NANOMEDICINE VITERBO 2016

Viterbo, 21-23 September 2016

University of Tuscia

DIAGNOSIS

THERAPY

THERANOSTICS

DRUG DELIVERY

TISSUE ENGINEERING

INVITED SPEAKERS

Luigi Ambrosio
Sophia G. Antimisiaris
Yechezkel Barenholz
Paola Luciani
Dusica Maysinger
Francesco Ricci
Ester Segal
Gert Storm
Vladimir P. Torchilin
Thomas J. Webster

Abstract Book

Congress Coordinators

Giovanna Mancini
Institute of Chemical Methodologies – CNR
Tel. 06 90672900
E-mail: giovanna.mancini@cnr.it

Agnese Molinari
Department of Technology and Health - ISS
Tel. 06 4990 3406
E-mail: agnese.molinari@iss.it

Scientific Committee

Annarica Calcabrini, Giuseppina Bozzuto, Maria Condello, Annarita Stringaro, Stefania Meschini
Department of Technology and Health - ISS

Cecilia Bombelli, **Francesca Ceccacci**
Institute of Chemical Methodologies - CNR

Felice Grandinetti, Stefano Borocci
University of Tuscia

Luisa Giansanti
University of L'Aquila

Organizing Committee

Monica Brocco, Marisa Colone, Giuseppe Formisano, Alessia Medici, Laura Toccacieli
Department of Technology and Health - ISS

Giorgio Giardini, Giuliana Gigli, Marco Pastore, Massimo Quici, Giampiera Porcu, Aldo Rosati, Enrico Rossi, Aurelia Stella, Stefania Tarquini
Institute of Chemical Methodologies - CNR

Maria Giordani
University of Tuscia

Abstract Book

NANOMEDICINE

VITERBO 2016

Viterbo

University of Tuscia

September, 21-23, 2016

The NANOMEDICINE VITERBO 2016
Conference is organized under the sponsorship of

Program

Wednesday, 21 September

09.00 Registration

10.00 **Opening Ceremony**

Walter Ricciardi - President of Istituto Superiore di Sanità
Tullio Pozzan - Direttore del Dipartimento Scienze Biomediche – CNR
Alessandro Ruggieri - Chancellor of Tuscia University

Chairpersons: ***Paola Luciani, Gert Storm***

Invited lectures

10.20 **Y. Barenholz**
"Doxil®, the First FDA Approved Nano-Drug: New Lessons Learned (from physical-chemistry and nanotechnology through metabolomics and tumor micro-environment to the clinics)"

10.50 **F. Ricci**
"Nature-inspired DNA-based nanodevices for diagnostic and drug-delivery applications"

11.20 *Coffee break*

Selected lectures

11.40 **L. Motiei**
"Communicating with fluorescent molecular sensors in biological environments"

12.00 **D. Alberti**
"Dual therapeutic agents to improve the effect of Boron Neutron Capture Therapy on cancer cells."

12.20 **E. Papini**
"The capture of PEG and POZ NPs by monocytes/macrophages in serum is mediated by complement activation"

Industry report

12.40 **A. Pigozzo**
"Microcalorimetry of Nanoparticles: a ground-breaking approach for drug delivery platforms design"

13.00 *Lunch and Poster session*

Chairpersons: ***Yechezkel Barenholz, Francesco Ricci***

Invited lecture

14.30 **D. Maysinger**
"Impact of nanomedicines on neurons and glia"

Selected lectures

15.00 **A. Clementino**
"Nanoparticles for an innovative administration of statins"

15.20 **J. W. Trzcinski**
"Bionanoparticles: versatile platforms for nanomedicine"

15.40 **S. Castellani**
"Safety and uptake of solid lipid nanoparticles by airway epithelial cells: an in vitro study"

16.00 **K. Turjeman**
"Liposome-based steroidal nano-drugs for the treatment of inflammatory diseases: from basic to the clinics"

16.20 *Poster session and Coffee break*

Thursday, 22 September

Chairpersons: ***Giovanna Mancini, Vladimir P. Torchilin***

Invited lecture

09.00 **P. Luciani**
"Design of a Ratiometric Fluorescent Probe for Specific Detection of Extracellular Reactive Oxygen Species"

