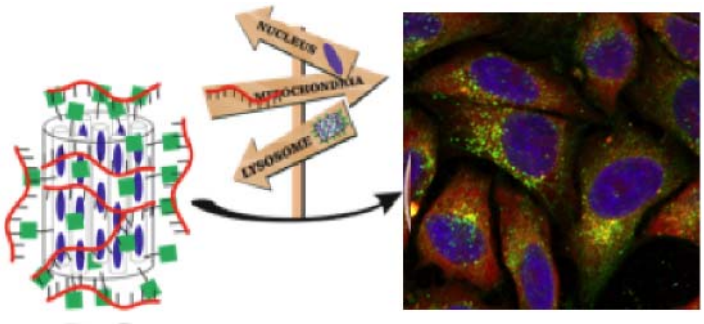

Figure. A schematic representation of a nano- container with dye molecules inside the pores and oligonucleotides on the surface. The kinetics of the release of the different components have been studied

1. R. Corradini, L. De Cola et al *Adv. Healthcare Mater*. **2014**, online.
2. L. De Cola et al. *Chem. Eur. J.* **2014** in press (cover of the issue).
3. L. Maggini, E.A. Prasetyanto, L. De Cola et al. to be submitted
4. F. Fiorini, L. De Cola et al. to be submitted

Acknowledgments: We kindly thank the ERC advanced grant award (number 2009-247365) for financial support.

Bio-inspired nanocarriers for siRNA delivery

Smadar Cohen

The Center for Regenerative Medicine and Stem Cell (RMSC) Research, The Ilse Katz Institute for Nanoscale Science and Technology, and The Avram & Stella Goldstein-Goren Department of Biotechnology Engineering, Ben-Gurion University of the Negev, Beer-Sheva 84105, Israel

Email of presenting author: scohen@bgu.ac.il

Sub-cellular targeting of siRNA can drastically improve its efficacy while minimizing side effects. Here, I will describe the development of anionic polyplexes formed between siRNA and hyaluronan-sulfate and mediated by calcium ion bridges. In addition to their potential biocompatibility compared to cationic carriers, the anionic siRNA polyplexes present several advantages; the simple preparation method under aqueous conditions ("green technology") enables mass production; and the use of hyaluronan-sulfate can enhance their targeting and uptake by certain cells carrying the HA receptor. Using this platform for delivering anti-EGFP siRNA, 85% silencing of EGFP was observed in EGFP-transfected CT26 mouse cell cultures. EGFP knockdown corresponded with the substantial cell uptake of these polyplexes and their accumulation and targeting to the cell cytoplasm. Similar levels of gene silencing of a more relevant therapeutic target (STAT3 transcription factor) were demonstrated in two human cancer cell lines: multiple myeloma (U266) and hepatocellular carcinoma (HepG2) as well as in primary cell cultures.

Fluorescent nanoplatforms for multimodal phototherapy

Salvatore Sortino

Laboratory of Photochemistry, Department of Drug Sciences, University of Catania, Viale A. Doria 95125, Catania, Italy; Email:

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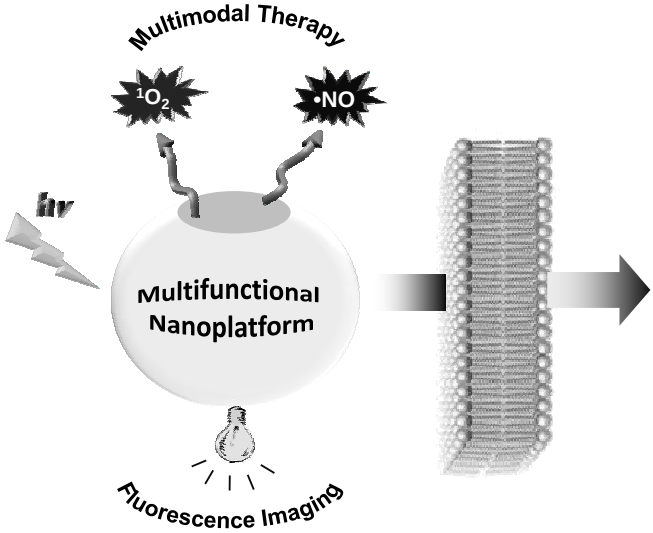
The achievement of molecular nanoconstructs able to release multiple therapeutic species in a controlled fashion is a major challenge in the burgeoning field of nanomedicine.[1] Light is a powerful tool for the introduction of bio-active agents in a cellular environment, mimicking an "*optical syringe*" with an exquisite control of three main factors, site, timing and dosage, which are determining for the therapeutic outcome.[2] Moreover light-triggering is biofriendly and offers the additional advantages of not affecting important physiological parameters such as temperature, pH and ionic strength. In this context, the use of photoactive compounds having intrinsic fluorescence properties or their integration with suitable fluorogenic units is a fundamental requisite for an imaging-guided phototherapy. This allows indeed the visualization of the phototherapeutic agent in cells through fluorescence techniques and can provide a highly localized "burst" precisely at the desired sites. Singlet oxygen ($^1O_2$) and nitric oxide radical (NO) are able to attack biological substrates of different nature (*i.e.,* lipids, proteins, and DNA) representing multitarget therapeutic agents and avoiding Multiple Drug Resistance problems encountered with several conventional drugs often target-specific. Due to their short half-life and lack of charge, both $^1O_2$ and NO radical diffuse in the cellular environment over short distances without inflicting systemic side effects common to general anticancer drugs. Finally, since NO release is very independent from O_2 availability, it well complements the photodynamic therapy based on $^1O_2$ at the onset of hypoxic conditions. For all these reasons, the combination of $^1O_2$ with NO has received growing attention in the last few years[3] with the exciting prospect to tackle cancer and bacterial diseases.



In our laboratories, we have been working on the design and fabrication of a number of nanoplatforms merging multiple imaging and therapeutic photofunctionalities. This contribution illustrates some of the most recent and representative examples including molecular and supramolecular conjugates, polymer nanoparticles, hydrogels and quantum dots, highlighting the rationale design and their potential relevance in biomedical research.

1. N. L. Komarova, C. R. Boland, *Nature* **2013**, *499*, 291.
2. S. Sortino, *J. Mater. Chem.*, **2012**, *22*, 301.
3. S. Sortino, *Chem. Soc. Rev.* **2010**, *39*, 2903.

Magnetic hyperthermia and cell death: the impact of physiological media on the heating efficiency and cell uptake.

Gerardo F. Goya,[a,b] M. Pilar Calatayud,[a,b] Laura Asin,[a] Beatriz Sanz,[a,b] Teobaldo E. Torres,[a,b] M. Ricardo Ibarra.[a,b]

[a] *Instituto de Nanociencia de Aragón, University of Zaragoza, M. Esquillor S/N, 50018, Zaragoza, Spain;* [b] *Departmento de la Materia Condensada, Facultad de Ciencias, Universidad de Zaragoza, Spain.*

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A central problem in nanomedicine is to understand the interaction of living organisms with man-made nanometric objects like magnetic nanoparticles (MNPs), nanowires or nanotubes, because these objects can interact with cellular structures and substructures in unprecedented ways. MNPs can disrupt or promote molecular exchange across cell membrane as well as to produce specific effects on the metabolic cell pathways, setting the basis for new biomedical therapies such as the intracellular heating known as Magnetic Hyperthermia (MH). This technique relies on the use of MNPs as heat generators to induce localized cell death by the application of external alternating magnetic fields (AMF). The interaction between the magnetic moment of the MNPs and the applied AMFs dissipates energy that can be used to provoke the death of any target cell.

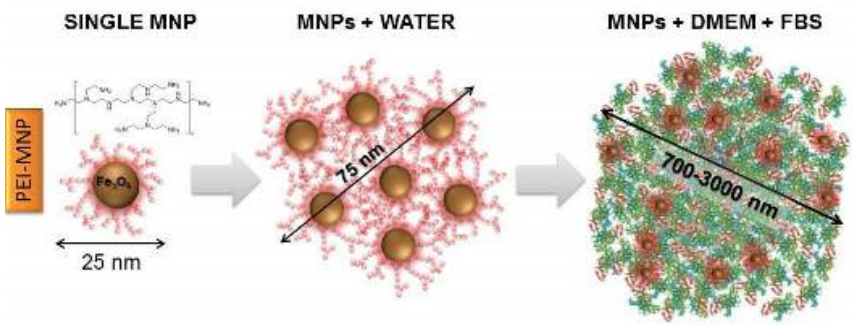

Figure 1. Evolution of the protein corona from the water-colloid state of MNPs to the protein-rich culture medium.

The main aspects about the heating efficiency of MNPs in colloids have been successfully explained by simple physical models. [1] However, for *in vitro* and *in vivo* applications the proteins in the physiological medium yield the formation of a 'protein corona' that influences not only the physicochemical properties of the particles but also the internalization into a given cell type. [2] We show here how the process of protein adsorption onto different MNPs affects the cellular uptake pathways, and its consequences for the efficiency of the MFH experiments. Additionally, novel approaches for observing new MNP-cell interactions at the single-cell level through physical measurements will be presented. The potential benefits and limitations of the 'Trojan horse' strategy for immune-related therapies using human-monocyte-derived dendritic cells (DCs), and the universality of the induced cell death mechanisms will be discussed.

1.Usov, N.A. and B.Y. Liubimov, *Dynamics of magnetic nanoparticle in a viscous liquid: Application to magnetic nanoparticle hyperthermia.* Journal of Applied Physics, 2012. **112**(2).
2.Calatayud, M.P., et al., *The effect of surface charge of functionalized Fe3O4 nanoparticles on protein adsorption and cell uptake.* Biomaterials, 2014. **35**(24): p. 6389-6399.

Thermoresponsive liposomal nanomedicine and hyperthermia for the treatment of tumors

Gerben A. Koning

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Liposomes have been successful as a nanomedicine for the delivery of chemotherapeutic drugs and are currently applied clinically in the treatment of various types of cancer. Efficacy of liposomal drug delivery is however hindered by limited levels of nanoparticle accumulation in tumors and by slow and uncontrolled drug release. Mild hyperthermia (HT), the heating of tumors to temperatures of 40-43°C, has been developed as a clinical treatment modality in combination with chemo- and radiotherapy. The aim of our work is to use HT in combination with novel liposome design to further improve solid tumor chemotherapy.
First, we studied whether HT can increase vascular permeability leading to increased intratumoral liposome deposition. Second, HT was used to trigger drug release interstitially from extravasated thermosensitive liposomes (TSL) [1]. Third, novel targeted TSL were designed to promote tumor targeting and cellular nanoparticle uptake in combination with triggered drug release [2]. Lastly, a novel intravascular drug delivery approach was applied, triggering drug release from TSL in tumor microcirculation [1].
Intravital microscopy and whole body optical imaging proved that TSL extravasation and interstitial penetration can be strongly promoted upon HT in different tumor models [4]. Besides depending on passive tumor accumulation we designed cationic TSL that targeted tumor vasculature and tumor cells, where they were triggered for content release [2,5], causing significant tumor vascular damage. Triggered drug delivery following an intravascular [1,3,6] or interstitial drug release approach [1,7] imaged by intravital microscopy demonstrated delivery of doxorubicin (Dox) to tumor and tumor endothelial cells. Treatment of BLM melanoma by intravascular Dox release from fast-releasing TSL outperformed a treatment with interstitial Dox release from slow-releasing TSL [7]. These novel fast-releasing TSL for intravascular Dox release proved more efficacious than lysolipid-Dox-TSL [4,6].
The combination of hyperthermia with thermosensitive liposomes provides a promising approach to further improve chemotherapy for solid tumors.

1. G.A. Koning, A.M. Eggermont, L.H. Lindner, T.L. ten Hagen, *Pharm Res,* ***2010***, 27, 1750.
2. B.M. Dicheva, G.A. Koning, *Expert Opin Drug Deliv,* **2014**, 11, 83.
3. A.A. Manzoor, L.H. Lindner, C.D. Landon, J.Y. Park, A.J. Simnick, M.R. Dreher, S. Das, G. Hanna, W. Park, A. Chilkoti, G.A. Koning, T.L. ten Hagen, D. Needham, M.W. Dewhirst, *Cancer Res*, **2012**, 72, 5566.
4. L. Li, T.L. ten Hagen, M. Bolkestein, A. Gasselhuber, J. Yatvin, G.C. van Rhoon, A.M. Eggermont, D. Haemmerich, G.A. Koning, *J Controlled Release,* **2013**, 167, 130.
5. B.M. Dicheva, T.L Hagen, L. Li, D. Schipper, A.L. Seynhaeve, G.C. van Rhoon, A.M. Eggermont, L.H. Lindner, G.A. Koning, *Nano Lett*, **2013**, 13, 2324.
6. L. Li, T.L. ten Hagen, M. Hossann, R. Süss, G.C. van Rhoon, A.M. Eggermont, D. Haemmerich, G.A. Koning, *J Controlled Release*, **2013**, 168, 142.
7. L. Li, T.L. ten Hagen, A. Haeri, T. Soullié, C. Scholten, A.L. Seynhaeve, A.M Eggermont, G.A. Koning, *J Controlled Release,* **2014**, 174, 202.

Acknowledgments: Presented work was funded by various grants from SEHK, St. Fondsen, DKF, NanoNextNL, and EU COST Action TD1004 Theranostics Imaging and Therapy.

Smart Implants Equipped with Active Nanoreservoirs for Regenerative Nanomedicine

N. Benkirane-Jessel

INSERM "French National Institute of Health and Medical Research"; UMR1109; Université de Strasbourg. "Osteoarticular and Dental Regenerative Nanomedicine" Laboratory, Strasbourg, France

Email of presenting author: Nadia.jessel@inserm.fr; http://www.regmed.fr

Recently, we have reported a "Smart Hybrid Materials Equipped by Nanoreservoirs of Therapeutics and stem cells ". This unique nanotechnology strategy is used to entrap, protect, and stabilize therapeutic agents into polymer coatings acting as nanoreservoirs enrobing nanofibers of implantable membranes. Upon contact with cells, therapeutic agents become available through enzymatic degradation of the nanoreservoirs. As cells grow, divide, and infiltrate deeper into the porous membrane, they trigger slow and progressive release of therapeutic agents that, in turn, stimulate further cell proliferation. This constitutes the first instance of a smart living nanostructured hybrid membrane for regenerative medicine. The cell contact-dependent bioerodable nanoreservoirs described here will permit sustained release of drugs, genes, growth factors, etc., opening a general route to the design of sophisticated cell-therapy implants capable of robust and durable regeneration of a broad variety of tissues.

ARTiOS NanoMedicine Start-up Project

1.F. Fioretti, C. Mendoza-Palomares, M. Helms, D.A. Alam, L. Richert, Y. Arntz, S. Rinckenbach, F. Garnier, Y. Hakel, S.C. Gangloff, N. Benkirane-Jessel, ***ACS Nano.*** 2010, *22*, 3277.

2.S. Facca, C. Cortez, C. Mendoza-Palomares, N. Messadeq, A. Dierich, A.P.R. Johnston, D. Mainard, J.C. Voegel, F. Caruso, N. Benkirane-Jessel, ***Proc. Natl. Acad. Sci. USA.*** 2010, *107*, 3406.

3.C. Mendoza, A. Ferrand, S. Facca, F. Fioretti, D. Mainard, N. Benkirane-Jessel, ***ACS Nano.*** 2012, *6*, 483.

Enzyme sensitive nanomedicines for delivery of platinum based therapeutics

Thomas L. Andresen, Anders E. Hansen, Fredrik Melander, Rasmus Eliasen, Rasmus Jølck, Jonas Henriksen, Rikke Brogaard

[a]*Department of Micro- and Nanotechnology, Technical University of Denmark, Building 423, 2800, Lyngby, Denmark;*

Email of presenting author: tlan@nanotech.dtu.dk

One of the main design challenges in liposome based chemotheraputics has been the productive delivery of drugs that are not able to escape the liposomes easily, where cisplatinum has been a hallmark of this challenge (1). We have been focused on finding methods for utilizing enzymes to enhance the drug release rate of platinum based drugs in tumor tissue, where we have had particular success with targeting peptidases. Drug release is achieved by surface coating the liposomes with molecules that are sensitive to enzymatic degradation. We have carried out biodistribution and PK studies, as well as tumor growth studies in mouse xenograft models with encouraging results. We furthermore utilized positron emission tomography (PET) for evaluating the developed drug delivery systems in vivo, which allowed us to study time resolved biodistribution of the drug delivery vehicles. PET imaging can furthermore be used to investigate therapeutic efficacy. We are currently investigating other platinum drugs using the developed formulation to compare differences in the therapeutic efficicacy. We also compared the peptidase activated drug delivery systems with PLA2 activated systems, which showed that the former was superior in all tested tumor models.

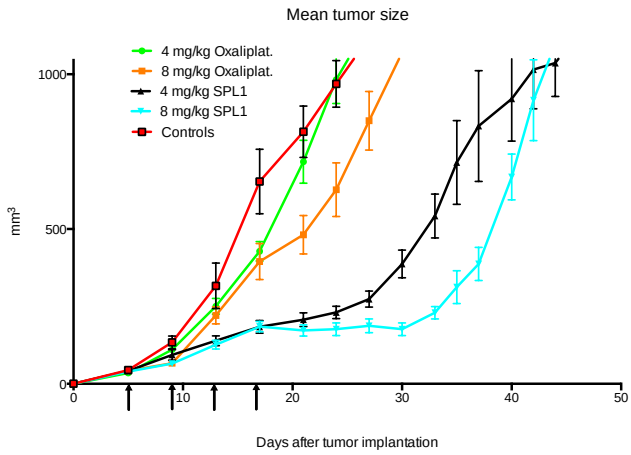

Figure 1. Tumor growth curve of an oxaliplatinum liposome formulation (SPL1) in comparison to free oxaliplatinum and controls.

1. Andresen TL, Jensen SS, Jørgensen, K. *Advanced strategies in liposomal cancer therapy: Problems and prospects of active and tumor specific drug release.* Prog. Lipid Res. 2005, 44(1), 68-97.

Selected Lectures

Poly(ethyl cyanoacrylate) nanoparticles for brain delivery of andrographolide

Clizia Guccione,[a] Cristina Grossi,[b] Fiorella Casamenti,[b] Benedetta Isacchi,[a] Vieri Piazzini,[a] Maria Camilla Bergonzi,[a] Anna Rita Bilia [a]

[a] *Department of Chemistry, University of Florence, via Ugo Schiff 6, 50019, Sesto Fiorentino, Florence, Italy;*
[b]*Department of Neuroscience, Pharmacology and Child's Health (NEUROFARBA), University of Florence, Viale Pieraccini 6, 50139, Florence, Italy*

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The limitations imposed by the blood-brain barrier (BBB) and the non-selective distribution of drugs in the brain have hindered the effective treatment of brain diseases and may result in severe side effects on the normal brains. Aim of this work was to deliver natural molecules into nanoparticles capable to provide sustained and controlled drug release, improved biodistribution and therapeutic efficacy for Alzheimer's Disease (AD) prevention and therapy. Novel fluorescent polymeric nanoparticles to target brain tissues were designed and optimised to follow their in vivo distribution as new strategies to cross the BBB, namely ethylcyanoacrylate nanospheres (ECA-NSs) coated with polysorbate 80. They were prepared by emulsion polymerization method using fluorescein isothiocyanate linked to dextran as a florescent probe [1]. NSs were characterized in terms of dimension, polydispersity, zeta potential, morphology, encapsulation efficacy, loading capacity and stability of probe by DLS, TEM and HPLC-DAD-FLD, respectively. A study after intracerebral injection of nanoparticles in nucleus basalis magnocellularis of rats, colynergic neuronal location, evidenced that NSs did not induce inflammatory response.
NSs were also administered intraperitoneally and intravenously to anaesthetized rats to evaluate their biodistribution by fluorescent microscope images. The biodistribution studies revealed that NSs were able to cross the BBB and reach brain tissue, as a consequence they were loaded with andrographolide, a major diterpenoid of *Andrographis paniculata* (Burm. f.) Nees, whose clinical utility for the treatment of inflammation-related neurodegenerative disorder has been demonstrated [2]. ECA-NSs were produced with good yields, suitable for the intraperitoneally administration (mean diameter ≤300 nm; PDI 0.2), with a sphere-like shape and a good encapsulation efficacy.
The developed nanocarriers represent a valuable targeting system for AD therapy.