Selected lectures

9.30 **A. Mazzaglia**
"Nanoassemblies based on folate-tailored amphiphilic cyclodextrin/pheophorbide complexes for targeted PDT"

9.50 **C. Ferroni**
"Near-infrared photoactivable poly-methylmethacrylate core-shell fluorescent nanoparticles"

10.10 **M. Comes Franchini**
"Nano-heaters for cancer therapy: a comparison between gold nanorods and magnesium nanoparticles"

10.30 **G. Varchi**
"Keratin-based nanoparticles: novel tools for drug delivery"

10.50 *Coffee break*

Chairpersons: ***Sophia G. Antimisiaris, Thomas J. Webster***

Invited lecture

11.10 **E. Segal**
"Porous silicon nanostructure for cancer therapy and diagnostics"

Selected lectures

11.40 **M. Capozza**
"In vitro and in vivo characterization of new nano-sized diagnostic probes for Photoacoustic Imaging"

12.00 **L. Tei**
"ICG-loaded Mesoporous Silica Nanoparticles as photoacoustic contrast agents"

12.20 **V. Maggi**
"Cyclic RGD functionalized PEGylated gold nanoparticles for tumor targeting"

12.40 **S. Lai**
"Organic-inorganic coated Gold Nanorods for biomedical applications"

13.00 *Lunch and Poster session*

Chairpersons: ***Luigi Ambrosio, Dusica Maysinger***

Invited lectures

14.30 **G. Storm**
"Liposomal anti-inflammatory treatment in atherosclerotic disease"

15.00 **T. J. Webster**
"15 years of commercializing nanomedicine into real medical products"

Selected lectures (special session on design and preparation - 15min talk)

15.30 **A. S. Rodrigues**
"Smart Hybrid Nanocontainers for Theranostic Applications"

15.45 **L. Chronopoulou**
"Biosynthesis of injectable gelling peptides for applications in bone tissue regeneration"

16.00 **S. Villa**
"Promising applications of multifunctional magnetic nanoparticles in biomedicine"

16.15 **F. Novelli**
"Self-assembling octapeptide-polymer conjugate to be used as drug carriers"

16.30 **T. Ribeiro**
"Small mesoporous silica nanoparticles for nanomedicine"

16.45 *Poster session and Happy hour*

Social Dinner *(poster prizes)*

Friday, 23 September

Chairpersons*:* ***Agnese Molinari, Ester Segal***

Invited lecture

09.00 **L. Ambrosio**
"Multifunctional templates and advanced technologies for tissue regeneration"

Selected lectures

9.30 **S. M. Dozio**
"3D Cell Cultures in Porous Scaffolds with Oriented Microtubules Designed for Dental Regeneration"

9.50 **V. Laghezza Masci**
"Ultrastructural investigation of different collagen-based scaffolds and their in vitro interactions"

10.10 **S. Centi**
"Development of cellular vehicles for delivery of gold nanorods to tumors"

10.30 *Coffee break*

Invited lectures

10.50 **S. Antimisiaris**
"Targeting the brain with liposomes or exosomes: What can each systems learn from the other?"

11.20 **V.P. Torchilin**
"Stimuli-sensitive combination nanopreparations for cancer"

Selected lectures

11.50 **Y. Yamakoshi**
"LDL for targeted delivery of MRI-CA to atherosclerosis"

12.10 **P. Oliva**
"Ex vivo plaque permeability evaluation in ApoE(-/-) mice using fluorescent blood pool agents"

12.30 **Flash presentations of awarded posters**

12.50 *Closing remarks and Lunch*

Invited Lectures

Doxil®, the First FDA Approved Nano-Drug: New Lessons Learned (from physical-chemistry and nanotechnology through metabolomics and tumor micro-environment to the clinics)

Yechezkel (chezy) Barenholz

Department of Biochemistry and Molecular Biology, IMRIC, Hebrew University-Hadassah Medical School, Jerusalem, Israel.

Email of presenting author: chezyb1@gmail.com

Nano-drugs such as Doxil® are superior to the same APIs when used conventionally "as-is". However it is clear that nano-drugs did not meet their high expectations. Doxil® the first FDA approved nano-drug in wide clinical use (>600,000 patient treated) sine 1996 shows much superior plasma pharmacokinetics over the API administered as-is. Doxil take advantage the tumor "Achilles' heel" the EPR effect. It resulting in passive targeting to tumors, which does not occur for the API administered in the conventional way. EPR effect was firstly demonstrated in cancer patients in the first Doxil clinical trial performed by us in Jerusalem (1991-1993, Gabizon and Barenholz, Cancer Res, 1994). EPR effect has a large impact on tumor physics, thus on nano-drug performance. While tumor EPR effect can be used to improve tumor therapy its clinical significance is still controversial. Numerous studies with various nano-drugs performed during the last 25 years clearly demonstrate that even though this passive targeting reduces (some-times even dramatically), the cardiotoxicity and other side effects, these are not sufficient to provide a " quantum leap" in the improvement of the therapeutic efficacy. Today we understand that the main reasons for these deficiencies are lack in considering sufficiently the obstacles imposed by the tumor micro-environment, especially the effect of the chaotic extracellular matrix in suppressing convection flow and contributing to the high tumor interstitial fluid pressure. These reduce intra-tumor distribution of the nano-drugs and its released API. Another so far ignored factor is the poor rate of API release at the tumor, reducing availability of the API to the tumor cells. "Out of the Box" approaches are required to overcome these obstacles in order to meet the high expectation from the performance of nano-drugs. My presentation will focus on pegylated liposomal doxorubicin (PLD) including Doxil.

We hypothesize and demonstrate that by improving the cross talk between the unique tumor microenvironment, interstitial fluid and metabolomics on one hand and the better physico-chemical characterization of the nano-drugs, we can improve the therapeutic efficacy of nano-drug anti-tumor therapy. We demonstrate that changing tumor micro-environment by radiofrequency (RF), or high intensity focused ultrasound (HIFU) we can effectively re-modulate the tumor microenvironment concomitantly enhancing nano-drug accumulation and API release at the tumor, leading to a significantly improved therapeutic efficacy. In addition we identified low molecular weight factors unique to tumor metabolism which seems to be a major factor in doxorubicin release of Doxil at the tumor interstitial fluid. This factor the ammonia which results of tumor specific glutaminolysis is lacking in the patient plasma. This selectivity in drug release between plasma and tumor explains the much faster drug release in the tumor than in the plasma explains reduction in Doxil toxicity without compromising therapeutic efficacy. Our studies give hope that it is possible to improve the performance of the anti-cancer nano-drugs to the level that will meet the expectation from nano-medicine.

Nature-inspired DNA nanodevices for sensing and drug-delivery applications

Francesco Ricci

Department of Chemistry, University of Rome, Tor Vergata, Rome, Italy.

Email of presenting author: francesco.ricci@uniroma2.it

Nature has invented a number of tricks and strategies by which the behavior of proteins and other biomolecular machines can be finely controlled. These highly optimized and evolved mechanisms allow to control biological pathways with different chemical and environmental stimuli and are at the basis of the high specificity and selectivity of biomolecular machines.
Motivated by the above arguments we have developed several DNA-based nanodevices for diagnostic and drug-delivery applications that mimic and exploit in-vitro naturally occurring recognition mechanisms. More specifically we have rationally designed different DNA-based nanodevices that, by taking inspiration from Nature, can be finely controlled by conformational change or allosteric strategies. This allows to modulate the activity of our DNA-based nanodevices in an extremely controlled way.
Moreover, we have also reported new recognition mechanisms that allow to control our DNA-based nanodevices with different chemical and environmental stimuli that include pH, antibodies, enzymes and small molecules.
An overview of the most representative and recent examples developed in our lab in the above research directions and a brief presentation of the new routes and possibilities that these results offer will be presented.

Impact of nanomedicines on neurons and glia

Dusica Maysinger,[a] Issan Zhang,[a] Jeff Ji,[a] Rainer Haag,[b] Gerhard Multhaup,[a] Anne McKinney [a]

[a] *Department of Pharmacology and Therapeutics, McGill University, 3655 Promenade Sir William Osler, H3G 1Y6 Montreal, Canada;* [b] *Institute of Chemistry and Biochemistry, Freie Universität Berlin, Takustr. 3, 14195 Berlin, Germany.*