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Selective Targeting, Sorting and Killing of Cancer-like Cells *in-vitro* with Magnetic Carbon Nanotubes

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With the aim to design addressable magnetically-active carbon nanotubes (CNTs) for cancer treatment, the use of Fe-filled CNTs (Fe@MWCNTs) as multifunctional scaffolds is reported for exohedrally anchoring a monoclonal antibody (mAb, Fig. 1) known to bind a plasma membrane receptor over-expressed in several cancer cells (EGFR).[1] Comprehensive microscopic (TEM, AFM, and SEM) and spectroscopic (Raman, ^{57}Fe Mössbauer, energy dispersive spectroscopy, XPS, X-ray diffraction) characterizations reveal the efficient confinement of magnetically-active Fe phases (α-Fe and Fe_3C), while compositional evaluations through XPS, thermogravimetric analysis and gel electrophoresis confirm that mAb immobilization onto Fe@MWCNTs occurs. Enzyme-linked immunosorbent assay (ELISA), confocal microscopy imaging and western blotting confirm the targeting action toward EGFR-overexpressing cell lines (EGFR+). In vitro magnetic filtration experiments demonstrate that a selective removal of EGFR+ cells from a mixed population of healthy cell lines could be obtained in very short times (≈10 min). Cytotoxicity evaluations by classic cell staining procedures after application of an electromagnetic radiation inducing magnetic fluid hyperthermia (MFH) show a selective suppression of the EGFR+ cell line. Molecular dynamics and docking simulations of the hybrid mAb/Fe@MWCNTs conjugates nicely show how the presence of the CNT framework does not sterically affect the conformational properties of the two antigen binding regions, further supporting the biochemical findings.

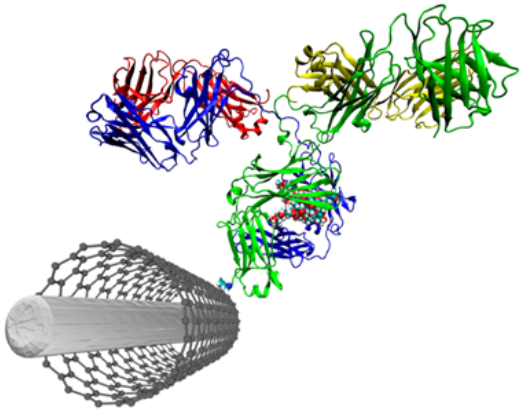

Figure 1. Schematic representation of Ab-functionalised Fe@MWCNTs.

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In vivo sonodynamic activity of TPPS loaded polymeric nanoparticles

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Although progress in basic research has led to the design of new generations of anticancer targeted drugs with some notable achievements further progress in cancer treatment may be accomplished through other existing, but still under-appreciated, therapeutic approaches.[1,2] Among these, sonodynamic treatment takes advantage from the use of non-thermal ultrasound to activate chemical compounds known as sonosensitizers.[3] The activated sonosensitizer agent is then able to kill cancer cells through the generation of highly reactive products, such as reactive oxygen species (ROS), through apoptotic and/or necrotic mechanism. The great advantage of this technique relies on its low systemic toxicity, the possibility of highly controlled non-invasive treatments/practices and the non-occurrence of drug resistance even after repeated treatment. Within this framework, we will present the use of biocompatible, polymeric core-shell nanoaparticles (NPs) as multi-functionalized carriers of a properly selected sensitizer for *in vitro* and *in vivo* tumor treatment.[4] In addition, PET and MRI *in vivo* bio-distribution data of our porphyrin loaded nanoparticles will be discussed.

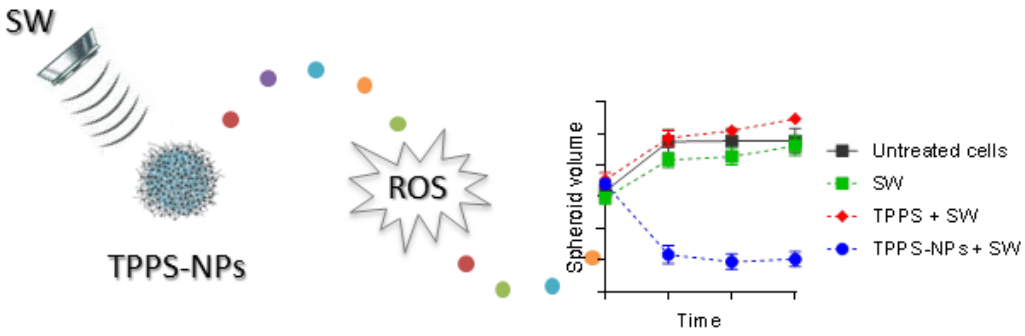

Figure 1. Schematic representation of the sonodynamic treatment mediated by TPPS-NPs and its effect on human neuroblastoma SH-SY5Y spheroids volume after different treatment conditions

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Acknowledgments: Italian Ministry of Health and Piemonte Region (grant "Giovani Ricercatori 2008," GR-2008-1138087) and the Associazione Italiana per la Ricerca sul Cancro (grant "MFAG 2012," MFAG-13048).

Lanthanide Doped Alkaline earth fluoride Nanoparticles as biocompatible, multifunctional materials for biomedical imaging

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Lanthanide doped fluorides are interesting hosts for their efficient luminescence in the optical (visible and near infrared) regions, make them interesting hosts in modern technological applications, such as in biomedical diagnostics. In particular, Er^{3+}/Yb^{3+} and Tm^{3+}/Yb^{3+} doped MF_2 (M=Ca, Sr) nanoparticles (NPs) have been investigated recently for their strong upconversion (anti-Stokes) properties [1-4].

A facile hydrothermal one-step procedure was used to prepare citrate capped CaF_2 NPs triply doped with Er^{3+}, Gd^{3+} and Yb^{3+} or Tm^{3+}, Gd^{3+} and Yb^{3+} ions. The obtained NPs are easily dispersible in saline or PBS solutions, important property for their use in biological fluids. The NPs are cubic single phase and well size monodispersed, with average sizes of 10-15 nm. The obtained NPs transparent colloidal dispersions show strong upconversion emission in the red (around 650 nm) and in the NIR (around 800 nm) for the Er^{3+} doped and Tm^{3+} doped nanoparticles, respectively, upon laser excitation at 980 nm in the $^2F_{5/2}$ level of Yb^{3+}. Both the excitation and the emitted radiation are close to or inside the biological window, suggesting a possible use for *in-vitro* and *in-vivo* optical imaging. Spin-echo measurements on saline colloidal NPs dispersions have also shown significant proton relaxivities, suggesting their possible use as MRI contrast agents. Considering also the high biocompatibility, the present NPs are suitable candidates to be efficiently used as nanoprobes for both *in-vitro* and *in-vivo* multimodal optical and magnetic resonance imaging.

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Acknowledgments: this work was supported by Fondazione Cariverona (Verona, Italy), project "Verona Nanomedicine Initiative". M.P and A.S. acknowledge financial support from "Performance in Lighting" company, Colognola ai Colli, Verona, Italy.

New Radiosensitizers agents from Nanotechnology: a novel approach for the synthesis of gold nanoparticles

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Compounds containing high atomic number (Z) atoms like Iodine (Z=53) or Gadolium (Z=63) are extensively used in radiotherapy for the increasing dose absorption at tumor site (1). The recent explosion of Nanobiotechnology, an interdisciplinary reasearch area between Physics, Chemistry and Biology, gives new tools for developing new therapeutic approaches and also for the improvement of the efficacy of radiotherapy by means of a new generation of radiosensitizers materials. Gold (Au = 79) Nanoparticles (AuNP) are the most promising candidate to become a suitable material for standard treatment in radiotherapy (2). Although the great success of gold nanoparticles in this framework, there is still need to opening new routes for the synthesis of more stable and functional nanoparticles.
In this research work we will discuss some results concerning with the synthesis and the characterization of new kind of stable AuNP (Figure 1) stabilized with the organic thiol 3MPS (3-mercapto-1-propansulfonate) and 1-β-thio-D-glucose (TG). The biocompatibility of the nanostructured system are tested with MTT assay in mammalian cell cultures while the internalizzation are followed by means of Atomic Absorption Spectroscopy (AAS).

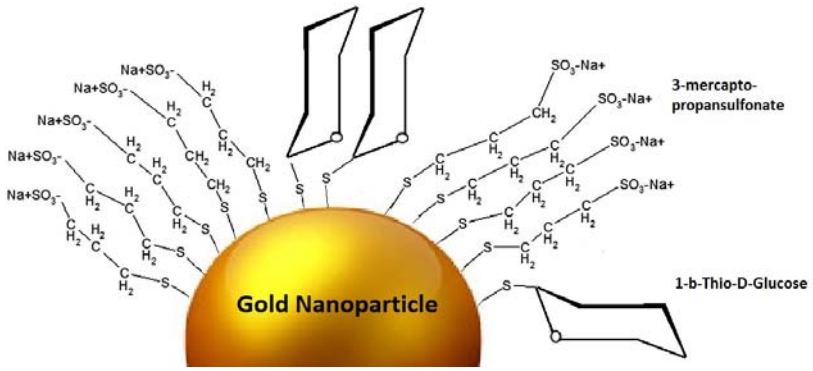

Figure 1. The molecule 3MPS gives waters solubility and stabilization to the nanoparticle while the TG gives important biological features for the uptake at tumor site. Tumor cell indeed have marked anaerobic metabolism, a phenomenon called Warburg effect; as consequence, they have a strong need for glucose. So the presence of glucose on the surface of nanoparticles can increase cellular uptake mainly in cancer cell and it can be use for a preferential targeting.

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Digestive ripening of pegylated Au nanoparticles in aqueous solution: their application as X-ray contrast agents

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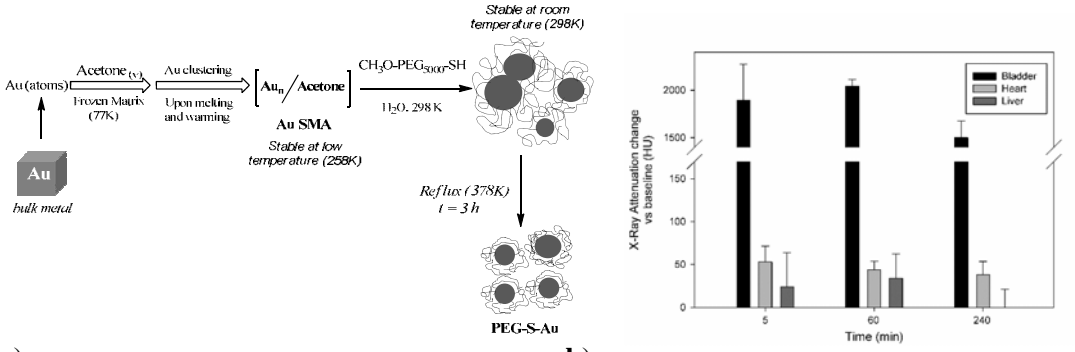
In the last 40 years medical imaging has undergone enormous improvements due to the development of non-invasive techniques such as computed tomography (CT), magnetic resonance imaging and positron emission tomography. Nano-material based contrast agents are quickly becoming valuable tools to enhance medical diagnostics for a wide range of in-vitro and in-vivo imaging modalities. In this context, gold nanoparticles (AuNPs) show interesting behavior as CT contrast agents owing to their high X-ray absorption coefficient and low toxicity.[1] Nevertheless, a routine application of AuNPs in diagnostics could be achievable only developing a reproducible, high yield and low cost procedure for their production. Recently, has been demonstrated that metal vapor synthesis technique (MVS)[2] combined with digestive ripening procedure, allows to prepare monodisperse Au colloids soluble in organic phase in large amount and reproducible quality, avoiding the presence of by-products of metal salts reduction in solution.[2] Here we report a novel synthesis of PEGylated AuNPs by combining MVS technique with aqueous digestive ripening process, using CH_3O-PEG5000-SH (PEG-SH) as capping agent (**Scheme 1a**). We demonstrate that the developed procedure allowed us to easily obtain a sufficient amount of **PEG-S-Au** acted as safety and efficient CT-contrast agents on a mouse model. Moreover, thanks to the innovative combination of MVS technique/surface functionalization, the hydrodynamic radius of the engineered **PEG-S-Au** permitted their efficient renal clearance, still retaining a prolonged blood circulation and a stealth capability (**Scheme 1b**).



a) **b**)

Scheme 1. a) Synthesis of PEG-S-Au NPs by combined MVS technique and aqueous digestive ripening process; **b**) X-ray attenuation change versus baseline in function of the time and organ accumulation of PEG-S-Au NPs.

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Controlled Photo-Release Drug from Nanoparticles for PDT Applications

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Nanoparticle-based systems have been developed for nanomedicine applications, in order to improve the efficacy of a wide range of drugs, reducing the toxicity for the normal tissues and the long-term side-effects. As a matter of fact, "classic" approaches to nanomedicine based on simple or even targeted drug-loaded particles, are showing strong limitations and very few nanomedicine agents are currently used in healthcare. Likely, dealing with such a complex environment as a living organism requires complex tools, capable to adapt their behavior to the different situations they encounter. Chemists can substantially contribute to the development of such responsive systems, not only by producing new chemical entities or materials, but also by implementing in the nanosystems smart behaviors, based on externally triggered chemical reactions. In this context, promising results have been accomplished in photodynamic therapy (PDT), a medical cancer treatment based on non-toxic photosensitizing drugs that become active producing cytotoxic reactive oxygen species (ROS, in particular singlet oxygen) by exposure to light. The major limitation of PDT is the lack of selectivity that cause the unfavorable accumulation of photosensitizer (PS) into healthy tissues, causing severe side effects. The introduction of the nanotechnology concept in the design of carriers for PDT may help in increasing the selectivity. Most important, the production of singlet oxygen combined with a photocleavable group offers a phototriggered mechanism that can be exploited in designing nanoparticles with photocontrolled behavior. Based on that, we here propose ORMOSIL (ORganicallyMOdifiedSILica)[1] nanoparticles where a porphyrin PS is covalently loaded through a β-enamino-ketone spacer, which is cleaved upon singlet oxygen exposure (Figure 1).[2] Preliminary results show that after light irradiation, the PS is released from the nanoparticles, and the extent of release increases by prolonging the exposure time. In this way, the release of PS may be controlled by an external input, and the same concept should be applied for releasing therapeutic molecules. Beside singlet oxygen sensitive linkers, we also investigated polysulphides-based nanoparticles to improve the nanoparticles behavior into biological systems.

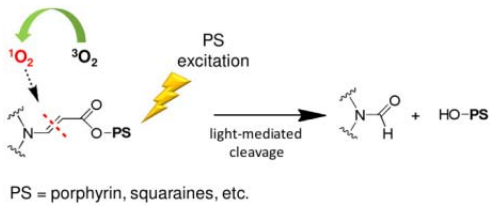

Figure 1: mechanism of phototriggered cleavage.

The data reported in this work paved the way for the creation of smart nanoparticles for theranostic applications.

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"NMR chemosensing": detection and identification of organic molecules and metabolites using nanoparticles as receptors

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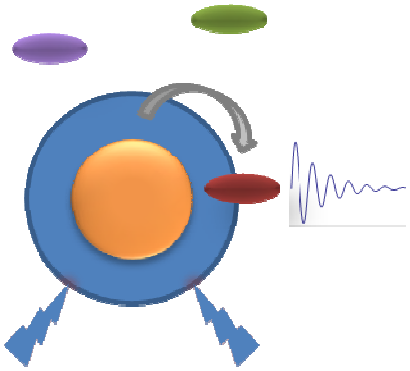
Chemosensors or chemical probes are molecular systems capable to recognized and signal the presence of an analyte of interest. Such systems, based either on molecular structrues or nanoparticles, have attracted over the last 30 years a huge interest for biological studies since they allow monitoring the levels of selected species in living cells with high sensitivity, spatial resolution and negligible perturbation. Albeit several advantages justify the widespread interest in chemosensors, one intrinsic limitation of such an approach is that the reliability of a chemosensor response depends crucially on its selectivity. Indeed, the signal produced arises from a property of the chemosensor itself and, as such, it does not provide any direct information on the identity of the analyte detected. The user must presume he is measuring the presence of the desired target, and not of a known or unknown interferent, because he trusts the recognition ability of the chemosensor.



Scheme 1. NMR chemosensing working scheme.

To overcome this problem we recently proposed a new chemosensing method based on NMR and gold nanoparticles protected by a monolayer as self-organized receptors.[1] This method, relies on the ability of monolayer-protected nanoparticles to bind substrates exploiting non-covalent interactions. After target recognition, the nanoparticle labels it by NOE magnetization transfer while the signals of the other species present are cancelled by a diffusion filter. In this way, only the interacting molecule appears in the NMR spectrum allowing its detection and identification.

In this communication we'll report on our recent results in the identification of organic anions (carboxylates, sulfonates and boronates) and cations (ammonium salts) by NMR chemosensing. The attention will be focused on the role played by the nanoparticle monolayer chemical structure in the analyte recognition. Indeed, different functional groups can be easily introduced and combined in the organic molecules forming the monolayer resulting in the formation of pre-organized and self-organized binding sites. Selectivity and sensitivity reached allowed the detection and identification of metabolites in biological fluids.

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Acknowledgments: Funded by the ERC Starting Grants Project MOSAIC (grant 259014).

Complex nanostructures based on a specific molecular beacon and PMMA nanoparticles for the detection and silencing of survivin mRNA in human cancer cells.

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Nanosensing by oligonucleotide optical switches coupled to nanoparticles is a highly promising and fascinating strategy for the intracellular detection of a plethora of targets, such as specific mRNA molecules and proteins. The use of an antisense oligonucleotide molecular beacon, able to generate a fluorescent signal when it hybridizes with the target mRNA, may represent an innovative strategy that conjugates the ability of sensing specific mRNA with the pharmacological silencing activity preventing the overexpression of proteins associated to pathologic conditions. In cancer research, this pharmacological approach has emerged because nonspecific toxicity can be minimized and the efficiency of transportation can be enhanced. In this study, we investigated the potential anticancer activity of a molecular beacon-oligo-deoxynucleotide (MB-ODN) targeting survivin mRNA and the ability of polymethyl-methacrylate (PMMA) nanoparticles (NPs) acting as MB nanocarriers in cancer cells. Survivin, a multifunctional protein that plays a role in cancer development and progression [1], is overexpressed in cancer cells and undetectable in most healthy tissues. Experiments were performed on the human melanoma A375 cell line and human lung carcinoma A549 cells, using human monocytes and human dermal fibroblasts (HDFa) as negative controls. MB functionality was firstly verified *in vitro* in solution and after adsorption onto the PMMA NPs. Its functionality and specificity were also examined in living cells on the human melanoma A375 cell line and on human monocytes as negative control. The MB was firstly transfected by the classical lipid agent, lipofectamine. Real-time PCR and Western Blot were used to analyse survivin mRNA and protein expression levels, respectively. A fluorescence increase in the cytoplasm was observed 1 h after the beginning the transfection without evidence of any fluorescence in the extracellular environment. Moreover, no fluorescence was observed in transfected cells not expressing survivin. Real-time PCR data and Western blot analysis demonstrated that the MB significantly decreased survivin expression in A375 cells, thus confirming its pharmacological silencing activity. A375 apoptotic cell death was observed after 48 and 72 h of MB treatment. PMMA NPs and MB-adsorbed onto NPs were also tested on adenocarcinomic human alveolar basal epithelial cells (A549) and human adult fibroblasts in terms of cell vitality and internalization. These experiments provided a clear evidence of the subcellular distribution of PMMA NPs in living cells as well as of their ability to promote the MB internalization. We have shown that oligonucleotide optical switches, together with NPs, can play a fundamental role in achieving quantitative information on intracellular events. The conducted analytical characterization has demonstrated that they can be used not only as simple on-off elements but also as real theranostic agents.