Email of presenting author: dusica.maysinger@mcgill.ca

Nanomedicines can impact both glia and neurons. In the central nervous system (CNS), neurons represent only a small proportion (about 10%) of cells, whereas glial cells are the most predominant cell type. Non-targeted nanomedicines are mainly internalized by glia, in particular microglia, and to a lesser extent by astrocytes. Internalized nanomedicines by glia indirectly modify the functional status of neurons. The mechanisms of biochemical, morphological and functional changes of neural cells exposed to nanomedicines are still not well understood. Certain classes of nanomedicines can profoundly alter the morphology and function of post-synaptic excitatory synapses and dendritic spines in the hippocampus. Effects of the pro-inflammogen lipopolysaccharide (LPS) and artificial dendritic structures on hippocampal dendritic spines and neighboring microglia will be shown and discussed in the light of neuroprotection and neural repair. We have investigated how the size, composition and surface properties of nanoparticles activate microglia, and how we can take the advantage of the phagocytic properties of these glial cells to benefit neural cell functions. Some agglomerated nanoparticles can over-activate microglia, leading to the release of pro-inflammatory cytokines and the initiation or propagation of neurodegenerative processes. Microglia homeostasis is critically important for normal brain functions and examples in this presentation will show how nanostructures can be exploited to "calm" the hyperactive microglia or stimulate those with deficient activity.

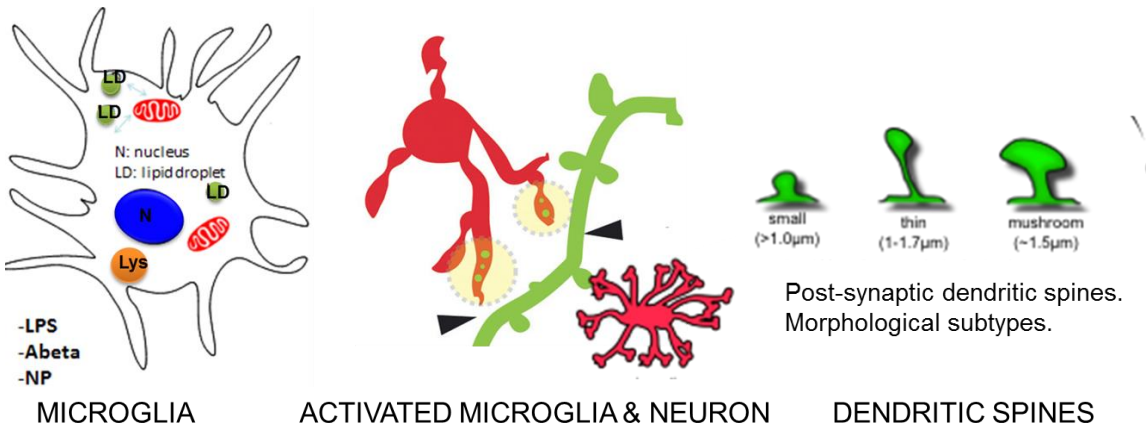


Figure 1. Impact of nanomedicines on glia and neurons. A. Nanoparticles (NP), Abeta and LPS can activate microglia and cause organellar remodeling. B. Hyperactive microglia adversely affect neurons. Some dendrimers can normalize LPS-induced abnormalities in both neurons and microglia, and maintain synaptic structures in organotypic hippocampal cultures.

Acknowledgments: The authors acknowledge financial support from Canadian Institutes for Health Research (CIHR) and Natural Sciences and Engineering Research, Canada (NSERC).

Design of a Ratiometric Fluorescent Probe for Specific Detection of Extracellular Reactive Oxygen Species

Diana Andina,[a] Davide Brambilla,[a] Jean-Christophe Leroux,[a] Paola Luciani [a,b]

[a] *Institute of Pharmaceutical Sciences, ETH Zurich, Switzerland;* [b] *Institute of Pharmacy, Friedrich Schiller University, Lessingstrasse 8, 07743, Jena, Germany.*

Email of presenting author: paola.luciani@uni-jena.de

Reactive oxygen species (ROS) are released during the oxidative burst, which is a first line defense mechanism of neutrophils during intestinal inflammation. The specific detection of the extracellular radicals has, however, remained a challenge. A diagnostic tool to monitor ROS-rich areas quantitatively and selectively might help to understand the progress of inflammation and evaluate the efficacy of antioxidant therapies. Thus, a new ratiometric fluorescent probe to detect extracellular ROS was designed.
The contrast agent consists of a ROS-sensitive dye connected to a negatively charged PEG-polyamino acid-peptide nucleic acid (PNA) and a ROS-insensitive dye coupled to the complementary PNA strand (Figure 1). Hydrocyanines were shown to selectively detect superoxide and hydroxyl radicals[1] and, therefore, were used in this project as ROS sensitive probe. The ROS-insensitive dye (Chromis dye series, Cyanagen) allows to calibrate the system and to trace the probe even when in a ROS-free environment. Hybridization of the two labelled complementary strand yielded a ratiometric fluorescent probe specifically responsive to ROS in cell-free assays as well as in a stimulated colon carcinoma cell line.

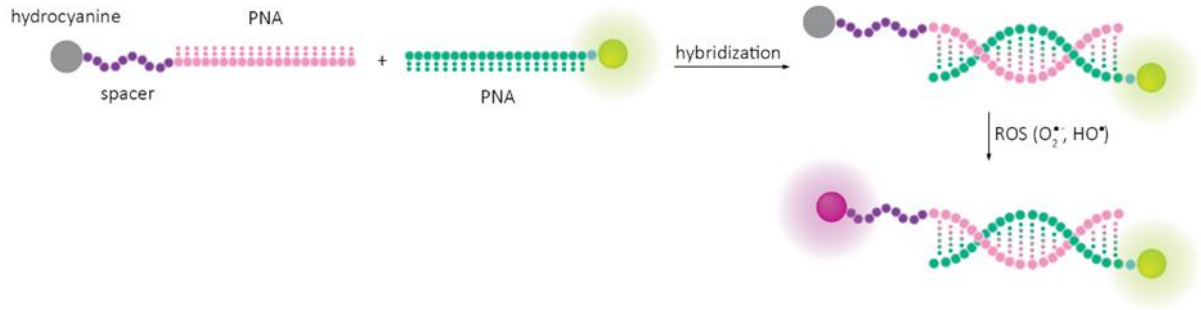


Figure 1. A hydrocyanine-spacer-PNA is hybridized with a ROS-insensitive dye connected to the complementary PNA strand.

1. K. Kundu, S. F. Knight, N. Willett, S. Lee, W. R. Taylor, N. Murthy, *Angew. Chem.-Int. Edit.* **2009**, *48*, 299.

Porous silicon nanostructures for cancer therapy and diagnostics

Ester Segal, Adi Tzur Balter, Sofia Arshavsky-Graham

Department of Biotechnology and Food Engineering, Technion – Israel Institute of Technology, Technion City, 32000, Haifa, Israel.

Email of presenting author: esegal@technion.ac.il

Nanostructured porous silicon (PSi) is a promising biomaterial for nanomedicine in general and cancer nanomedicine in particular. PSi offers a unique combination of properties, including large surface area and porous volume, biocompatibility, degradability in vivo into non-toxic silicic acid species, as well as its wealth of intrinsic optical properties. These properties together with the ability to tailor the PSi nanostructure and surface characteristics have led to an immense research effort directed at the development of PSi-based platforms for biomedical applications (1).

Although PSi degradation largely determines the release of embedded drugs, its erosion profile under pathological states that are of clinical relevance was not established. Understanding the factors affecting PSi degradation in vivo is critical to facilitate its development and clinical application as a drug carrier. Our work provides the first mechanistic study that examines the potential impact of tissue microenvironment on PSi degradation (2). We show that PSi particles, which are increasingly used to deliver chemotherapeutics, undergo enhanced degradation in cancerous environments compared with healthy environments. The particles in vivo degradation kinetics is determined by their continuous optical tracking in a breast cancer murine model, while tumor state is monitored simultaneously, as shown in Figure 1.

We confirm that upregulation of reactive oxygen species (ROS) in the tumor vicinity oxidizes the silicon nanostructure and catalyzes its degradation. PSi degradation profiles in vitro and in vivo correlates in healthy and diseased states when ROS-free or ROS-containing media are used in vitro, respectively. Thus, this work demonstrates that material performance is contextual and hence should be studied in light of the relevant intended clinical use.

The approach presented is generic and could be adapted for any material and clinical scenario to attain predictive performance. Yet, specific clinical scenarios may require further material optimization but would facilitate successful clinical outcome and translation of new materials to the clinic.