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Acknowledgments: Work supported by the national flagship project NANOMAX

Diolein Based Nanostructures for Theranostic Applications

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Dioleins are well known monomers used in the preparation of highly ordered two or three dimensional mesophases in aqueous solution. (1) They could be used to obtain: 1) nanostructures for diagnostic applications, when doped with metal-based contrast agents, or 2) nanodevices for drug delivery when filled with active drugs, or, finally, 3) theranostics when the two above reported functions (drug and contrast agents) are combined together in the same diolein based supramolecular aggregate.
We describe new nanostructures obtained by co-assembling diolein and the amphiphilic gadolinium complex $(C18)_2$-DTPA(Gd), at 90/10 w/w. Pluronic PF127 is added as stabilizer agent. Moreover, target selective nanostructures are obtained by adding to the previous formulation a small amount (3% w/w) of the amphiphilic folate derivative $(C18)_2$-Peg3000-folic acid.
The obtained nanostructures are loaded with Doxorubicin to give, respectively, theranostics and target-selective theranostic agents for IGROV-1 cells overexpressing folate receptors.
All aggregates are fully characterized for their structure by DLS and TEM, for drug loading ability and for doxorubicin release.
Relaxometric studies suggests their potential application in MRI as target selective contrast agents, while in vitro experiments (citotoxicity studies and confocal microscopy images) confirm that target diolenin based nanostructures show a higher uptake and citotoxicity versus IGROV-1 cells respect to liposomial doxorubicin.

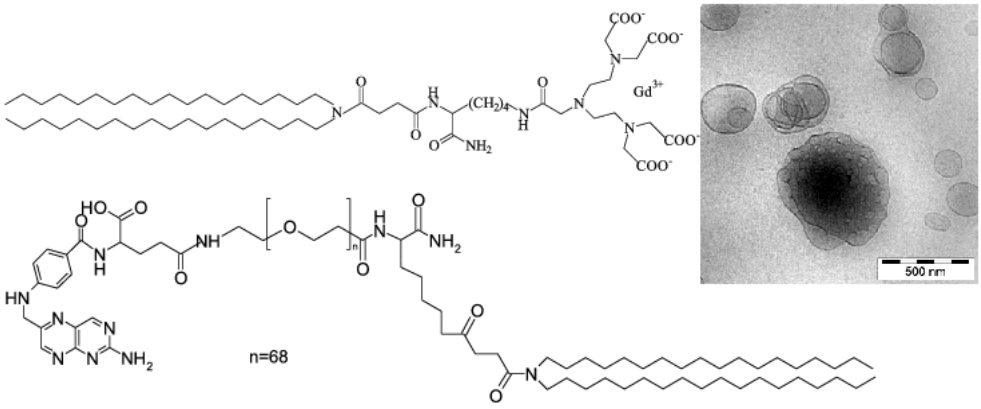

Figure 1. Schematic representation of [$(C18)_2$DTPA(Gd)] and [$(C18)_2$Peg3000-FA] monomers mixed with the commercially available diolein (DO) and the Cryo-TEM image of the resulting nanostructures

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Sensitive MicroRNA Quantification Using DNA-Gold Nanoparticle Probes

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MicroRNAs (miRNAs) are small noncoding RNAs involved in gene regulation. Dysregulated miRNA levels are typical of pathological conditions such as cancer.[1] Therefore, the detection and quantification of miRNA expression levels has high clinical relevance and the development of new, direct, sensitive, and rapid quantification strategies is essential for the exploitation of these biomarkers in diagnosis and prognosis.

We have developed a sensitive assay for the absolute quantification of miRNAs based on enzymatic processing of DNA-functionalized gold nanoparticles (AuNPs), resulting in a fluorescence signal (Figure 1).[2] The limit of detection of our assay is as low as 0.2 fmol of miRNA, which corresponds to approximately 100 copies/cell. The assay is direct, avoiding the conversion of miRNA into cDNA, and technologically easy to implement, not requiring thermal cycling, and can be completed in only 2 □ 5 hours.

In this contribution, we will demonstrate why and how the molecular design of our DNA-AuNP probes is essential for the efficiency of this sensing platform. We will also present the application of our assay to the determination of hsa-miR-21 and hsa-miR-203, representing high- and low-abundance miRNAs respectively, in samples of total RNA extracted from cell cultures. The sensitivity, simplicity and rapidity of our strategy hold great promises for establishing this assay as valuable alternative for absolute and direct miRNA quantification.

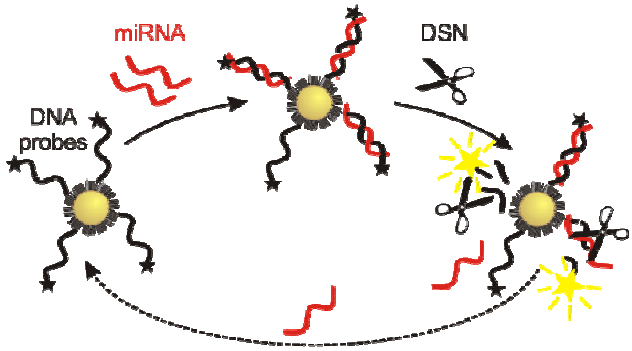

Figure 1. Assay strategy: the fluorescence of labeled DNA-probes is quenched by their vicinity to the AuNP surface. Enzymatic cleavage of the DNA-probes is triggered by hybridization with target miRNA and results in fluorescence recovery. The enzyme duplex specific nuclease (DSN) allows for miRNA target recycling.

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Curcumin bio\nano-template for building layer by layer capsules

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Layer-by-layer (LbL) assembly is a very simple and versatile technique for the fabrication of nanostructured architectures with high molecular order. The assessment of this process depends mainly on adsorption of polyanion–polycation polymers alternatively assembled onto the surface of template. In spite of its important in biomedical application LbL process is not completely understood because it depends mostly on template for building multilayers. Most used templates are derived from inorganic (manganese carbonate, calcium carbonate and cadmium carbonate) and organic (melamine formaldehyde and silicon oxide) materials. The dissolution of these templates in solvents might affect capsules assembly. Additionally, solvent like EDTA can dissolve easily curcumin from $CaCO_3$ carrier after pre-loading during core removal. Therefore our aim is to breakdown curcumin to nano-scale and used it as bio-template for building layer-by-layer assembly onto its surface .
Curcumin nanoscale template provides unique properties for carrier: it can be encapsulated by using LbL, keeping its physical and chemical properties. Since core removal is not needed, this procedure keeps curcumin without releasing it. Moreover, curcumin nanoscale template have allowed us to create capsules after curcumin removal. Capsules size and morphology were investigated by using SEM and TEM. The charge of curcumin template and layer-by-layer assembly was assessed by zeta potential. The cytotoxicity of curcumin bio-template nanocarrier was also investigated confirming its potential as novel template for nano-drug delivery.

Liposomes encapsulating doxorubicin and conjugated with cell penetrating peptides overcome drug resistance in human cancer

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Despite the design of several anticancer drugs that induce cancer cell death or the retardation of their growth, the success of pharmacological treatment is often hampered by the onset of the drug resistance. Several mechanisms are used by tumor cells to protect themselves from the antitumor compounds currently used in the clinical setting. New delivery systems including liposomes have been developed to circumvent drug resistance. In fact, drug encapsulation in liposomes markedly changes pharmacokinetic and pharmacodynamic properties and reduces systemic toxicity; moreover, drug is prevented from early degradation and/or inactivation[1].To enhance the antitumor efficacy of liposomes encapsulating anti-cancer agents, we have used liposomes externally conjugated with the 19 residues of gH625 peptide, previously identified as a membrane-perturbing domain in the gH glycoprotein of Herpes simplex virus type I [2,3]. We have evaluated the growth inhibition of either wild type (A549) or doxorubicin (Doxo)-resistant (A549 Dx) human lung adenocarcinoma cell line treated with increasing concentrations of liposomes encapsulating Doxo (LipoDoxo), liposomes encapsulating Doxo conjugated with gH625 peptide (LipoDoxo - gH625), empty liposomes (Lipo) or free Doxo for 72h. The growth inhibition was assessed by MTT viability assay. Thereafter, we have studied the Doxo uptake in both cell lines by spectrophotometric assay and flow cytometry. We have also evaluated the cell death mechanisms induced by 50% growth inhibitory concentrations (IC_{50}) of pharmacological agents and the oxidative stress status by flow cytometry. We found that the growth inhibition induced by LipoDoxo-gH625 was more potent than that one caused by LipoDoxo with an IC_{50} of 2.7 and 5μM, respectively, in A549 Dx cells. Interestingly, empty liposomes had no cytotoxic effects. The data on cell growth inhibition were paralleled by an increased uptake of Doxo induced by LipoDoxo - gH625 of compared to LipoDoxo in A549 Dx cells. These data were confirmed by flow cytometric analysis. Moreover, cytometric analysis revealed that the antiproliferative effects of each drug treatment were mainly due to the induction of apoptosis. Moreover, the data obtained on oxidative stress suggested a greater internalization of LipoDoxo-gH625 than LipoDoxo after 72h of treatment. In conclusion, we have demonstrated that the functionalization of liposomes with gH625 viral peptide increases Doxo accumulation in Doxo resistant A549 cell line and this effect is paralleled by an increased cytotoxicity.

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Multifunctional biomaterials based on nanostructured poly(lactic acid): a way to drive stem cell responses.

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The possibility to control specific cell functions by modulating the polymer properties represents a key step on material science in tissue engineering. This work reports the promising perspectives of poly(L-lactic acid), (PLLA) based nano-engineered biomaterials and their relevance in tissue engineering (1,2). The effects of nanocomposite properties are here extensively investigated and reported in terms of stem cell responses. PLLA nanocomposite films were obtaining by solvent casting technology. Multiwalled carbon nanotubes (MWCNTs) were used as conductive nanostructures (0-3wt.%). Morphological, mechanical, electrical and surface properties of PLLA/MWCNTs nanocomposites were investigated and correlated to the MWCNT content. Human bone marrow mesenchymal stem cells (BM-MSCs) were obtained from washouts of the medullary cavities of the femurs, while human umbilical cord mesenchymal stem cells (UCMS) were isolated from umbilical cord. Stem cell/material interactions were evaluated analyzing the plasma membrane integrins, cytoskeleton organization (α-tubulin/F-actin), nuclear laminin A/B of seeded stem cells. PLLA conductive nanocomposite films show the formation of a three-dimensional nanotube network in the percolated formulations. Interestingly hBM-MSCs and hUCMS differently interacted when seeded on nanostructured PLLA based biomaterials. While BM-MSCs showed a canonical mesenchymal morphology with F-actin-containing fibers arranged on the major cellular axis, UCMS changed their fibroblast-like morphology, acquiring a sphere-like structure (Figure 1). Our data demonstrates that nanostructured PLLA is a good candidate to study the effect of the biomaterial physical properties on the stem cell fate. The physical and chemical properties of the PLLA, including size, shape, mechanical properties, surface texture, etc. can regulate biological responses and provide mechanical stimuli to stem cells. The identification of critical mechano-sensitive molecules and cellular components that contribute to the mechanotransduction response is basic for medicine regenerative applications.

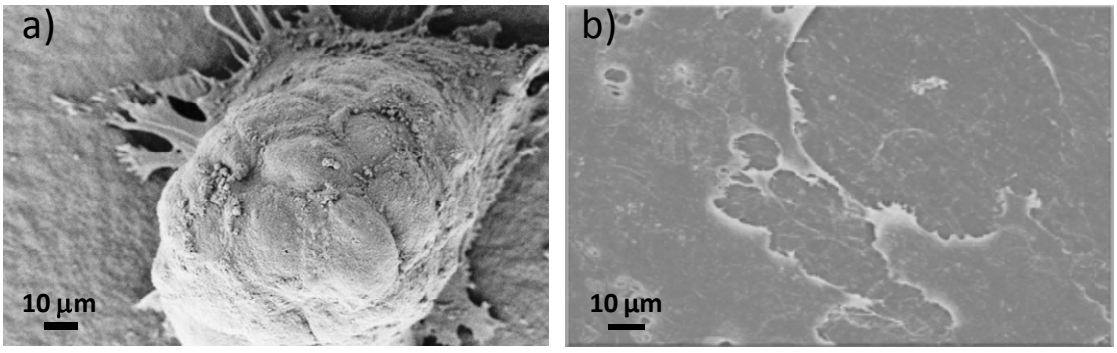

Figure 1. FESEM images of UCMS (a) and BM-MSCs (b) grown on PLLA nanocomposite.

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Chitosan-tannin hybrid nanoparticles as potencial nanomedicine to prevent urinary tract infections

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Oligomeric plant polyphenols (tannins) are present in many foods, botanicals and nutritional supplements. A growing body of research indicates that increased dietary consumption of tannins is associated with a decreased risk of diseases such as coronary artery disease, cancer, urinary tract infections, and ulcers.

Chitosan (a random co-polymer of N-acetyl-D-glucosamine and N-glucosamine) binds to negatively charged tannins by an electrostatic interaction driven by its positively charged amino group. This interaction allows developing stable hybrid nanoparticles via ionotropic gelation with tripolyphosphate (TPP), suitable as a therapeutic controlled release system for urinary tract infections.

We study the effect of chitosan-GSE (grape seed extract) hybrid nanoparticles on the invasion of Caco-2 cells by Uro-pathogenic *Escherichia coli* (UPEC) strain 5011, supplied by the UW-University Hospital, Madison, WI, USA.

When the pathogen was exposed to a chitosan nanoparticle preparation, no disruption of the normal structure of flagella is observed (Fig. 1, Panel B). When the pathogen was exposed to the tannin-chitosan hybrid nanoparticles, extensive coating and cross-linking of flagella on multiple cells is seen (Fig. 1, Panel C). It was also noted that this interaction created numerous aggregates of UPEC. Figure 1 (panel D) is a SEM micrograph of the tannin-chitosan hybrid nanoparticles alone. These results indicate that the GSE tannin-chitosan hybrid nanoparticles physically coat the flagella of UPEC, which in turn prevents invasion of the intestinal epithelial cell.

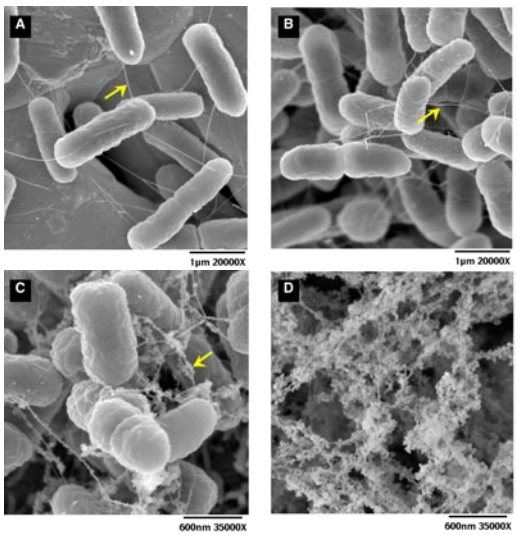

Figure 1. SEM micrograph exploring the interactions of GSE-chitosan composite nanoparticles with UPEC 5011 and its effect on cell invasion.

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Liposome-encapsulated plant alkaloid voacamine improves the efficacy of chemotherapy on osteosarcoma resistant cells

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Cancer chemotherapy is often effective only on drug sensitive tumor cells, leaving unaltered a high portion of drug resistant cells. Consequently, patients with osteosarcoma that do not respond to therapy for drug resistance induction, have metastatic diseases and a poor diagnosis. For these reason, it is very urgent to find efficacy therapeutic combinations with few side effects. In vitro studies demonstrated that voacamine (VOA), a bisindolic alkaloid isolated from *Peschiera fuchsifoliae* plant, enhanced cytotoxic effect of doxorubicin (DOX) on drug resistant tumor cells, such as osteosarcoma and melanoma.[1,2] The chemosensitizing effect of VOA on osteosarcoma cells was due to the P-gp inhibition, the main efflux pump responsible of drug resistance.[3] Moreover, the dose of VOA used to sensitize tumor cells to DOX was not toxic to normal cells.[2] These properties make VOA an attractive candidate for the development of targeting liposomal formulations more biologically effective.
The cationic liposomes VOA may increase the biomedical applications favoring its solubility in water, increasing accumulation in target tissues, and reducing its accumulation in healthy tissues. We included VOA into liposomes composed of DOPC (1,2-dioleoyl-sn-glycero-3-phosphocholine), gemini surfactant, and cholesterol by a remote loading technique. Preliminary flow cytometric analysis using liposomes labeled with fluorescent probe (NBD) clarified the best conditions of treatment (concentration and exposure time) on osteosarcoma resistant cells (U-2/OS DX). Trypan blue uptake assay by flow cytometry indicated that these formulations induced transient plasma membrane permeabilization. Then we evaluated the chemosensitizing effect of VOA-loaded in liposomes against VOA-free on U-2/OS DX cells. DOX intracellular uptake, assessed by flow cytometry, was higher in liposomes-VOA than free-VOA treated cells. MTT assay showed a growth inhibition of about 10% in cells pre-treated with VOA loaded into liposomes than VOA-free. Optical microscopic evaluations, 24 h after recovery in drug free medium, showed that osteosarcoma cells pre-treated with liposomes-VOA before DOX treatment were dead compared to cells treated with free-VOA and DOX. UIC2 flow cytometry shift assay showed that liposomes do not alter the functionality of P-gp, and that VOA loaded into liposomes exerts the inhibitory effect against P-gp as the free VOA.
These results showed that the encapsulation of the VOA into liposomes improved its delivery in resistant tumor cells and its chemosensitizing efficacy. Further studies will clarify if there are different molecular targets involved in VOA loaded liposomes and VOA free.

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Collagen-based Scaffolds loaded with nanoparticles

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During the last decade the employment of collagen-based scaffolds in the biomaterials' field has been intensively growing. Collagen is well-known in tissue engineering for its excellent biocompatibility, low antigenicity and capacity to regenerate different tissues: bone and cartilage, , skin, and peripheral nerves. Peripheral nerve and spinal cord regeneration are very important topics in regenerative medicine. Conventional tissue engineering strategies utilize acellular biodegradable scaffolds combined with the systemic administration of bioactive molecules [1] (i.e. neurotrophic factors) to promote bio-mimicking processes of tissue regeneration. However, this approach has several limitations [2]. One of the most common methods to achieve controlled and localized release of biomolecules is to incorporate them within biomaterials during the scaffold's fabrication [3]. According to this approach, the scaffold degradation affects the biomolecule release rate over a prolonged time period. In this study collagen-based scaffolds showing an oriented porous structure, suitable for peripheral nerve regeneration, were embedded with biodegradable polymeric nanoparticles loaded with fluorescent active biomolecules. Confocal laser scanning microscopy (CLSM) analysis allowed to assess the degradation of the nanoparticles in physiological conditions and the release kinetic of biomolecules from the scaffold.

Scaffold synthesis. An aqueous collagen suspension was prepared and added with the nanoparticles. Several concentrations of nanoparticles were tested. The final suspensions were cast into tubes and processed by means of uniaxial freezing and freeze-drying, in order to obtain porous collagen-based scaffolds with longitudinally oriented pores. EDAC crosslinking method was also performed to preserve the structure of the collagen matrix. ***Characterization experiments.*** The resistance to thermal degradation was evaluated *in vitro*, by incubating the scaffolds in PBS at 37°C. At different time points the scaffolds were analysed by means of CLSM.

CLSM allowed to assess the distribution of the nanoparticles loaded with a fluorescent antibody inside the collagen scaffolds by detecting the fluorescence signals arising from the fluorophore (FITC). Scans at different heights and at the transversal sections of the scaffolds were collected in order to define the 3D localization of the nanoparticles. The nanoparticles were embedded inside the collagen matrix and localised to the inner regions of the tubular collagen sheets for up to 30 days. The concentration of nanoparticles seemed to affect their distribution within the scaffold structure: at lower concentrations, they were homogeneously distributed within the collagen matrix, whereas at higher concentrations the nanoparticles formed large clusters, likely due to strong interparticle interactions. This in turn influenced the degradation time of the nanoparticles. Indeed, when using high nanoparticle concentration, their degradation was slowed and the fluorescent signal was still well visible in the scaffold after 30 days. Another critical parameter is the capacity of the scaffolds to maintain the oriented porous structure. We observed that after two weeks in PBS collagen scaffolds started to degrade, but still maintained their lamellar-like structure after 30 days.

The experimental results from the current study show that accurate control of the crosslinking treatment was essential to preserve the structure of collagen. Further, the degradation kinetic of the nanoparticles seems to be in the optimal time window to have complete regeneration in peripheral nerves [4].

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Zoledronic acid encapsulated in self-assembly pegylated nanoparticles in combination with radiotherapy on glioblastoma cell lines.