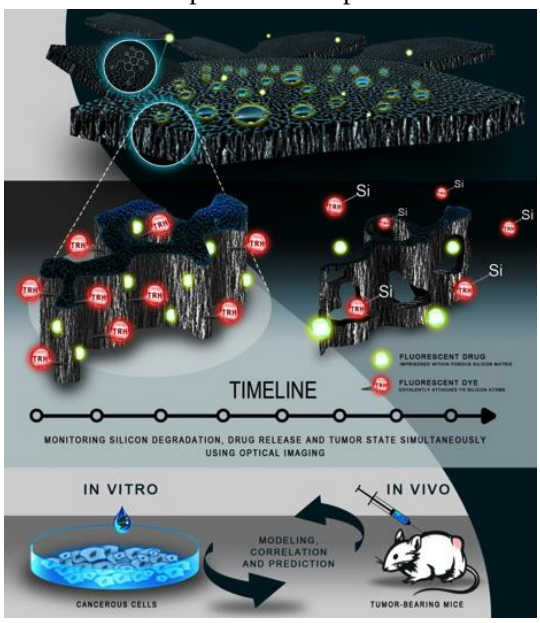


Figure 1. PSi particles (labeled with a fluorophore) are monitored *in vitro* and noninvasively *in vivo*, by tracking the changes in fluorescence intensity with time.

1. A. Tzur-Balter, G. Shtenberg, E. Segal, *Reviews in Chemical Engineering*, **2015**, *31*, 193.
2. A. Tzur-Balter, Z. Shatsberg, M. Beckerman, E. Segal, N. Artzi,. *Nat Commun*, **2015**, 6.

Liposomal anti-inflammatory treatment in atherosclerotic disease

Gert Storm,[a,b] Josbert M. Metselaar,[b] Mark E. Lobatto,[c,d] Zahi A. Fayad,[c] Erik S.G. Stroes,[d] Willem J.M. Mulder [c,d]

[a] *Department of Pharmaceutics, Utrecht Institute for Pharmaceutical Sciences (UIPS), Utrecht,Universiteitsweg 99, 3584 CG Utrecht, The Netherlands;* [b] *Department of Targeted Therapeutics, MIRA Institute, University of Twente, The Netherlands;* [c] *Translational and Molecular Imaging Institute, Icahn school of Medicine at Mount Sinai, New York;* [d] *Department Vascular Medicine, Academic Medical Center, Amsterdam, The Netherlands.*

Email of presenting author: g.storm@uu.nl

Atherosclerosis, the final common pathway underlying cardiovascular disease (CVD), is a predominantly lipid-driven inflammatory disease affecting the arterial wall/ Lipid lowering strategies, mainly statins, have shown to reduce the cardiovascular (CV) event risk by 25%, though a large residual CV-risk remains unaddressed. Complementing statins with other lipid-modulating agents, such as cholesteryl-ester transfer protein (CETP) inhibitors, nicotinic acid derivatives and fibrates, has failed to further reduce this residual risk. In line with the insight that atherosclerosis is a low-grade inflammatory disease, anti-inflammatory agents have emerged as additional therapeutic strategies. A major hurdle prior to clinical implementation is to select compounds that combine a robust anti-inflammatory effect with the absence of an effect on systemic immune protection.

Targeted drug delivery to plaques via nanomedicine offers a novel paradigm to circumvent the systemic, adverse effects of anti-inflammatory agents by combining enhanced drug accumulation within the arterial plaque with decreased systemic exposure. The accumulation of nanoparticles exploiting permeable microvasculature within arterial plaques has been demonstrated to occur in animal models of advanced atherosclerotic lesions. To date, however, this opportunity has been unexplored in patients at increased CV-risk due to atherosclerosis. Glucocorticoids (GC) are amongst the most commonly prescribed anti-inflammatory agents in the treatment of inflammatory disorders. Whereas these anti-inflammatory effects should have a beneficial impact in CV-patients, the adverse effect of GC on glycaemic control, blood pressure and lipids has been suggested to increase the CV risk. However, the relation between GC and CVD is still at equipoise. Nanomedicinal formulations of GC can be expected to minimize the systemic adverse effects, while exerting a local anti-inflammatory action within the atherosclerotic lesion. In support, we recently substantiated a potent, anti-atherosclerotic effect of liposomal prednisolone (LN-PLP) in an atherosclerotic rabbit model, which was evaluated with novel non-invasive clinical imaging methods.