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Glioblastomas are the most common and aggressive brain tumors in adults. The treatment of central nervous system tumors is limited by the presence of blood–brain barrier (BBB), which commonly prevents many drugs or infectious agents from entering the brain (1). Currently the chemotherapy drug used is temozolomide (TMZ) in combination with radiotherapy but glioblastoma cells develop resistance to this drug.
Zoledronic acid (ZOL) is a drug whose potent anticancer activity is limited by its short half-life and accumulation within bone (2). On these bases, there is a need to develop a delivery system to avoid ZOL accumulation into the bone, thus improving extra-skeletal bioavailability. Nanotechnology utilized to enhance drug activity consists in PEGylated nanoparticles (NPs), self-assembling, obtained by mixing an aqueous solution of calcium phosphate with ZOL (CaPZ NP) and cationic liposomes (3). The functionalization of these nanoparticles with transferrin (Tf) could allow their crossing tight endothelial cells of BBB by internalization transferrin receptor (TfR) –mediated.
The encapsulation of ZOL in Tf functionalized NPs (Tf-NPs) caused higher *in vitro* cytotoxic activity than free ZOL on LN-229, U-373 MG and in U-87 MG glioblastoma cell lines. Same strengthening of Tf-NPs-ZOL was obtained in in mice intramuscularly bearing glioblastoma tumors.
Moreover, we performed experiments with TMZ, a gold standard for the treatment of glioblastomas and the three cell lines showed resistance to drug because the half maximal inhibitory concentrations (IC50) were very high (95 μM for U-87 MG, 110 μM for U-373 MG and 176 μM for LN-229 cells).
On these bases, we used radiotherapy in combination with Tf-NPs to overcome resistance to TMZ and to potentiate ZOL activity.
We evaluated the effects of different radiotherapy dosages with concomitant treatment of functionalized NPs-ZOL or free ZOL on growth inhibition of glioblastoma cell lines by MTT assay. Preliminary results showed that drug activity was increased by radiotherapy. Moreover, the growth inhibition induced by radiation therapy alone did not reach 50% even at higher dosages (12 Gy).
In conclusion, the use of nanoparticles represents a new strategy for the delivery of drugs and these experiments lay the foundation for a more efficient cancer therapy.

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Functional dissection of the role of *hard* corona proteins in the interaction of SiO_2-NPs with cells in human plasma

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Multiple layers of host proteins on nanoparticles (NPs) influence the bio-activity of nanotheranostics. Some proteins stably bind to NPs (*hard corona)*, while others interact reversibly (*soft corona)*. We characterized the human plasma (HP) proteins bound to amorphous SiO_2-NPs (Ø 26 nm), and studied their effect on the NPs interaction with human lymphocytes, monocytes and macrophages. Histidine Rich Glycoprotein (HRG), a plasma 64 KDa protein having a characteristic Histidine Rich Region (HRR), is a major component of the SiO_2-NPs hard corona in excess HP, together with minor amounts of the HRG homologous High Molecular Weight Kininogen (HMWK). HRG competes with other HP proteins for the NPs surface so inhibiting their uptake by macrophages. In lymphocytes and monocytes several plasma proteins can interchange in this inhibitory activity. Upon depletion of HRG from HP, a heterogeneous hard corona forms including fibrinogen, HDLs, LDL and VLDL , IgG, Human serum albumin and complement H factor. HRG is the main component of the HP-derived SiO_2-NPs hard corona (Figure 1), uniquely conferring to these particles the ability to evade macrophages capture.

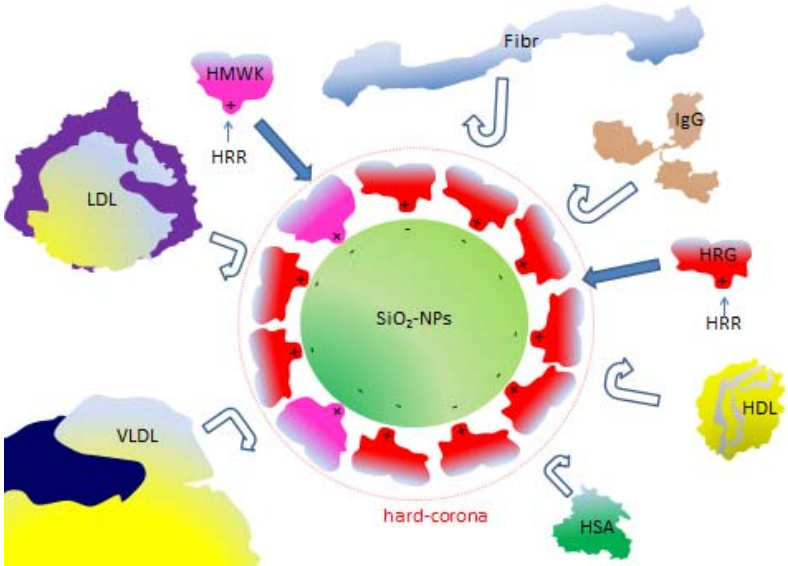

Figure 1. Scheme of hard corona coated SiO_2-NPs in human plasma

Transferrin-conjugated self-assembled nanoparticles incorporating ZOL as a tool for the targeting of glioblastoma

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Glioblastomas are highly aggressive brain tumors of adults with poor clinical outcome. The blood–brain barrier (BBB) is the most important limiting factor for the development of new drugs and drug delivery for the central nervous system (CNS). Different methods have been used to facilitate the drug transportation into the brain (1). Here, we propose a new strategy to treat glioblastoma based on self-assembling nanoparticles (NPs) encapsulating Zoledronic Acid (ZOL) (2). In order to improve the internalization rate of ZOL into the brain tumor, these NPs have been functionalized with transferrin (Tf), maintaining the possibility to prepare them before use by self-assembling procedure. NPs-ZOL-Tf have been assessed on the glioblastoma cell line U373MG-LUC that showed a refractoriness *in vitro* to anti-cancer agents conventionally used for the clinical treatment of glioblastoma, namely temozolomide (TMZ) and fotemustine (FTM). NPs-ZOL-Tf treatment resulted in higher *in vitro* cytotoxic activity than free ZOL. However, the potentiation of anti-proliferative activity of NPs-ZOL-Tf was superimposable to that one induced by NPs-ZOL (not armed with Tf). On the other hand, NPs-ZOL-Tf showed a higher antitumor efficacy if compared with that one caused by NPs-ZOL in immunosuppressed mice intramuscularly bearing U373MG-LUC xenografts, inducing a significant tumor weight inhibition (TWI) of 41%, a tumor growth delay (T-C) of 10 days and an increase of mice survival (ILS) of 23%. Interestingly, while NPs-ZOL treatment induced a stable disease in 1/6 treated mice, NPs-ZOL-Tf produced a complete tumor regression in 1/6 treated mice. The experiments performed on mice with intracranial U373MG-LUC xenografts confirmed the efficacy of NPs-ZOL-Tf. Indeed, NPs-ZOL administration elicited the stabilization of the disease in 2/8 treated mice and an ILS of 13%, NPs-ZOL-Tf treatment resulted in an improved therapeutic efficacy as 3/8 mice showed a stable disease and, impressively, one mouse displayed a complete tumor response. Moreover, an ILS of 23% was observed. These effects were paralleled by a higher intratumour localization of fluorescently-labeled-NPs-Tf both in intramuscular and intracranial xenografts. In conclusion, our results demonstrate that the encapsulation of ZOL increases the antitumor efficacy of this drug in glioblastoma through the acquisition of ability to cross the BBB, thus suggesting the application of this strategy in clinical setting.

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New nano-carriers based on Graphene Oxide (GO) derivatives.

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In this work, we demonstrated two important aspects of this new nanostructured GO, highlighted below: **1**. The high biocompatibility of GO with human cells[1]. The human endothelial-like immortalized cell line EaHy926, derived from the fusion of human umbilical vein endothelial cells (HUVEC) with the bronchial carcinoma cell line A549, and expressing an endothelial-like phenotype, after "in vitro" exposure for 24hrs to different concentrations of GO (0,2-20μg/ml) did not show any significant morphological or functional modification. The cell viability was not affected. TEM and Confocal Laser Scanning Microscopy (CLSM) analysis demonstrated the uptake of GO into cells and their localization in the cytoplasmic compartment; **2.** GO can be "in vitro" conjugated to several bioactive molecules, such as hormones, cytokines or bio-mediators, so that they can be easily delivered into cells. Thus could be particularly relevant for those agents which normally play their action exclusively through the linking to specific receptors. In this study, we also shown the possibility to manipulate spatially inhomogeneous, non-uniform conductivity patterns across a flake of graphene. In this way, the auto-fluorescence into the total reflectance phenomena for GO, performed by using the Confocal Laser Scanning Microscopy (for "in vitro" and "in vivo" measurements), could be very promising for molecular imaging in Nanomedicine[2].

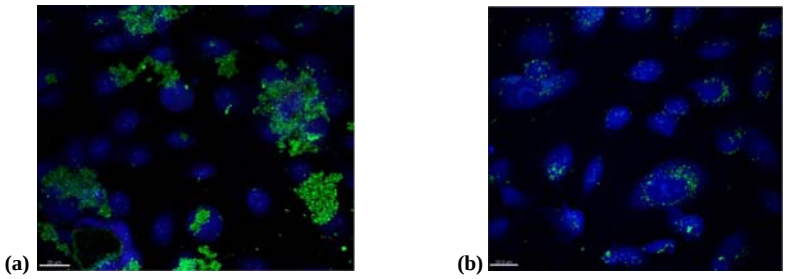

Figure 1. CLSM images of **(a):** Ea.Hy926/SWCNTs (Single-Wall Carbon Nanotubes, as graphene precursors[3]) treated cells; **(b):** Ea.Hy926 /GO.

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Industry Report

Monitoring the stability of a colloid for Magnetic Hyperthermia by Specific Power Absorption measurements

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Magnetic nanoparticles (MNPs) are bringing novel and promising ways to treat deadly diseases such as cancer. They have multiple applications that range from magnetic hyperthermia, localized drug delivery and release, to tissue engineering and new materials. Magnetic nanoparticles can be heated when they are exposed to alternating magnetic fields (AMF) due their properties. Iron oxides nanoparticles (Fe_2O_3/Fe_3O_4) are currently one of the magnetic materials most used as heating agents. The physical properties of the core material and chemical properties of the coating surface are the key parameters for successful biological applications. Colloidal stability against precipitation and agglomeration of the constituent nanoparticles is a key factor to keep the heating efficiency of the ferrofluid in the long term. Within the scope of the ongoing collaboration between nanoScale Biomagnetic (nB) and the Instituto de Nanociencia de Aragón (INA) we have developed a highly stable water-based colloid composed of PEI-coated Fe_3O_4 magnetic nanoparticles that is commercially known as Magno, which specific goal is to serve as a basic elementary supply for many magnetic heating procedures, including in vitro and in vivo experiments. We have exploited and studied Magno's physical and chemical properties to track the evolution of the heating efficacy of the particle, particularly measuring the specific power absorption (SPA) along one year using a DM1 device, delivering an AMF up to 24 kA/m and frequencies $260 < f < 830$ kHz. We demonstrated that the SPA parameter is highly sensitive to minute changes in the colloidal state of the samples calculating the value according several fit methods. This fact is particularly relevant in relation to the biological applications magnetic heating of nanoparticles, like magnetic hyperthermia or magnetically induced drug release due to the known fact of that MNPs tend to form aggregates once they penetrated the cell membrane and remain inside the cell.

Nanoparticle-based approaches to push the limits of Surface Plasmon Resonance imaging for the detection of diagnostic biomarkers

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Diagnostic biomarkers for neurological disorders, cardiovascular diseases and cancer are often in low abundance in bodily fluids, presenting many challenges for their detection.
Surface Plasmon Resonance imaging (SPRi) is a label-free optical technology used to monitor biomolecular interactions in real-time and in a multiplex format. It is thus possible to detect specifically and simultaneously multiple molecules from the same sample. SPRi is compatible with the analysis of complex solutions such as bodily fluids which makes it an ideal technique for the development of fast diagnostic tests.
Nanoparticle-based sandwich assays create a mass loading effect on the SPRi sensor chip surface, generating a significant enhancement of the SPRi signal. This strategy was used to push the limits of SPRi for the detection of ultra-low levels of ssDNA (down to 100 fM) and C-reactive protein in human serum (down to 43 aM).

ELECTRON MICROSCOPY BRADENS THE HORIZONS OF TOXICOLOGY: The role of nanoparticles vehiculated by bacteria

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This communication shows the ways in which nanoparticles, originated by the biodestruction of plastic materials carried out by microbes, are able to access human organs by means of microorganism acting as facilitators. The aim of this work is to highlight how electron microscopy is a fundamental and versatile technique of high value in the investigation of the interaction between bacteria and polymeric materials.

Assessment of the efficiency of encapsulation of a fluorescent drug using Nanoparticle Tracking Analysis

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The use of nanoparticles in drug delivery continues to grow rapidly. Nanoparticles offer excellent pharmacokinetic properties, controlled and sustained release, and targeting of specific cells, tissues or organs. When considering a nanomaterial drug delivery system, size is clearly a key parameter as it directly influences the processes of delivery, uptake, degradation and clearance from the body.
Complementary to classical light scattering techniques, NTA (Nanoparticle Tracking Analysis) allows nanoparticles to be sized on a particle-by-particle basis, enabling high resolution profile. On the same analysis, NTA also delivers concentration measurement through a direct count (particles per ml), helping the understanding of aggregation or other particle behaviour in complex systems. Finally, a fluorescence mode allows differentiation of suitably labelled particles.

Posters

Inclusion of Voacamine in cationic liposomes by active loading

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Voacamine, a bisindolic alkaloid extracted from Peschiera fuchsiaefolia, displays several pharmacological properties, such as cardiotonic effect, antimalarial activity and enhancement of the cytotoxic effect of doxorubicin (DOX) on multidrug resistant tumour cells [1].

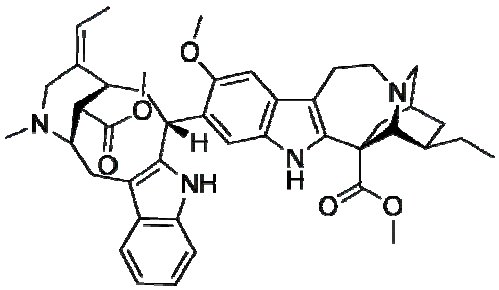
The inclusion of VOA in liposome formulations could increase its potential application by i) rendering it soluble in water, ii) controlling its pharmacokinetics and biodistribution, iii) increasing its accumulation in target tissues and iv) avoiding or reducing its accumulation in healthy tissues, thus reducing toxic effects.

Actually the two tertiary amine groups of VOA could be exploited to load it in the internal aqueous compartment of liposomes by a remote loading technique.

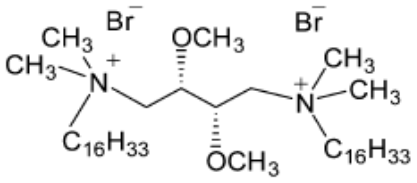
Here we report the inclusion of VOA into gemini based cationic liposomes by a remote loading technique where an ammonium sulfate gradient generates a transmembrane pH imbalance necessary a transmembrane pH imbalance necessary to trigger loading of VOA [2].

The gemini surfactant **1**, was chosen as cationic component of liposomes, due to the fact that it is characterized by low toxicity and was shown able to attribute to phospholipid formulations high delivery efficacy [3].

VOA loaded in liposomes composed of DOPC (1,2-dioleoyl-sn-glycero-3-phosphocholine), gemini and cholesterol (in a 1.6:0.4:1 ratio) with high efficacy was evaluated against free VOA on an osteosarcoma cell line.



Voacamine



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Cationic SLN for siRNA and DNA plasmid delivery in hepatocellular carcinoma

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Hepatocellular carcinoma (HCC) is the sixth most common cancer worldwide and the third leading cause of cancer-related deaths. For the treatment of HCC several drugs are under development, but the only one with proven survival benefit is sorafenib. This agent is a multikinase inhibitor that blocks Raf signaling and VEGF, PDGF and c-Kit. It has antiproliferative and antiangiogenic activity and delays tumor progression [1,2]. Moreover, systemic tumor-targeted gene delivery is attracting increasing attention as a promising alternative to conventional therapeutic strategies. At this purpose a large number of viral and non-viral vectors have been studied and applied as systems of stable transfection with low toxicity. Although cationic polymers and liposome are promising systems, solid lipid nanoparticles (SLN) have been recently proved to be a really useful vehicle for gene therapy [3,4]. The aim of this work was to design and to obtain cationic SLNs containing sorafenib capable of forming complexes with siRNA and DNA plasmid for the treatment of HCC, in order to combine the effects of drug and nucleic acids.
The physical binding between cSLN and nucleic acids was confirmed by the study of complexes' zeta potential values that became more positive as higher was the amount of cSLN and via the electrophoretic mobility of the samples in agarose gel 0.8%. Transfection studies on different tumor cell line are in progress.

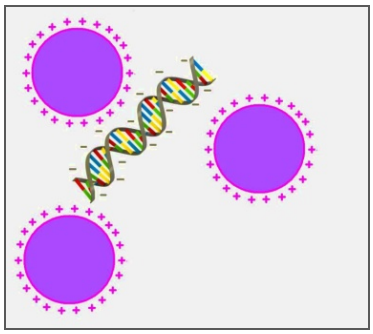

Figure 1. Cationic SLN-DNA interaction.

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In vivo activity of new dressing materials for wound healing

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Skin wound healing is a complex and highly-regulated sequential biological process, which is temporally divided into three distinct phases: early acute exudative, fibro-proliferative, and tissue remodeling. A variety of cytokines, growth factors, and chemokines are known to be involved in different aspects of the skin wound healing process.
In the present work we investigated a single active wound dressing model composed by a water soluble chitosan derivate, N-methacrylate glycol chitosan (MGC), cellulose fibers as support, and a water soluble drug, 2% eosin. The samples impregnated with the antibacterial agent were tested in a wound healing animal model, measuring the activity of the pro-inflammatory cytokine IL-6 in the early acute exudative phase.
Mice (male C57BL/J6, gr. 22-24) were anesthetized with isoflurane 1.5–2% from a nose cone. After shaving the dorsal hair and cleaning of the exposed skin, one full-thickness (including the *Panniculus carnosus*) excisional wound was punched in the middle of the dorsum (Gerharz et al., 2007).
The wound induced a significant increase of IL-6 levels 6 hours after its induction. When the wound area was covered with the polymer impregnated with eosin, IL-6 levels were significantly reduced by at least 50%, suggesting a potent anti-inflammatory action of the wound dressing.
Our preliminary data demonstrate that the polymer's application is safe and biocompatible for the animal skin and it is able to release eosin within the first 6 hours after the wound induction. Furthermore, both polymer and eosin reduce IL-6 expression in this mouse model of punch wound. Further studies are ongoing to better clarify the combined action of polymer plus eosin in the wound healing model.

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A molecular dynamic simulation to identify the physicochemical parameters that might control the cell internalization pathway of liposome drug carriers.

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The main objective in the development of new liposomes as drug delivery systems is obtaining a control over their biodistribution, thus achieving selective and sufficiently high localization of their cargo at disease sites such as tumors and inflamed tissues. A complete understanding of the liposome parameters that might control internalization and intracellular trafficking pathways is fundamental to enable optimal nanomedicine design.
It was reported that the cell uptake and the intracellular distribution of a photosensitizer delivered by liposomes composed of 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine, DMPC, and one of two stereoisomeric cationic gemini amphiphiles, **1a** and **1b** is controlled by the stereochemistry of the gemini component **1**.[1]
Herein we report on the molecular dynamic simulation of DMPC/**1a** and DMPC/**1b** lipid bilayers aimed at giving an atomistic description of the molecular organization in the two diastereomeric lipid bilayers. The results obtained show that the different orientation of polar groups at the surface of lipid bilayers controls the organization and the mobility of the water bound to the lipid systems, parameters that could indeed influence the interaction with biological systems.

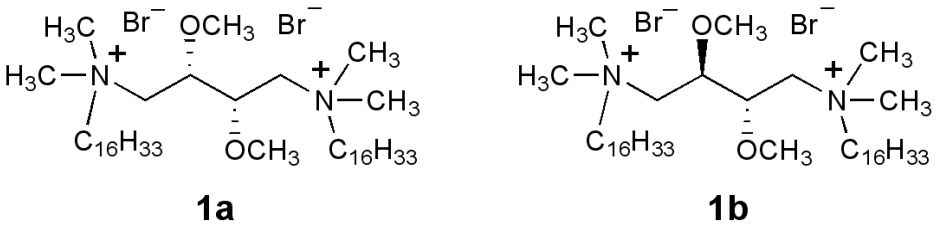

Figure 1. Diasteromeric gemini amphiphiles.

1. C. Bombelli, A. Stringaro, S. Borocci, G. Bozzuto, M. Colone, L. Giansanti, R. Sgambato, L. Toccaceli, G. Mancini, A. Molinari *Mol. Pharm.* **2010**, *7*, 130.

Stereochemistry of the gemini surfactant influences the final fate of cationic liposomes in human tumor cells

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We have developed cationic liposomes, formulated with 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine, DMPC, and one of two stereoisomeric cationic gemini amphiphiles, **1a** and **1b**, to deliver a photosensitizer to cancer cells. The stereochemistry of the gemini component was shown to affect cell uptake of the photosensitizer, as well as its intracellular distribution.[1]
Herein we report a detailed biological investigation aimed at elucidating the pathways of internalization and the intracellular trafficking of DMPC/**1a** and DMPC/**1b** liposome formulations. The study was carried out on human and murine glioblastoma cells by using inhibitor assay along with flow cytometry and laser scanning confocal microscopy (LSCM). Transmission electron microscopy (TEM) observations on ultrathin sectioned samples and on PT-C replicas by freeze-fracturing contributed to elucidate at higher resolution the intracellular trafficking of the two liposome formulations.
The results obtained demonstrated that the stereochemistry of the gemini component controls the subcellular fate of the two liposome formulations. Actually, after binding to the plasma membrane, DMPC/**1a** liposomes are internalized trough caveolae-coated vesicles to be preferentially transferred to early endosomes. On the other hand, clathrin-mediated endocytosis of DMPC/**1b** leads to the formation of early endosomes, which are acidified and fuses with prelysosomal vesicles to give rise to late endosomes and, finally, to lysosomes.

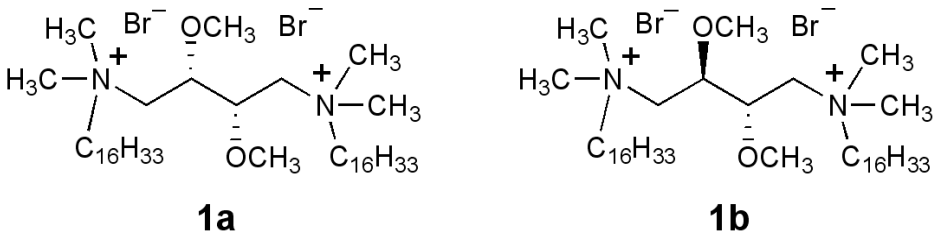

Figure 1. Diasteromeric gemini amphiphiles used in the liposome formulations.

1. C. Bombelli, A. Stringaro, S. Borocci, G. Bozzuto, M. Colone, L. Giansanti, R. Sgambato, L. Toccaceli, G. Mancini, A. Molinari *Mol. Pharm.* **2010**, *7*, 130.

Nanocarriers encapsulated with Zoledronic acid and RNA interference: two combined strategies to improve pharmacological treatment of glioblastoma

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INTRODUCTION: Glioblastoma (GBM) is the most common brain cancer in adults. It is also, unfortunately, the most aggressive type, characterized by rapid progression and poor prognosis, and the least responsive to therapy

Protein isoaspartyl carboxyl *O*-methyltransferase (PCMT1; EC 2.1.1.77) is a *S*-adenosylmethionine-dependent methyltransferase, which specifically recognizes and methyl esterifies the free α-carboxyl groups of isoaspartyl residues, arising from asparaginyl deamidation. The tissue distribution of PCMT1 is ubiquitous, although maximal expression has been reported to occur in brain and blood tissues. This enzyme promotes the conversion of the abnormal L-isoAsp residue into L-aspartyl, thus "repairing" the isopeptide bond and preventing the accumulation of conformationally altered, dysfunctional proteins.

Our previous work demonstrated the antiapoptotic action of PCMT1 and proposed a mechanism of action, based on the ability of this methyltransferase to maintain the structural stability of some crucial antiapoptotic proteins, including Bcl_{XL} (1). Recently we demonstrated that PCMT1 silencing induces increased susceptibility to apoptosis in an hepatocarcinoma cell line (2).

Zoledronic acid (ZOL) is a potent amino-bisphosphonate used for the treatment of bone metastases with recently reported antitumor activity. One of the most important limits of ZOL is the pharmacokinetic profile. In fact, ZOL is rapidly eliminated from plasma upon intravenous administration, due to renal excretion and rapid uptake and accumulation within bone (about 55% of the administered dose) (3). In the light of these considerations, a new formulation able to change ZOL pharmacokinetic and pharmacodistribution, inducing a lower drug accumulation into the bone and a longer half-life into the blood, would be of great potential usefulness to take advantage of the ZOL anti-apoptotic and anti-proliferative effect in peripheral tumours. Nanotechnologies offer a very powerful tool to improve pharmacokinetic profile of drugs, we designed a new delivery system for BPs, consisting in new self-assembly PEGylated nanoparticles (NPs) based on calcium/phosphate NPs and cationic liposomes (4). The new self-assembling method developed in our lab assured a ZOL encapsulation efficiency of about 66%, about 12-fold greater than that obtained with LIPO-ZOL.

AIM: In this work we analyzed the correlation between PCMT1 expression and activity and the grading of GBM in several cell line. We explored the rationale for the use of PCMT1 silencing, as a means to increase susceptibility of GBM cells to apoptosis, combined with treatment with free ZOL and encapsulated ZOL.

RESULTS: PCMT1 expression rate and subcellular distribution in various GBM cell line reflects grade and malignacy of originating tumor. PCMT1 silencing improves sensitivity to chemotherapic drugs through enhanced susceptibility to apoptosis. Particularly we found that PCMT1-silenced GBM cell lines showed enhanced sensitivity to the apoptosis induced by several drugs, such as doxorubicin and themozolomide. PCMT1-silenced GBM cell lines treated with ZOL vehicolated in nanoparticles showed an increased susceptibility to apoptsis which is faster and stronger than the cells treated with free ZOL,

Nano-sized systems for medical imaging

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It is well known that passive accumulation of nano-sized systems in tumor and inflamed tissue is due to the enhanced permeability and retention (EPR) effect. Nano-sized systems with different size and shape can efficiently target tissues having aberrant microvasculature characterized by an enhanced blood vessels permeability. In order to accumulate the nano-sized systems in tumor tissues by passive targeting it is mandatory to improve their blood half-life and in vivo stability by means of a suitable stealth coating. However the physio-pathological state of the tumor can significantly change the amount and the tissue distribution of a nano-sized system. Our research project is aimed to characterize the EPR effect using in vivo imaging modalities over several tumor-bearing mice models. In particular we would like to understand how the dimensions affect extravasation, the retention time and the depth of permeation in the tumor. To this end we will use imaging modalities like Optical Imaging (OI) and Photo-Acoustic Imaging (PAI). OI that exploits invisible near-infrared (NIR) fluorescent light (700–900 nm) has the potential to improve cancer surgery outcomes, minimize the time patients are under anaesthesia and lower health-care costs largely by way of its improved contrast and depth of tissue penetration relative to visible light. PAI has been developed extensively over the last decade. Possessing many attractive characteristics such as the use of nonionizing electromagnetic waves, good resolution and contrast, portable instrumentation, and the ability to partially quantitate the signal.
In order to consider two nano-sized systems in a quite large range of size we chose albumin and Solid Lipid Nanoparticles (SLNs).Albumin was chosen because is the most abundant plasma protein and it may be simply tagged both by a fluorescent moiety using a covalent labelling, and by non covalent labelling using small fluorescent dyes having a high affinity for albumin. We also developed multi-modal SLNs loaded with a amphipilic Gd(III) complex for Magnetic Resonance Imaging and Indocyanine Green (ICG) or a cyanine dye like Cy5.5 to enable OI or PAI. These nanoparticles offer the advantage of higher stabilities with respect to nanoemulsions or liposomes due to the presence of a solid core in their inner structure. The physicochemical properties of different batches of SLNs were investigated in term of particle size distribution, zeta-potential, DSC and fluorescent properties such as quantum yield and photostability. First in vitro Photo-Acustic experiments with SLNs uploaded with ICG, showed encouraging results, while some preliminary in vivo experiments were done with multi-modal SLNs in tumor bearing mice characterizing the nanoparticles bio-distribution and the tumor targeting properties. In conclusion, we developed two nano-sized systems to get an overview on passive accumulation in tumor by NIR fluorescent imaging and photoacoustic techniques. This knowledge could be important in the future to develop imaging tools enabling the stratification of patients and tumors eligible for therapies based on macromolecular or nano-sized drugs.

The use of novel PLGA-g-PVP amphiphilic copolymers for fabrication of nanostructured materials.

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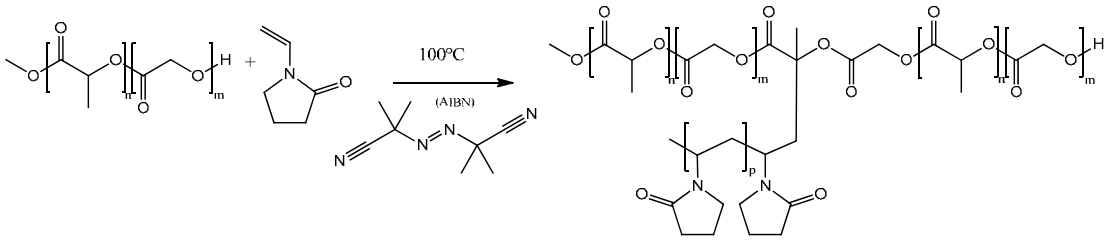
The aim of this work is to present a one-pot synthetic process leading to poly(lactide-co-glycolide)-g-poly(vinylpyrrolidone) (PLGA-g-PVP) copolymers consisting of high molecular weight PLGA carrying oligomeric PVP side chains. The title copolymers were prepared by radical polymerization of N-vinylpyrrolidone in the presence of 50:50 PLGA acting as polymeric chain transfer agent in the absence of solvents. All copolymers were characterized by ^{1}H-NMR (400 MHz), FT-IR, SEC, MALDI-TOF, DSC, TGA and DLS.



Scheme 1. Synthesis of poly(lactide-co-glycolide)-g-poly(vinylpyrrolidone) (PLGA-g-PVP) copolymers. Multiple PVP grafts are present on a single PLGA chain.

PLGA is a lipophilic biodegradable polymer, whereas PVP is hydrophilic, biocompatible and also bio-eliminable for molecular weights < 40.000.[1,2] Both polymers have been approved for human use by the U.S. Food and Drug Administration, therefore the PLGA-g-PVP copolymers are eligible for medical applications. The water-soluble PVP portion imparts amphiphilicity to the otherwise hydrophobic PLGA, thus modifying its behavior in aqueous systems. In particular, PLGA-g-PVP samples spontaneously formed nanoparticles when dispersed in water. These nanoparticles, besides dissolving hydrophobic drugs, for instance antimalarial drugs, in the inner core, show higher compatibility than native PLGA towards many drugs known to interact with PVP. In addition, PLGA-g-PVP samples were co-extruded with PLGA to give nanofibrous meshes with dramatically improved wettability (Figure 1). These nanofibers can be used in applications involving contact with the body fluids.

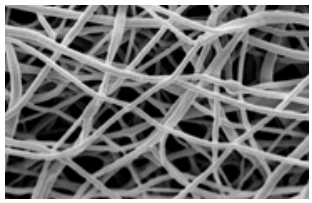


Figure 1. Electrospun nanofibers of PLGA with a shell of PLGA-g-PVP copolymer

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Artificial neural networks applied to biodegradable nanoparticles for Noscapine delivery

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Biopolymer based nanocarriers have been extensively studied as delivery systems for different therapeutics, due to their multiple favorable properties - e.g. small size, long circulation time, reduced opsonization, drug stability and controlled release [1-2].
In this study a series of biodegradable block copolymers based on polyethylene glycol (PEG) and poly lactide (PLA) were synthesized by ring opening polymerization (ROP).
Successively, biocompatible PLA nanoparticles loading Noscapine were prepared using the nanoprecipitation method and characterized for their size, drug entrapment efficiency and morphology.
Noscapine, an alkaloid derived from opium, has been widely used as antitussive agent for many years. However, more recently, it was discovered that it owns a tubulin binding activity. It affects the dynamics of microtubules resulting in arresting the metaphase of cell cycle which eventually leads to apoptosis of dividing cancer cells [3].
Artificial neural networks (ANNs) [4] were applied to predict particle size and Noscapine entrapment efficiency within the formed nanoparticles using different factors - i.e. copolymer molecular weight, ratio of polymer to drug and the number of blocks present in the copolymer backbone.
Using these networks it was found that the copolymer molecular weight has the greatest effect on the nanoparticle size distribution. On the other hand, polymer to drug ratio was found to be the most influential factor on drug entrapment efficiency.
ANNs may have a great impact on the design of PEG/PLA based copolymers, and they can be used to customize the formulations of nanoparticles that can fit required targets.

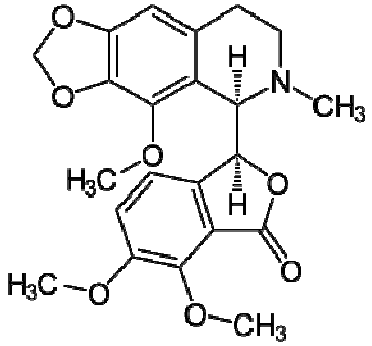

Figure 1. Noscapine chemical structure.

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Novel mitochondriotropic liposomes formulated with an alkylphosphonium bolaamphipile.

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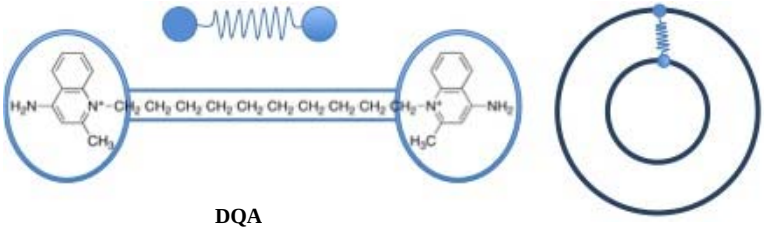
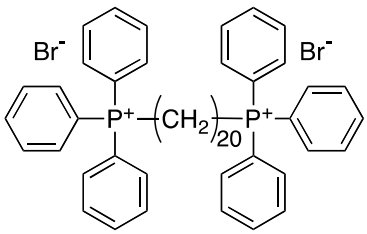
Mitochondrial delivery of drugs is a key issue for the treatment of several diseases related to oxidative stress, but it is hampered by the peculiar structure of the two mitochondrial membranes. An efficient strategy for the delivery of active compounds to mitochondria exploits liposomes composed by a cationic natural Bolaamphipile, DQA, produced by Plasmodium Falciparum.[1] Because of the extended delocalization of the positive charge on the headgroups, this Bolaamphipile can easily cross the mitochondrial membranes guided by the negative potential of the inner membrane. Following this strategy, we decided to synthesize and characterize the novel Bolaamphipile **1** featuring triphenylphosphonium headgroups, aiming at formulating mitochondriotropic liposomes.
The aggregation properties of **1** were investigated by conductivity measurements, Dynamic Laser Light Scattering (DLS), and Transmission Electron Microscopy (TEM); mixed liposomes formulated with **1** and a natural phospholipid, 1,2-dioleoyl-*sn*-glycero-3-phosphocholine (DOPC) or 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC), were characterized by DLS and the toxicity of some formulations was assessed on a murine skeletal muscle cell line.



DQA **DQA-some**



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Acknowledgments: CB, VM, SS are grateful for the financial support from FIRB 2012 RBFR12BUMH.

Antitumor activity of aloe-emodin in breast cancer cells

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Aloe emodin (AE), a natural hydroxyanthraquinone from Aloe vera leaves, is reported to have cytotoxic activity against various cancer cell lines (1). Interestingly, AE showed an affinity for human breast cancer. With the aim of developing novel anticancer drugs characterized by selective targeting and low toxicity for normal dividing cells, we have devoted our attention to a number of natural compounds that have traditionally been used to treat a variety of diseases. We have assayed only those natural compounds showing no toxicity to normal cells, and we evaluated their efficacy against cancer cells.

Our recent study clearly demonstrated the *in vitro* anti-proliferative effect of AE on the breast adenocarcinoma cell line SKBR3. Cell viability assay (MTT test) showed that AE inhibited SKBR3 cell proliferation in a concentration- and time-dependent manner. Moreover, cell cycle was analyzed by flow cytometry to elucidate the mechanism of AE-induced cell growth inhibition. Cell cycle modifications and alteration of its regulatory proteins are frequently associated with induction of apoptosis. Cancer cells were incubated with or without different AE concentrations for 24, 48 and 72 h. To determine whether AE activated apoptosis in SKBR3 cells, morphological and biochemical parameters were evaluated. Höechst staining and flow cytometric analysis of annexin V-FITC-positive cells showed that AE treatment induced apoptotic death in SKBR3 cancer cells. The morphological alterations were evaluated by scanning electron microscopy (SEM), (Figure 1). Studies are in progress to evaluate different drug delivery systems (cationic liposomes, lysozyme-shelled hollow nano/microbubbles and nano/microcapsules) in order to increase antitumor AE efficacy on breast cancer, as model of solid tumor.

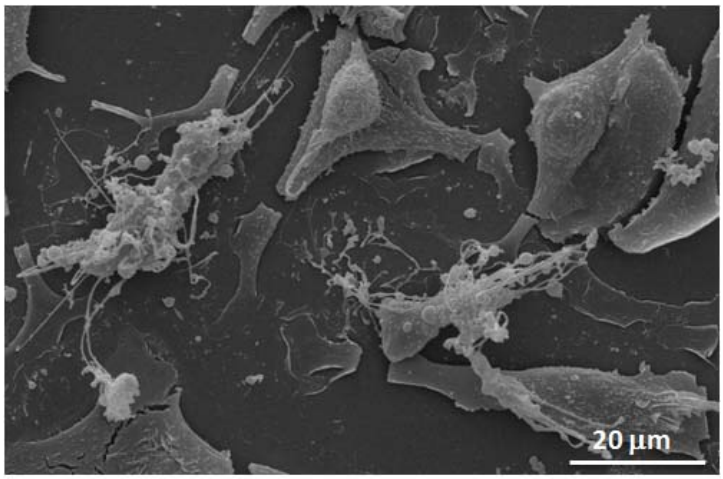

Figure 1. SKBR3 cells showed evident morphological alterations induced by AE treatment.

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Chitosan-Tannin hybrid 3D-scaffolds as potential biomaterials for tissue engineering

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Chitosan has been applied to promote extracellular matrix (ECM) formation in tissue regenerative therapy. The superior tissue compatibility of chitosan may primarily be attributed to its structural similarity to glycosaminoglycan in ECM. Chitosan has been reported to be biocompatible, bio-absorbable and particularly, is considered a good wound-healing accelerator. Dermis and scaffolds made from chitosan exhibit weak antigenicity, biodegradability, and superior biocompatibility (hemostatic and cell-binding properties) by comparison to the synthetic polymers, such as poly(lactic acid) (PLA), poly(glycolic acid) (PGA), and polyethylene terephthalate (PET). As a scaffold, chitosan-based materials in the form of a sponge have been considered the most popular 3D-scaffolds for dermal regeneration. Recently, much attention has been given to utilize tannins (natural polyphenols) as adjuvants in biomedical applications, for example, a wound healing agent, bandage material, skin grafting template, hemostatic agent, hemodialysis membrane and drug delivery vehicle.
We isolated chitosan from native shrimp waste streams and tannins from grape and cranberry waste by-products. Hybrid 3D- scaffold biomaterials were successfully obtained by mixing chitosan with tannins at different molar ratios. Chitosan-tannin hybrid composites were formulated as a 3D sponge-like scaffold, applying previously developed methodologies involving solvent casting and freeze drying. Chitosan-tannin hybrid 3D-scaffolds were characterized according to its water uptake capacity, thermal behavior (DSC) and morphology (SEM).