This lecture will report on the development of GMP-grade LN-PLP. Preclinical pharmacokinetics and anti-inflammatory effects on the vessel wall of atherosclerotic rabbits will be shown. Subsequently, the pharmacokinetics of LN-PLP in humans, and the targeting of intravenously administered LN-PLP to plaque macrophages in patients with symptomatic peripheral artery disease will be discussed.

Stimuli-sensitive combination nanopreparations for cancer

Vladimir Torchilin

Center for Pharmaceutical Biotechnology and Nanomedicine, Northeastern University, 360 Huntington Avenue, 02125 Boston, MA, USA.

Email of presenting author: v.torchilin@neu.edu

Tumor therapy of multidrug resistant cancers could be significantly enhanced by using siRNA down-regulating the production of proteins involved in cancer cell resistance, such as Pgp or survivin. Even better response is achieved if such siRNA could be delivered to tumors together with chemotherapeutic agent. This task is complicated by low stability of siRNA in biological surrounding. Thus, the delivery system should simultaneously protect siRNA from degradation. We have developed several types of lipidic systems based on PEG-phospholipid or PEI-phospholipid conjugates, which are biologically inert, demonstrate prolonged circulation in the blood and can firmly bind non-modified or reversibly-modified siRNA. Additionally, these nanopreparations can be loaded into their lipidic core with poorly water soluble chemotherapeutic agents, such as paclitaxel or camptothecin. In experiments with cancer cells monolayers, 3D spheroids and animals, it was shown that such preparations can significantly down-regulate target proteins in cancer cells, enhance drug activity, and reverse multidrug resistance.
To specifically unload such nanopreparations inside tumors, those can be designed sensitive to local tumor-specific stimuli. Using pH-, hypoxia-, or MMP2-sensitive bonds between different components of nanopreparations co-loaded with siRNA and drugs, one is able to specifically deliver biologically active agents in tumors, inside tumor cells, or even to individual cell organelles providing improved therapeutic response.
Intracellular drug delivery also opens new opportunities in overcoming MDR. The use of cell-penetrating peptides (CPP, such as TAT peptide) and combinations of CPP with targeting ligands within the same nanopreparation allows for effective intracellular delivery of pharmaceuticals into target cells. Intracellular drug delivery via the mechanism of an endosomal escape can also be achieved by using target-specific phage coat proteins as targeting ligands for nanopreparations.
Specific targeting of individual cell organelles responsible for the initiating apoptosis in cancer cells including MDR cells opens new opportunities for treating MDR tumors. Examples of specific targeting of lysosomes and mitochondria in cancer cells with chemotherapeutic agents illustrate the benefits of this new approach.

1. E.Koren, V.P.Torchilin, *Trends Mol. Med.*, **2012**, *18*, 385.
2. L.Zhu, V.P.Torchilin, *Integr. Biol.*, **2012**, *5*, 96.
3. S.Biswas, N.S.Dodwadkar, P.P.Deshpande, V.P.Torchilin, *J. Control. Release*, **2012**, *159*, 393.
4. A.Koshkaryev, A.Piroyan, V.P.Torchilin, *Cancer Biol. Ther.*, **2012**, *13*, 50.
5. L.Zhu, T.Wang, F.Perche, A.Taigind, V.P.Torchilin, *Proc. Natl. Acad. Sci. USA*, **2013**, *100*, 17047.
6. F.Perche, S.Biswas, T.Wang, L.Zhu, V.P.Torchilin, *Angew. Chemie*, **2014**, *53*, 3362.
7. L.Zhu, F.Perche, T.Wang, V.P.Torchilin, *Biomaterials*, **2014**, *35*, 4213.
8. G.Salzano, G.Navarro, M.S.Trivedi, G.De Rosa, V.P.Torchilin, *Mol. Cancer Ther.*, **2015**, *14*, 1075.
9. G.Salzano, D.F.Costa, V.P.Torchilin, *Curr. Phar. Des.*, **2015**, *12*, 4566.
10. F.Perche, S.Biswas, N.R.Patel, V.P.Torchilin, *Methods Mol. Biol.*, **2016**, *1372*, 139.