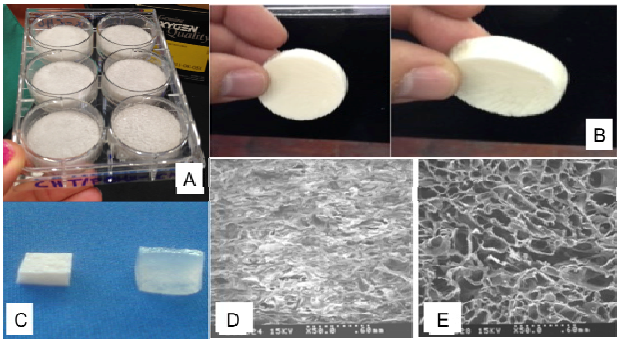

Figure 1. Illustrative picture of Chitosan-tannin hybrid 3D-scaffolds. A- Preparation of hybrid scaffolds. B- Physical appearance of hybrid scaffolds. C- Swelling behavior of hybrid scaffolds. D, E- SEM micrographs of chitosan alone (D) and chitosan-tannin (E) hybrid scaffolds.

Chitosan-tannin hybrid 3D-scaffolds for tissue engineering have been successfully optimized and characterized according to its thermal behavior, water absorption capacity, porosity and morphology. We are currently starting cell growth studies on the scaffolds using model epithelial cells.

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Acknowledgments: Authors are deeply thankful to Prof. Jess D. Reed (University of Wisconsin-Madison, USA) for providing the tannin fractions from grape seed extract and cranberry presscake.

Interaction between functionalized magnetic core-shell nanoparticles and microglia cells

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Magnetic nanoparticles (MNPS) are important contrast agents used as theranostic tools to monitor a wide range of disease processes. In particular, the MNPs have shown a strong tendency to be internalized by phagocytic cells, such as microglia, which constitute the macrophages of the central nervous system (CNS). Microglia are the first line of defense against invading pathogens and are also involved in the recruitment of immune cells from the periphery in areas of pathology. These cells are ubiquitous distributed throughout the CNS and constitute about 10% of the total population of glial cells, with a high motility and, even under the so-called 'rest' conditions, constantly monitor the local microenvironment for endocytosis of nutrients and clearance of cellular debris[1].
We synthesized core shell MNPs (***Fe_3O_4@Cu @ Au***)[2] coated with isolectin B4 from Griffonia simplicifolia as this protein binds with a large affinity the α-D-galactose residues of glycosylated proteins present on the cell membrane of microglia[3], thus allowing a specific interaction between nanoparticles dispersed in the culture medium and cells. Because of this interaction, the microglia showed a rapid and an extensive MNP uptake under basal conditions. Experimental evidence of MNPs internalization has been obtained by collecting AFM, MFM*, SEM and confocal fluorescence microscopy images of the cells, before and after incubation with the nanoparticles activated with isolectina B4.
Taking advantage of the great mobility of the microglia in physiological environment, we hypothesize that the internalization of MNPs may be a new approach to deliver functionalized nanoparticles within the tissue affected by pathology (i.e. infection, cancer).

(*) MFM: magnetic force microscopy

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Mesoporous Biosilica from Thalassiosira weissflogii: diatoms application for Ciprofloxacin Delivery and Bone Cell Adhesion

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Catalysis, separation and sensing extensively exploit mesoporous silica adsorption properties. Micro- and nano-texturing are known to be remarkably useful for biological applications. Frustules, coming from diatom biosilisification, specific and really polymorphic silica shells produced by diatoms, are used in photonics, molecular separation and detection and biosensing [1]. We used frustules from Thalassiosira weissflogii, a pelagic centric diatom from Oceans, for building a chemical model for bio-application. Here we report frustules-based scaffold systems obtained by chemical modification of the outer surface of the diatom shells with a specific anti-oxidant moiety (TEMPO radical trap) via the APTES-method and the resulting material was also used as biosilica "sponge" for drug loading/delivery of ciprofloxacin (antibiotic). We then performed cell adhesion experiments to compare fibroblasts and osteoblasts-like cells proliferation and adhesion attitude on nano-textured diatom "glass" and decorated diatom "glass" with TEMPO-APTES moiety.

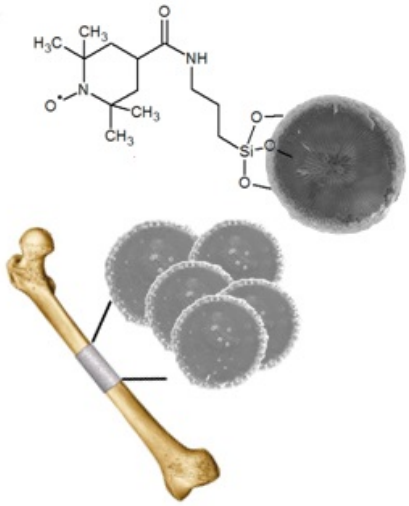

Figure 1. Frustules with specific chemical modification (TEMPO moiety) represent a promising candidate for bone cell adhesion.

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Acknowledgments: This work was financially supported by MIUR (PON 02_00563_3316357 Molecular Nanotechnology for Health and Environment MAAT and PRIN 2011 F81J12000380001 Nanomolecular Technology for Drug delivery NANOMED).

In-depth characterization of cationic nanovector for delivery of small RNA and DNA fragments.

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One of the major challenges of gene therapy is the development of non viral vectors for genetic material, such as plasmids and small interfering nucleic acid, to obtain efficient delivery systems without immunogenic effect respect to viral vectors. Currently, the most promising non viral vectors are cationic self –assembly aggregates, such as liposomes or micelles.[1,2] The molecular properties of complexes obtained by interaction between carriers and nucleic acids are of fundamental importance to obtain efficient transfection agents.

In this contribution we describe the kinetics of oligonucleotide (siRNA[3] or siDNA) complexation with micelles formed by two types of divalent cationic surfactants, *i.e.* three Gemini bis (quaternary ammonium) bromide with variable spacer length (12-3-12, 12-6-12, 12-12-12) and one weak electrolyte surfactant bearing a triazine polar head. The kinetics of complexation were studied by time resolved Small Angle X Ray Scattering performed at the European Synchrotron Radiation Facility (Grenoble, France). All systems contained a fixed amount of surfactant and different concentrations of oligonucleotide at charge ratios (+/-) 0.75 and 1.25. Each experiment consisted on the recording of a total of 80 or 60 curves, collected in three different time groups: first group each 0.15 s, second group each 2-3 s and third group each 40-60 s.

SAXS intensity diagrams were analyzed to investigate the interactions leading to complex formation and the final structure of the aggregates. This analysis allowed us to highlight differences in the kinetics of complexation and in the structure of the nucleic acid/micelles complexes due to the nature of the nucleic acid. The complexes formed by siRNA and siDNA with the micellar aggregates of gemini surfactant 12-6-12 were also studied by molecular dynamics simulations to obtain an atomistic description of the interaction between the nucleic acid molecules and the surfactant aggregates.

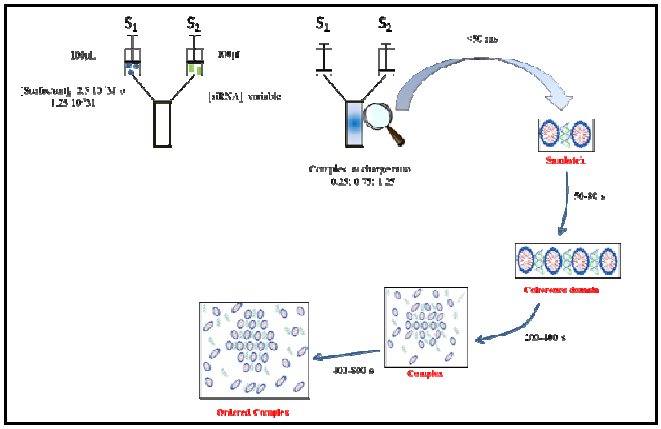

Figure1. Sketch of the main steps involved in complex formation

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Self-assembled Squalene-based Fluorescent Hetero-Nanoparticles

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The recent advances in nanotechnology and nanomaterials have been integrated into analytical chemistry for the design of large numbers of fluorescent chemical and biological probes. In particular, nanoparticles have some advantages because they are much brighter than the single dyes since one particle contains several dyes molecules and their molecular size minimizes physical perturbation of living cells.Our continuous interest in the field of chemical approaches to target cancer cells moved us to study the preparation of a novel class of squalene conjugates with paclitaxel, podophyllotoxin, camptothecin and epothilone A. All of them were characterized by a squalene tail that makes them able to self-assemble in water, and to secure the release inside the cells by a disulfide-containing linker.[1] The need to trace the delivery of the nanoassemblies and to demonstrate the internalization of the drugs pushed us toward the formation of heterogeneous fluorescent nanoassemblies by mixing a paclitaxel-squalene conjugate and fluorescein-squalene conjugate.

Figure 1. Self-assembling of fluorescent hetero-nanoparticles

To prove the capability of nanoassemblies to enter the cell, A549 cells were incubated with suspensions of heterogeneous nanoparticles and we noticed that fluorescent entities accumulate in the perinuclear region. The following application of hetero-nanoparticles is in the combined therapy. Mixing a paclitaxel-squalene conjugate and a cyclopamine-squalene conjugate we were able to obtain again hetero-nanoparticles. The preparation of fluorescent hetero-nanoparticles containing a paclitaxel-squalene conjugate, a cyclopamine-squalene conjugate and tetramethylrhodamine-sqalene conjugate serves to demonstrate the internalization of the nanoassemblies.

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Novel Epigenetic Target Therapy for Prostate Cancer: A Preclinical Study

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Epigenetic events are critical contributors to the pathogenesis of cancer, and targeting epigenetic mechanisms represents a novel strategy in anticancer therapy. Classic demethylating agents, such as 5-Aza-29-deoxycytidine (Decitabine), hold the potential for reprograming somatic cancer cells demonstrating high therapeutic efficacy in haematological malignancies. On the other hand, epigenetic treatment of solid tumours often gives rise to undesired cytotoxic side effects. Appropriate delivery systems able to enrich Decitabine at the site of action and improve its bioavailability would reduce the incidence of toxicity on healthy tissues. In this work we provide preclinical evidences of a safe, versatile and efficient targeted epigenetic therapy to treat hormone sensitive (LNCap) and hormone refractory (DU145) prostate cancers. A novel Decitabine formulation, based on the use of engineered erythrocyte (Erythro-Magneto-Hemagglutinin Virosomes, EMHVs) drug delivery system (DDS) carrying this drug, has been refined. Inside the EMHVs, the drug was shielded from the environment and phosphorylated in its active form. The novel magnetic EMHV DDS, endowed with fusogenic protein, improved the stability of the carried drug and exhibited a high efficiency in confining its delivery at the site of action in vivo by applying an external static magnetic field (1) Here we show that Decitabine loaded into EMHVs induces a significant tumour mass reduction in prostate cancer xenograft models at a concentration, which is seven hundred times lower than the therapeutic dose, suggesting an improved pharmacokinetics/pharmacodynamics of drug. These results are relevant for and discussed in light of developing personalised autologous therapies and innovative clinical approach for the treatment of solid tumours.

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PLoS One. 2011 Feb 23;6(2):e17132.

Remote loading of Aloe-Emodin in gemini based cationic liposomes

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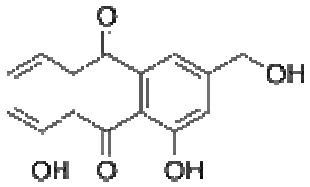
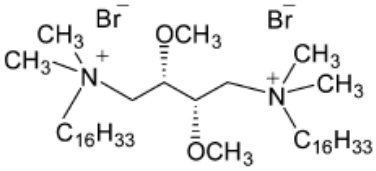
Aloe-Emodin (1,8-Dihydroxy-3-(hydroxymethyl)-9,10-anthracenedione) (Figure 1), AE, is an anthraquinone compound extracted from Aloe leaves. This anthracene derivative is a polyphenol that has shown many therapeutic properties, such as antineoplastic, antibacterial, antiviral, anti-inflammatory and scavenging activity on free radicals acting through several cellular mechanism and pathways[1]. Hence, AE, due to its properties, attracts increasing interest and could be applied in the treatment of many diseases. Its inclusion in liposome formulations could solve the problem of its scarce solubility in water and allow i) controlling its pharmacokinetics and biodistribution, ii) increasing its accumulation in target tissues and iii) avoiding or reducing its accumulation in healthy tissues, thus reducing its toxicity and side effects. The weak acid nature of AE, due to its two phenolic functions, could be exploited to remotely load it in the internal aqueous phase of liposomes.
In fact weak acids can accumulate in the internal aqueous compartment of lipid vesicles in response to a difference in pH between the inside and the outside of the liposomes, $pH_{in}>pH_{out}$[2].
Here we report the inclusion of AE in gemini based cationic liposomes by the acetate gradient method[3] (remote loading). A number of different cationic liposome formulations composed of different ratios of natural phospholipids, namely DMPC (1,2-dimyristoyl-sn-glycero-3-phosphatidylcoline), DPPC (1,2-dipalmitoyl-sn-glycero-3-phosphocholine), DOPC (1,2-dioleoylsn-glycero-3-phosphocholine), or POPC (1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine), and one of two diastereomeric gemini surfactants (Figure 1), (2S,3S)-2,3-dimethoxy-1,4-bis(N-hexadecyl N,Ndimethylammonium)butane bromide, **1**, or (2R,3S)-2,3-dimethoxy-1,4-bis(N-hexadecyl-N,Ndimethylammonium)butane bromide, **2**, and cholesterol were explored for the inclusion of AE.



Aloe Emodin



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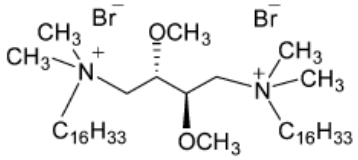

2

Figure 1

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Bola-shaped Glicocalix[4]arenes for efficient and specific drug delivery systems

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The protein-carbohydrate recognition phenomena can be used for the targeted drug delivery, exploiting the numerous specific carbohydrate receptors present on the cell membrane.[1] Liposomes are well known as drug delivery systems and, in some cases, functionalized with sugar units, they indeed show targeting properties on the basis of these processes.[2] In other examples, the functionalization of the lipid bilayer with bolaamphiphiles gives rise to liposomes with higher rigidity and lower permeability, improving their ability in entrapping molecules.[3]
We recently prepared two new bolaamphiphiles characterized by the presence of active polar heads for targeting, based on calixarenes in the so-called 1,3-alternate geometry and functionalized with four units of glucose and cellobiose (glycocalix[4]arenes).[4] Experiments to determine the incorporation of these glycocalixarenes into lipid bilayers of DOPC liposomes, permeability and entrapment properties of these mixed liposomes, and interaction with a glucose recognition lectin (ConA) were successfully performed.[4] Moreover, targeted delivery experiments were performed towards human breast cancer cells (MDA-MB-231) over-expressing the glucose receptor GLUT1, by using DOPC and DOPC/calixarene mixed liposomes fluorescently labelled with a N-(7-nitrobenz-2-oxa-1,3-diazol-4-yl) (NBD) tagged lipid. The preliminary results collected by laser scanning confocal microscopy indicate that the uptake of liposomes containing the glucosylated calixarenes is significantly higher respect to the uptake of DOPC liposomes suggesting the occurrence of a receptor mediated process. The use of bola-shaped glycocalix[4]arenes could be then a novel successful strategy to functionalize liposomes and obtain drug delivery systems able to target specific lectins, cells and tissues.

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Regular structure formation in photoactive liposomes.

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Liposomes are lipid vesicles that can be functionalized with fluorescent surfactants in order to apply them as sensors. We have exploited such approach trying to verify the potential of using lipid's structure reorganization (for different formulations) in order to develop specific sensors. Structure features (like internal reorganization) can be probed by the ratio between excimer and monomer fluorescence, according to the fluorescent surfactant concentration.
We investigated the excimer/monomer ratio formulated with an amphiphilic fluorescent molecule and phospholipid to elucidate the lipid organization. The fluorescent amphiphilic is characterized by a pyrrolidinium headgroup and a hydrocarbon tail tagged with a pyrene residue[1](1). We have investigated the influence of vesicle size and temperature on the lipid organization.

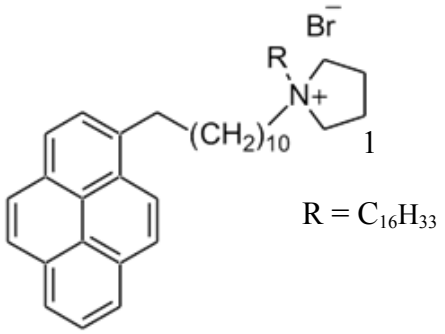

Figure 1. Molecule of fluorescent surfactant.

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Acknowledgment: D.G. Villalva thanks *Ciência sem Fronteiras* - CAPES Foundation, Ministry of Education of Brazil and L. Giansanti thanks for financial support the project FIRB 2012 RBFR12BGHO.

Chitosan-coated surfactant vesicles: a novel approach to cellular targeting

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Among all the currently employed nanovectors, surfactant vesicular systems are rapidly gaining popularity as vectors for delivering drugs to target organs and cells [1].
Chitosan-coated surfactant vesicles were proposed as targeting vectors for myogenic differentiation. Chitosan's key properties are its biocompatibility, bioactivity, nonantigenicity and nontoxicity (its degradation products are known natural metabolites).
Surfactant vesicles coated with a chitosan shell were prepared and characterized in terms of size (dynamic light scattering), shape (electron microscopy after freeze-fracture), zeta potential (electrophoretic mobility), bilayer fluidity and polarity (fluorescence studies), formulation stability (dynamic light scattering). Carrier stability in fetal bovine and human serum were evaluated and release kinetics of model molecules from selected formulations (fluorescence studies, HPLC) were performed. Chitosan-coated surfactant vesicles, containing a fluorescent dye, were added to C2C12 skeletal muscle cell lines and fluorescence was measured by flow cytometric analysis.

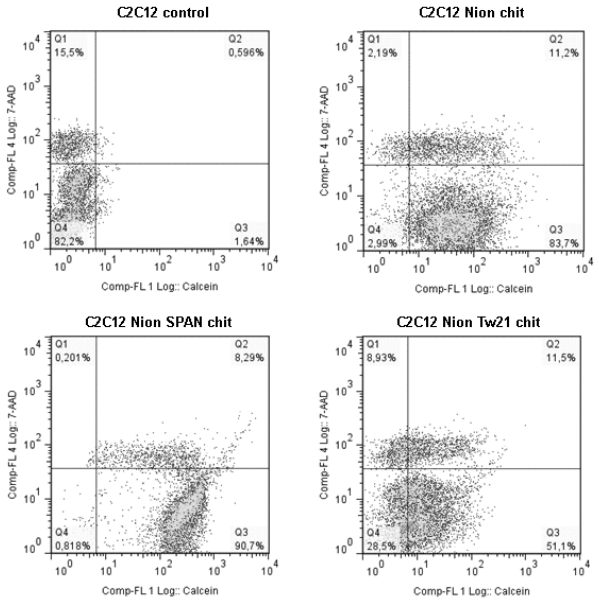

Figure 1. Flow cytometry analysis of nanoparticles cellular uptake in C2C12 cells.. The Q3 quadrant indicates percentage of calcein positive and 7 Amino-Actinomycin D (7-AAD) negative live cells.

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Acknowledgments: This work was supported by CLNS IIT@Sapienza

Recognition of Concanavalin A by novel glucosylated amphiphiles included in liposome lipid bilayer

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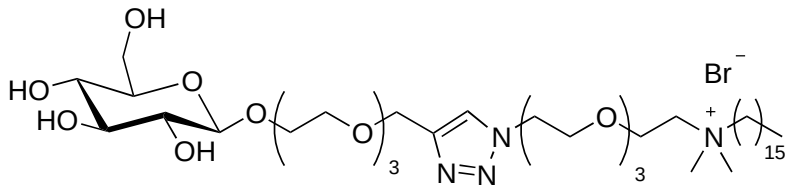
The highly specific interactions of carbohydrates with lectins, a class of sugar-binding proteins without enzymatic activity, are involved in a wide variety of cellular recognition processes including bacterial and viral infections, immune response and tissues growth.[1] Since lectins have been found to locate on various cell surfaces, including microbial and viral ones, targeting devices exploiting the recognition of sugar moieties have been frequently investigated in recent years.[2] Glycosylated liposomes (glycoliposomes), obtained by functionalization of vesicles with glycosylated amphiphiles, represent a promising approach to improve the selective targeting of drugs to diseased tissues *in vivo*, leading to reduction in drug toxicity and improved therapeutic outcomes.
Herein it is reported on the capability of the new glucosylated synthetic amphiphiles **1** and **2** to interact with the plant lectin Concanavalin A (Con A), used as model system, as pure components and in formulation with dimyristoyl-*sn*-glycero-phosphocholine (DMPC) at 5:95 molar ratio. Experiments on breast cancer line cells showed an enananched cellular uptake of the glycoliposomes with respect to DMPC vesicles.



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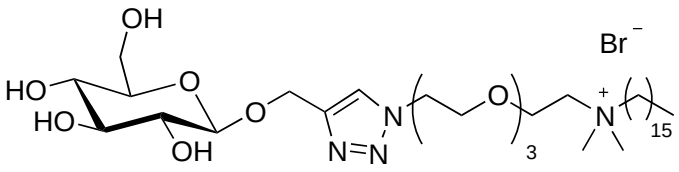

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Calixarenes as versatile and tunable scaffolds for biologically active, polyglycosylated ligands

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In many biological processes, based on molecular recognition phenomena, carbohydrates play a central role. The interactions between saccharides and proteins are in fact important for different cell activities like proliferation or intercellular communication, but they also mediate the beginning and development of pathological events. These interactions are strengthened by the glycoside cluster effect: multiple identical binding sites simultaneously bind multiple identical saccharide units. The design and synthesis of molecules for the interaction with cells or microorganisms can take advantage by this concept of the multivalent glycoside-protein recognition in order to improve the specificity and efficiency of the binding.
In this field, calixarenes, macrocycles obtained by phenol-formaldehyde oligomerization, constitute a perfect scaffold for the synthesis of polyglycosylated ligands.[1] In fact, valency and geometry of the sugar units display can be easily tuned with the possibility of obtaining small libraries of potential biologically active compounds.
We present here some recent works of our laboratory, in which the functionalization of calixarenes with specific saccharide units selected on the basis of the biological target, pointed out interesting results. The inhibition of two different galectines has been reached with two isomers of a calix[4]arene, both functionalized with four lactoside units.[2] A calix[5]arene bearing five units of GM1 tetrasaccharide has been developed for the inhibition of the cholera toxin.[3] A 1,3-alternate glucocalixarene, able in binding Concanavalin A, was successfully introduced in a liposome bilayer.[4]

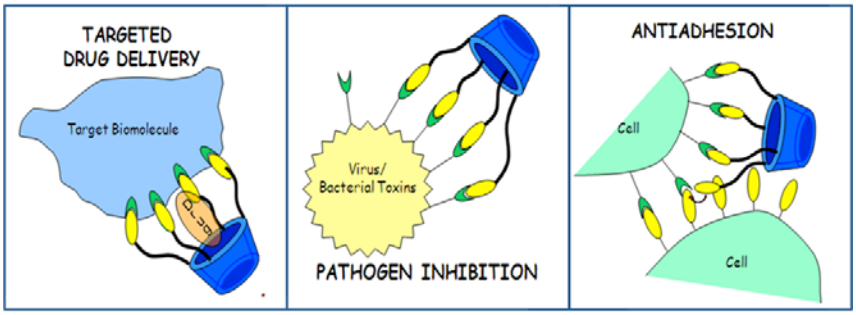

Figure 1. Possible biological applications of glycocalixarenes.

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Acknowledgments: COST Action CM1102 “MultiGlycoNano” and MIUR PRIN projects 200858SA98 and 2010JMAZML “MultiNanoIta”

Generation of low-cost Virus-Like Particles from rotavirus VP6 inner capsid protein in *Escherichia coli*

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[1]Istituto di Microbiologia, Università Cattolica del Sacro Cuore, [2]Istituto di Fisica, Università Cattolica del Sacro Cuore, [3]ICRM CNR Sec.Rome, 4Istituto di Biochimica e Biochimica Clinica, Università Cattolica del Sacro Cuore, Largo Francesco Vito, 00168, Rome, Italy.

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Expression of viral structural proteins, such as those of the pericapsid or capsid, may cause the self-assembly of protein oligomers in Virus Like Particles (VLPs) or nanotubes (NTs). In recent years VLPs were produced in Eucharіotic cells like insect cells and mammalian cells with the complexity and high costs associated with such expression systems. The expression of these viral proteins in bacterial cells has been limited by their poor solubility and their subsequent accumulation within the inclusion bodies. The recent development of a new prokaryotic expression system that uses the fragment SUMO (Small Ubiquitin-like Modifier) as a fusion partner, opens new scenarios in the production of these particles. Using this system we developed and optimized a biotechnological platform in Escherichia coli for the expression and purification of the soluble viral VP6 protein from human rotavirus. We produced VLPs and NTs from VP6 at different pH values in an efficient and reproducible way, verified and characterized by means of TEM and AFM. Both nanoparticles administrated to different groups of mice showed strong immunogenicity with high levels of systemic IgG response with the tubular structural conformation as the most immunogenic one. The interest in developing and refining new approaches to manipulate the mechanical properties of low-cost VP6 nanoparticles is rapidly increasing, in order to improve their suitability for biomedical applications. In this regard, in addition to deepen the immunogenic properties of these particles, our work plans to use these nanoconstructs as a carrier of bioactive molecules. The increasing number of multidrug resistant (MDR) bacteria is rapidly becoming a global problem. Since the difficulty to obtain new effective antibiotics, the development of new drugs, beside antibiotics, is becoming of great relevance. In order to face this problem, the aim of our future work is to design, synthesize and test in vitro and in vivo, a new type of multivalent drug based on VP6 Virus Like Particles functionalized with Anti-Microbial Peptides (AMPs), normally produced by the Human Innate Immune System.

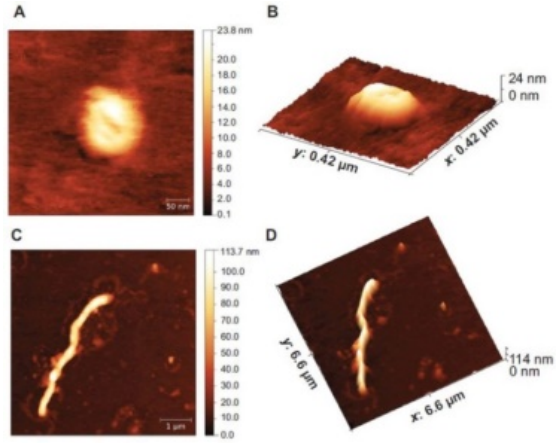

Figure 1. Atomic force microscopy images collected in contact operation mode, at different pH and ionic strength.
(**A**) Representative 2-D image of a spherical virus-like particle detected at pH4.5.
(**B**, **D**) 3-D representation of the same images in (**A**) and (**C**), respectively.
(**C**) Representative 2-D image of an elongated wormlike nanotube detected at pH6.

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Liposome-based sensor for the detection of bacteria

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The identification and quantification of bacteria, that affects human life both directly causing diseases and indirectly contaminating natural ecosystems, are mostly carried out with culture plating, enzyme-linked immunosorbent assay (ELISA) and Polymerase Chain Reaction (PCR)[1]. These methods are quite expensive and time-consuming, as a consequence the number of analysis to be performed is usually kept to a minimum. Therefore, there is the need for rapid and economic methods for detecting bacteria in the environment and in diagnostics.
Within the AQUALITY[2] project (funded by FP7-SME-2011-1) we propose an innovative approach, the use of engineered liposomes for detecting bacteria in drinkable water.
Our idea consist in preparing cationic liposomes functionalized with a proper probe, that will produce an optical signal (changes in the emission) due to an interaction between liposomes and bacteria. We have prepared and characterized a large number of liposomes formulated with a natural phospholipid and a synthetic cationic component. In particular, we have evaluated their dimension, their capability of retaining the probe and their Zeta potential in the presence and in the absence of bacteria (Figure 1) in order to select the formulations most suitable for the interaction. Finally, the most promising formulations were tested against specific bacterial strains (*Staphylococcus aureus*, *Escherichia coli*, *Enterococcus faecalis)* and the change in the fluorescence emission of the entrapped probe was evaluated as a function of bacterial concentration. One of the tested formulations is able to detect up to 10^2 CFU/mL of bacteria.
These encouraging results could also be exploited for the formulations of liposome for the identification of bacteria in biological fluids.

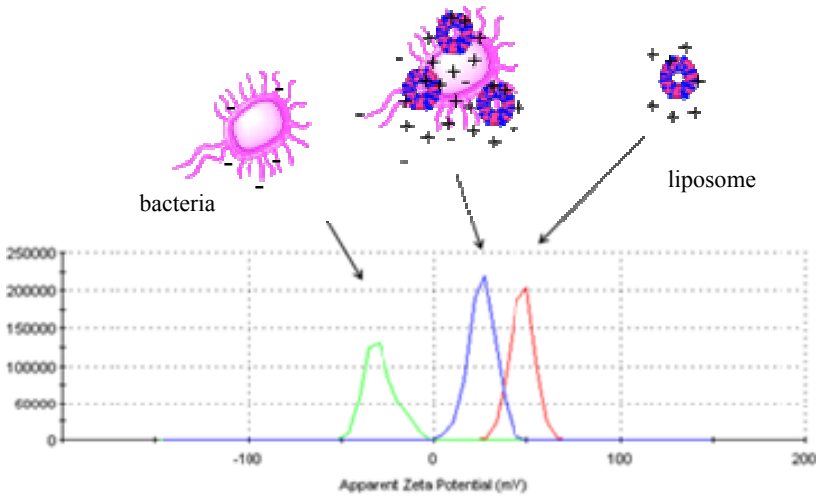

Figure 1.

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In vitro evaluation of the effects of graphite nanoplatelets on breast cancer cells

Gennaro Salvatore Ponticelli,[a,b] Marisa Colone,[a] Annarica Calcabrini,[a] Ilaria Rago,[b] Giovanni De Bellis,[b] Maria Sabrina Sarto,[b] Annarita Stringaro[a]

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Graphene, a single, tightly packed layer of carbon atoms bonded together in a hexagonal honeycomb lattice, is stimulating research on related structures, such as Graphite NanoPlatelets (GNPs), a 1-15 nm thick flake, constituted of 3-48 layers of graphene, because of their unique structures, properties and possible applications. Graphene and its derivatives have shown outstanding potentials in many fields, such as nanoelectronics, composite materials, energy technology, sensors, and catalysis. Beyond the applications aforementioned, the biomedical application of graphene is a relative new area with significant potential[1]. Due to the expanding applications of nanotechnology, human and environmental exposures to graphene-based nanomaterials are likely to increase in the future. However, the prospective use of graphene-based materials in a biological context requires a detailed comprehension of their toxicity.

Herein, we report on the interaction of stable and evenly dispersed exfoliated GNPs obtained using an ultrasonic bath for different times (30, 50 and 70 min) with human breast adenocarcinoma cells (SKBR3 and MDA-MB-231) for 24-48-72 hrs. Biocompatibility of nanoplatelets has been evaluated by MTT while cell viability has been detected using Trypan Blue assays. Our results showed that GNPs particles were more cytotoxic in SKBR3 than MDA-MB-231 cells suggesting a cell phenotype-dependent effect.

Furthermore, light microscopy observations and scanning electron microscopy analysis were used to gain understand on the mechanism of cell-nanoplatelets interaction.

Our studies carried out with interdisciplinary approaches among chemistry, biology and engineering, can contribute to the mechanistic understanding of graphene-based platforms for bio- and nanomedicine applications.

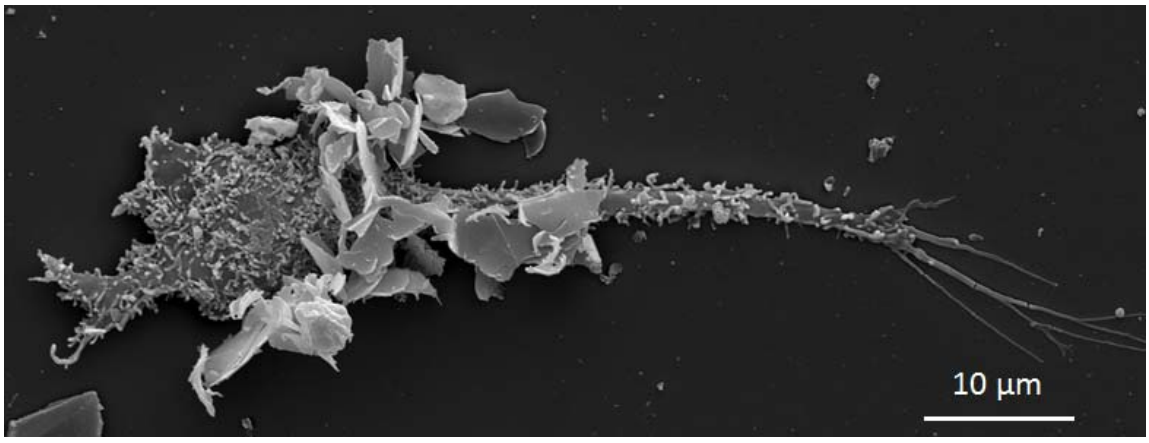

Figure 1. SEM micrograph of cancer cell-GNPs interactions

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Chitosan-coated surfactant vesicles: a novel approach to cellular targeting

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Among all the currently employed nanovectors, surfactant vesicular systems are rapidly gaining popularity as vectors for delivering drugs to target organs and cells [1].
Chitosan-coated surfactant vesicles were proposed as targeting vectors for myogenic differentiation. Chitosan's key properties are its biocompatibility, bioactivity, nonantigenicity and nontoxicity (its degradation products are known natural metabolites).
Surfactant vesicles coated with a chitosan shell were prepared and characterized in terms of size (dynamic light scattering), shape (electron microscopy after freeze-fracture), zeta potential (electrophoretic mobility), bilayer fluidity and polarity (fluorescence studies), formulation stability (dynamic light scattering). Carrier stability in fetal bovine and human serum were evaluated and release kinetics of model molecules from selected formulations (fluorescence studies, HPLC) were performed. Chitosan-coated surfactant vesicles, containing a fluorescent dye, were added to C2C12 skeletal muscle cell lines and fluorescence was measured by flow cytometric analysis.

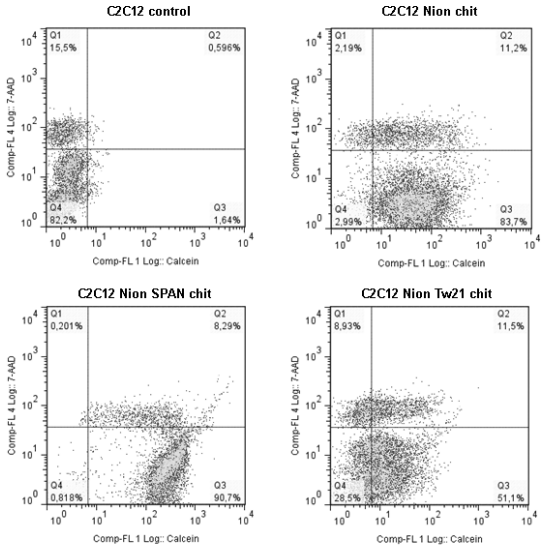

Figure 1. Flow cytometry analysis of nanoparticles cellular uptake in C2C12 cells.. The Q3 quadrant indicates percentage of calcein positive and 7 Amino-Actinomycin D (7-AAD) negative live cells.

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Electron (TEM) and probe microscopy (AFM) vs ultrastructure characterization in targeted drug delivery systems

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In the field of nanomedicine, the characterization of functionalized drug delivery system, represents a pivotal aspect of great importance. In this study, PLGA nanoparticles functionalized with three different kinds of ligands (carbohydrate ligand, antibody and quantum dots crystals) were intentionally designed, created and tailored with specific physico-chemical properties to meet the needs of specific applications (cell targeting or imaging). We applied and combined two advanced microscopies as AFM and TEM, optimizing the preparative procedure to discriminate amongst the ligands conjugated on the NP surface. Despite the high resolution of the two microscopical techniques, low molecular weight molecules, such as sugar residues (Neu5Ac), anchored to the surface of polymeric NP were not detectable. On the contrary, due to the different nature of the ligands with respect to the polymer (PLGA) of the NPs, the CdSe/CdZnS core of the PEG-QD anchored on NPs surface was clearly detected by using TEM. TEM analysis of complex and high molecular weight molecules, such as antibodies conjugated to the surface of NPs, required a preliminary procedure with gold particles staining and labeling of the samples. In contrast, the AFM allowed to discriminate on the qualitative evaluation of both QD and antibody, without any additional treatment of the samples and without operating in a vacuum environment. Moreover, AFM images shows 3D-data allowing the height of the observed object to be measured. Also the heterogeneity/discontinuity of the coverage or the surface alteration obtained during the functionalization of NPs can be defined. Hence, AFM can support additional analytical techniques allowing a detailed sample characterization.

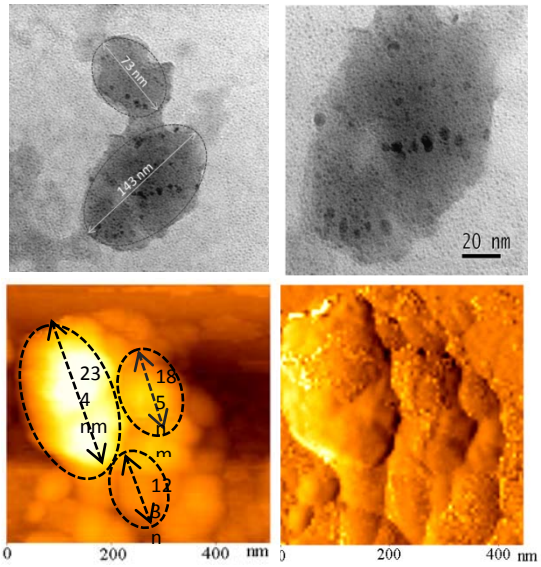

***Figure1**: TEM and AFM images of antibody (Ab) conjugated nanoparticles (NPs)*

A photo-physical characterization of optimized multimodal QD-PLGA nanoparticle

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Combinations of different nanostructured materials are considered promising tools to allow the development of multifunctional nanomedicines for multimodal imaging [1]. QDs and polymeric nanotechnology revealed to be a good binomial for these applications. In fact, the sensitive and fast responsive fluorescent imaging method using QDs as probes can dynamically monitor the behavior of labeled nanocarriers in both cells and animals. Moreover, fluorescence quenching of quantum dots is one of the most interesting and useful photochemical phenomena [2, 3], but still poorly investigated. The comprehension of polymer interaction with the metallic QD surface must be absolutely considered to finally display of a detailed chemico-physical and biological characterization of these systems.

In this study, we compared two different synthetic procedures to obtain QD-labeled polymeric nanoparticles (NPs), investigating if the optical properties of QDs (i.e. the intensity of fluorescence) may change accordingly to the formulation methods, as a consequence of the different polymeric environment.

By AFM and TEM characterization and by confocal experiments, we demonstrated that NPs modified with QDs after the formulation process (post-NPs-QDs) conserved the photophysical features of QD probe. On the contrary, by using a polymer modified with QDs to formulate NPs (pre-NPs-QDs), a significant quenching of QD fluorescence and a blue-shift in its emission spectra were observed.

Our results suggest that fluorescence quenching phenomenon appears to be due to the packaging of QDs into the polymeric matrix. The dissolution of the polymeric matrix in DMSO reverted the quenching phenomenon, allowing a recovery of the fluorescence and, in part, no-shifted emission peak.

These results can be exploited to develop nanosystems for imaging and biological applications as NPs labeled with QDs, if safe and characterized in depth, can represent useful tools for treatment, diagnosis and monitoring of biological systems at the level of single molecule or molecule assemblies.

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Modified-PLGA nanoparticles for CNS delivery of cholesterol in Huntington's therapy

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Alterations in the homeostasis of brain cholesterol (Chol) have been described in neurodegenerative diseases such as Huntington's disease (HD). Reduction of brain cholesterol (Chol) biosynthesis and level has been described in Huntington's disease (HD) *[1]*, a neurodegenerative genetic disorder that leads to motor defects and cognitive decline. The implementation of Chol in brain, through delivery of exogenous Chol, has thus been reported could be a feasible approach to HD. Unfortunately, Chol does not cross the blood-brain barrier (BBB) in suitable amounts after systemic administration. Several *in vivo and in vitro* studies demonstrated that NPs based on FDA approved poly(D,L-lactide-co-glycolide) when functionalized with a glycopeptide (g7-NPs) are able to cross the BBB delivering drugs into the brain *[2-4]*. Starting from these premises, we aimed to obtain g7-NPs able to release Chol in brain after having optimized the technological conditions and parameters of their preparation. We investigated both the physical and technological parameters (the mean nanoparticle size, the morphology, the architecture and the surface properties) of the g7-NPs loaded with Chol, demonstrating the stability of drug embedded into the matricial structure. The Chol release was evaluated by means of *in vitro* studies analyzing its concentration in buffer at a pH 7.4 (under perfect sink conditions) by a gas chromatography (GC)/mass spectroscopy (MS) method. The release of Chol was characterized by an initial "burst effect" attributed to the fraction of Chol adsorbed or close in contact with the surface of the NPs, and by a second phase, from day 5 and day 10, with slow linear release of Chol ascribable to the NPs degradation. Also the uptake of NPs by brain cell cultures expressing mutant huntingtin (NS-hdhQ140/7) and the Chol release in these cells, were evaluated by loading the fluorescent NBD-Chol probe, confirming the slow kinetic profile of drug release from nanoparticles. Finally, *in vivo* experiments were carried out. g7-NPs loaded NBD-Chol were intra-peritoneal (i.p.) administered in a transgenic mouse model of HD (R6/2) *[5]* to monitor the uptake, the qualitative biodistribution and the release of drug in animal brains. Few hours after administration, g7-NPs were localized in several cerebral areas. Confocal studies demonstrated that within 14 days, degradation of particles started and the release of Chol occurred.

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Efficient BLIMP-1 regulation in PEL cells by new siRNA-pegylated lipoplexes

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Recently experimental evidences demonstrated that BLIMP-1 is a possible target for down-regulation by micro RNA in diffuse large B-cell lymphomas of the activated B-cell type as Primary Effusion Lymphoma (PEL), a B cell non-Hodgkin lymphoma involving the serous cavities invariably associated with Human HerpesVirus-8 (HHV8) characterized by poor prognosis [1, 2]. However, in vivo systemic delivery of micro RNA or siRNA-based therapeutics to tumour cells remains a challenge. The major limitations against the use of siRNA as a therapeutic tool are its degradation by serum nucleases, poor cellular uptake and rapid renal clearance following systemic administration. In this contest we proposed a pegylated cationic lipoplexes to administer therapeutic siRNA in PEL therapy. Near the physicochemical properties (size, zeta potential, morphology and structure), siRNA loading and the efficacy to mediate gene silencing in PEL cells were evaluated. The post pegylation via DSPE-PEG micelles of preformed lipoplexes produced the organized and stable structures that maintained siRNAs protect exerting both an efficient transfection and release of active fragment into PEL cells with an high gene silencing.

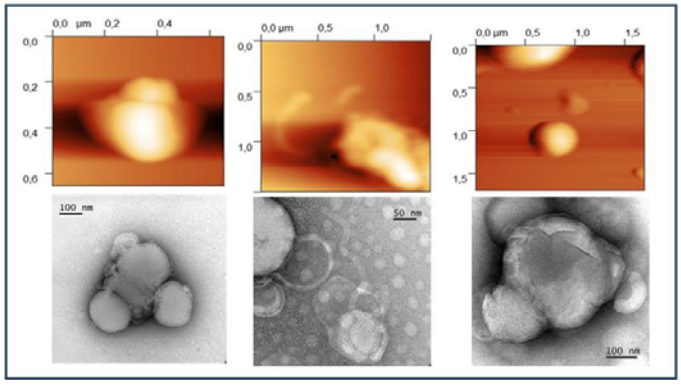

***Figure1**: AFM and TEM images of lipoplexes and PEG lipoplexes.*

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A self-assembling linear L,D -oligopeptide-poly (ethylene glycol) conjugate as promising candidate for drug delivery.

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Self-assembling peptide-based nanotubes are widely investigated for the development of novel biomaterials.[1] Among the strategies developed for peptide nanotube engineering, those characterized by regularly alternating enantiomeric sequences are particularly attractive. In fact, they self-assemble in stacks directed and stabilized by hydrogen bonds forming hollow tubular architectures where the side chains are located on the periphery, as a consequence of the residue configuration.
The preparation and structural organization of a new bioinspired nanomaterial are described. The hybrid conjugate, Cbz-(L-Ala-D-Val)$_4$-NH-(CH$_2$-CH$_2$-O)$_{45}$-CH$_3$ (PEP8-PEG) (Figure 1A) was obtained end-linking the linear peptide with regularly alternating configurational sequence, Cbz-(L-Ala-D-Val)$_4$-OH, synthesized via solid-phase peptide synthesis, to a poly(ethylene glycol) (PEG) chain derivatized as amine. NMR, CD and fluorescence measurements and DLS and TEM results show that this conjugate is able to self-assemble in water forming well-defined nanorods having core/shell morphology with an internal peptide single channel (Figure 1B) that has been clearly visualized by TEM images (Figure 1C). The synthetic strategy and the structure of the conjugate ensure a controlled and regular pegylation of the aggregates and high density PEG blocks in the corona making the nanoparticles more resistant to phagocytosis and able to prevent biofouling. Such features along with biocompatibility and stability make these nanoparticles highly promising candidates for drug delivery.

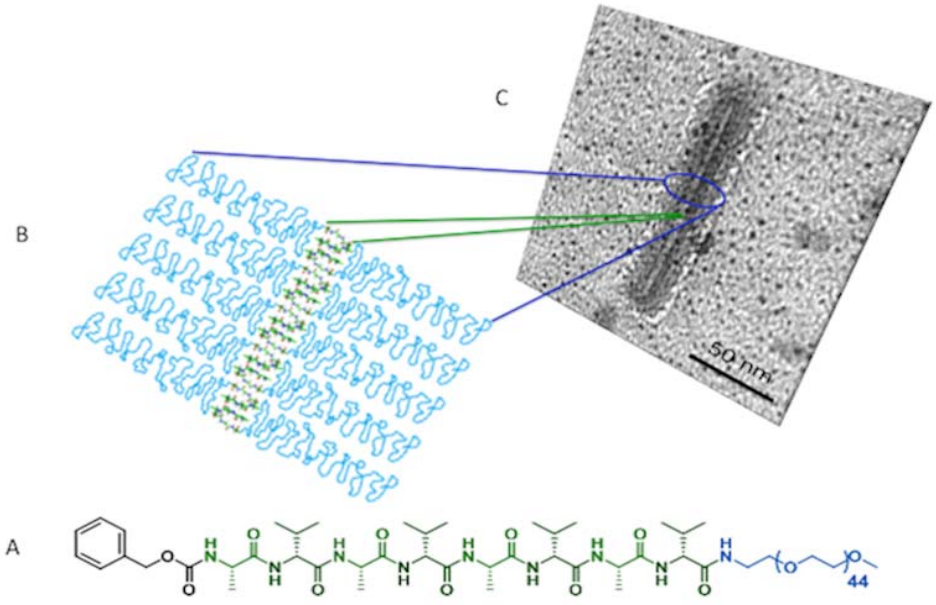

Figure 1. A) Molecular formula of the pegylated L,D-peptide: B) structural model of the nanoparticle obtained for self-assembling of PEP8-PEG; C) a selected TEM image of the self-assembled PEP8-PEG.

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Investigation and characterization of self-assembling peptides for regenerative medicine

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There is a great deal of interest in regenerative medicine due to its capability to generate powerful new treatments for a wide range of diseases and injuries.
In this context, self-assembling peptides (SAPs) are an appealing class of materials due to their ability to organize in nanostructured hydrogels that can be successfully anchored to appropriate substrates (such as for example the biocompatible TiO_2 [1]), or directly injected into a lesion. SAPs scaffolds are able to mimic the structure of the extra-cellular matrix (ECM), offering tridimensional support for cell growth and/or becoming vehicles for the delivery of stem cells or drugs [2]. Indeed nanomaterials, eventually combined with growth factors, may constitute a biomimetic matrix with the ability of surrounding cells and promoting specific interactions with them, in order to control and conduct their behavior by mimicking their native environment. The ideal matrix must have a 3D geometry similar to the extracellular matrix and must be able to promote cell adhesion, proliferation, infiltration and differentiation aimed at new tissue formation.

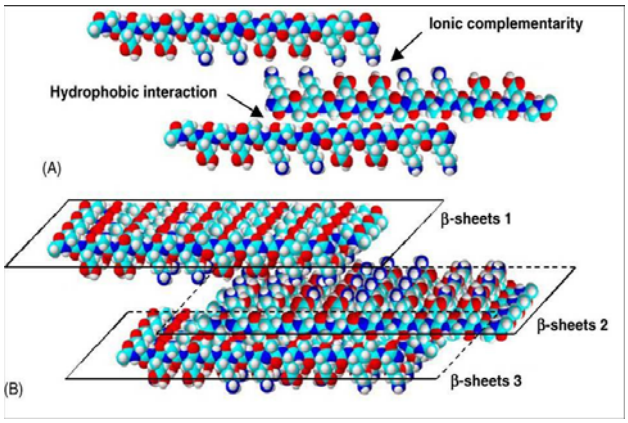

Figure 1. Scheme of self-assembling peptide (EAK16-II)

The realization of self-assembling peptides should include a first step of chemical and structural characterization, to check the stability of the molecular structure following the scaffold's development. In this work, we present an accurate spectroscopic investigation of different classes of hydrogel materials using X-ray photoelectron spectroscopy (XPS) and Fourier-Transform Infrared spectroscopy (FT-IR) techniques. XPS and FT-IR are successfully used to probe the chemical composition, molecular structure and conformation of the proposed materials. Specifically we focus on verifying the stability of the antiparallel β–sheet structure after self-assembling.

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Controlled release from magnetoliposomes exposed to a low intensity magnetic field

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Recently, in a pilot study [1] has been demonstrated the feasibility of a smart controlled delivery from magnetic vesicles through an alternating magnetic field (AMF) with intensity significantly lower than the usual ones reported in literature unable to generate heat. Therefore, the drug release from the magnetically responsive vesicles (MLs) is controlled by mechanical oscillations induced alone on the magnetic nanoparticles by the AMF action. This could represent a clear advantage for therapeutic applications: the tissues are not affected neither by the temperature elevations nor by the eddy currents induced by the field, thus preventing damage and safety secondary effects [2]. Based on these encouraging results, to establish whether a non-thermal magnetic field could be effectively used to remotely control the release of an entrapped cargo from phospholipid vehicles, commercially available magnetic carboxymethyl-dextran coated Fe_3O_4 nanoparticles (MnPs) have been embedded in the aqueous core of saturated soybean phosphatidylcholine (HSPC)/cholesterol liposomes which exhibit high *in vitro* stability with respect to temperature (T_m higher than physiologically acceptable temperature), fig. 1A. Samples were exposed for different time intervals at 37.0±0.5°C to a low amplitude AMF (20 kHz, H field 60 A/m), and the release of the co-entrapped hydrophilic model drug, 5(6)-carboxyfluorescein (6-CF), was compared to the one obtained in isothermal conditions in the absence of the magnetic field application (sham situation), fig. 1B. Results provide evidence of AMF mechanical destabilization effects.

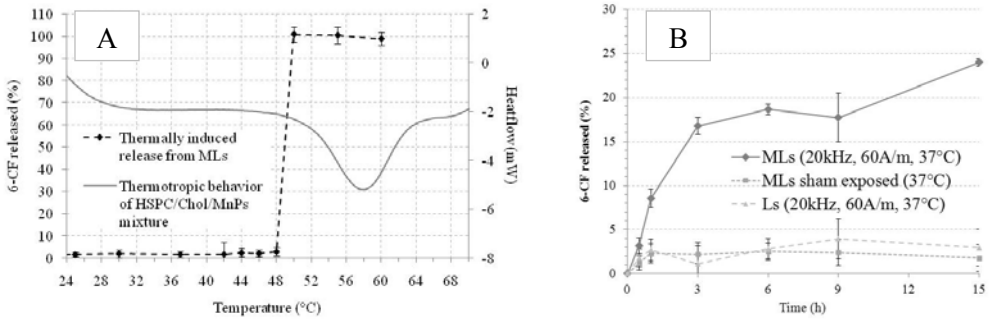

Figure 1. A. Release amount of 6-CF from MLs after 3h of heating (dashed line) and differential scanning calorimetric profile (solid line) of HSPC/cholesterol/MnPs mixture as a function of temperature. B. Experimental comparison of the release of the 6-CF loaded MLs both for AMF exposure and for the sham condition, at increasing exposure times. Release profile of control liposomes (without MnPs) is also reported.

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Interaction of Lysozyme-Shelled Microparticles with Human Breast Adenocarcinoma Cells

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The ultrasound-assisted self-assembly and cross-linking of lysozyme at the water–air and water–perfluorohexane interfaces are shown to produce lysozyme-shelled hollow microbubbles (LSMBs) and microcapsules (LSMC), respectively. The interaction of LSMBs and LSMCs with human breast adenocarcinoma cells (SKBR3) is studied (1). The cellular internalization kinetics of LSMBs and LSMCs and the effects on cell cycle are evaluated using flow cytometry. Evidence for the internalization of microparticles and degradation within the cell are also monitored by confocal and scanning electron microscopic analyses. The integrity of cell membrane and cell cycle are not affected by LSMBs and LSMCs uptake. These studies show that the positively charged LSMB and LSMC are not cytotoxic and can be readily internalized and degraded by the SKBR3 cells. LSMBs and LSMCs show a different uptake kinetics and intracellular degradation pattern due to differences in the arrangement of the protein at the air–liquid or oil–liquid interfaces (Figure 1).

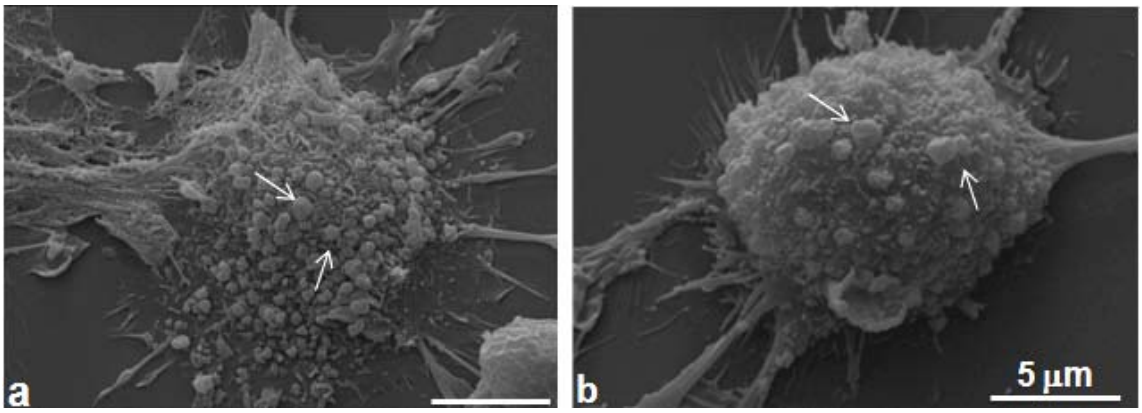

Figure 1. After cell treatment for 60 min with LSMBs (a) and LSMCs (b) SEM observations reveal the strong adhesion of MCs on cell surface with the formation of ruffles (arrows) without inducing cytotoxic effects.

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Dye doped silver and gold nanoparticles for biomedical applications

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Nanostructured materials are particularly interesting due to their high surface area-volume ratio and synergetic effects, which are useful for medicine, optics, and sensing applications.[1-4] In particular gold and silver nanoparticles (AuNPs, AgNPs) are promising materials in nanomedicine applications. In this work we present recently results obtained for the development of water-soluble gold and silver nanoparticles stabilized with hydrophilic ligands: a dye is bonded on particles, as schematically shown in figure 1, in order to obtain dye doped metal nanosystems, in analogy to polymeric systems, exploitable in biomedical applications.[5,6]

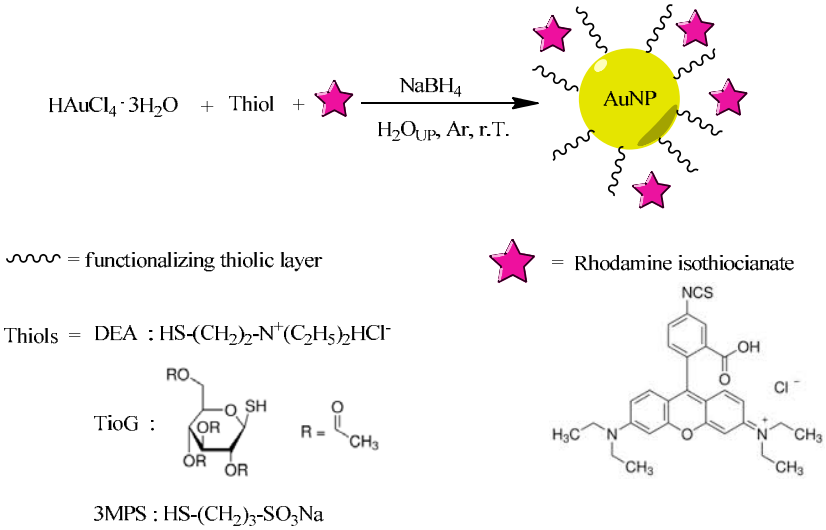

Figure 1. Reaction scheme to obtain dye doped AuNPs.

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6. A. Laganà, I. Venditti, I. Fratoddi, AL. Capriotti, G. Caruso, C. Battocchio, G. Polzonetti, F. Acconcia, M. Marino and MV. Russo, *J. Coll.Interf. Sci.* **2011**, 361, 465

Self-assembled fluorogenic nanoparticles for photo-tunable multicolour fluorescence imaging.

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The research for new fluorescent materials recently became even more urgent because of the advent of new imaging techniques that gave a strong impulse to the development of "smart" nano-systems such as environment sensitive (fluorogenic) and photo-activable nano-probes. Aggregation induced emission (AIE) is surely an attractive mechanism for designing "smart" responsive probes.[1] Nevertheless this process is peculiar of a limited range of dyes while most fluorophores undergo aggregation caused quenching (ACQ). Here we demonstrate that the use of nano-aggregates, non-fluorescent because of ACQ, can become a very general strategy for the design of new fluorogenic nano-probes exploiting a process specular to AIE namely disaggregation induced emission (DIE). In particular we will describe the efficient microwave assisted synthesis of a new strongly fluorescent perylene derivative that can be dispersed in water in the form of self-assembled non fluorescent NPs.[2] We will demonstrate that these NPs are efficient fluorogenic imaging agents in the case of model cells and that controlling the dosage either green or red fluorescence can be obtained. The same NPs can be used to achieve multi-colour fluorescence imaging by photo-activation of the sample. Thanks to these features, to the lack of toxicity and to the versatility of the proposed synthetic methodology we believe that our approach can inspire the design of new smart imaging agents for application in life sciences.

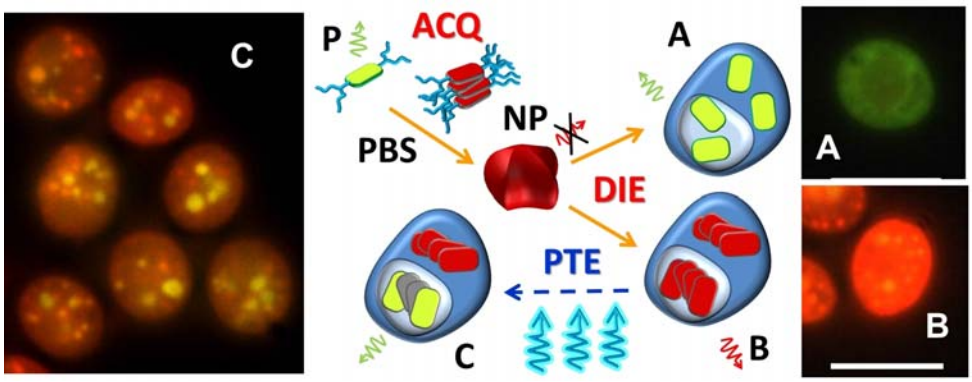

Figure 1. The perylene derivative P aggregates in PBS and forms NPs quenched by ACQ. Uptake by cells leads to disaggregation of the NPs and to fluorescence recovery (DIE). The cells become green fluorescent (A) or redfluorescent (B) respectively at low and high NPs dosage. Exposition to strong visible livisible light allows the photo-tuning of the emission (PTE) of specific cellular compartments (C).

[1] Y. Hong, J. W. Y. Lam and B. Z. Tang, *Chem. Soc. Rev.*, 2011, *40*, 5361-5388.
[2] M. Montalti, G. Battistelli, A. Cantelli and D. Genovese, *Chem. Commun.*, 2014, *50*, 5326-5329.
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