Multifunctional templates and advanced technologies for tissue regeneration

Luigi Ambrosio, Roberto De Santis, Antonio Gloria, Vincenzo Guarino, Maria Grazia Raucci

Institute of Polymers, Composites & Biomaterials and Department of Chemical Sciences & Materials Technology, National Research Council of Italy, P.le Aldo Moro, 7 - 00185 Rome, Italy.

Email of presenting author: luigi.ambrosio@cnr.it.

In tissue regeneration it is growing the interest of alternative routes to synthesize/process instructive biomaterials with biologically recognized functionalities. A large variety of processes and tools are currently investigated to develop engineered scaffolds able to provide an active support matrix to reproduce “in vitro” all the main functionalities exerted “in vivo” by health or pathological tissues microenvironment. Among them, Electrofluidodynamic techniques (EFDTs) and 3D Printing are emerging as highly flexible and low-cost processes to manipulate biomaterials to design 3D ECM-like platforms for tissue repair/regeneration processes.

By a rational selection of polymer solution properties and process conditions, EFDTs allow producing fibres and/or particles at micro and/or sub-micrometric size scale which may be variously assembled by tailored experimental setups, to generate a plethora of different 3D devices including specific topological (i.e,. surface roughness) or biochemical signals in the form of biopolymers (i.e, proteins, polysaccharides) and/or active molecules (e.g., drugs, growth factors) for different applications. Electrospinning may be successfully used to design temporary extracellular matrix (ECM) analogue able to support in vitro cell proliferation/differentiation for tissue regeneration. The process of synthetic - i.e, PCL– and natural polymers - i.e, Gelatin - allows to develop bicomponent electrospun scaffolds with improved bioactivity, able to guide regeneration processes in the skeletal system (i.e., bone, nerve, skeletal muscle). In this perspective, EFDTs have been properly used to design more complex platforms for molecular delivery by combining mono- or bi-component fibres with loaded nanoparticles produced via additive electrospraying (AES).

3D Printing, utilizing biomaterials, cells, proteins, and/or other biological compounds as basic building blocks to fabricate 3D structures or in vitro biological models encompasses a broad application to tissue-engineered substitutes for regenerative medicine. Here, will report some advances, covering from bio-modeling and computer-aided tissue engineering to design and fabrication of 3-dimensional tissue scaffolds for bone tissue engineering.

A 3D rapid prototyped magnetic scaffold made by poly(ε-caprolactone)/iron-doped hydroxyapatite (PCL/FeHA) 80/20 w/w is proposed to provide a morphologically controlled and tailored structure with interconnected pores of specific scale, and the possibility to magnetically "switch-on/switch-off" to stimulate cell adhesion, proliferation and differentiation.

1. V. Guarino, A. Gloria, M.G. Raucci, R. De Santis, L. Ambrosio, *Intern. Mat Rev* **2012**, *57* (5), 256.
2. V. Guarino, V. Cirillo, R. Altobelli, L. Ambrosio, *Expert Review of Medical Devices* **2014**, *12*, 113.

Acknowledgments: Financial support by projects: MERIT, MIUR-RBNE08HM7T-001, NEWTON (FIRB-RBAP11BYNP), REPAIR (PON01-02342), MAGISTER – FP7-NMP3-LA-2008-214685 and EU FP7 project “Disc Regeneration”, Grant agreement no. NMP3-LA-2008-213904.

Targeting the brain with liposomes or exosomes: What can each systems learn from the other?

Sophia G. Antimisiaris,[a, b] Konstantia Papadia,[a] Anastasia Basta [a]

[a] *Department of Pharmacy, University of Patras, University Campus Rio, 26504, Patras, Greece;*
[b] *Institute of Chemical Engineering & Sciences, FORTH/ICE-HT, Platani, 26500 Greece.*

Email of presenting author: santimis@upatras.gr

Exosomes and liposomes have many similarities since they are both vesicles composed of phospholipid membrane bilayers. Their complementarities in terms of the advantages and disadvantages of each system as drug delivery systems (DDSs) can be explored with the aim to produce superior systems for drug targeting. In view of the recent findings on the organotropism of exosomes towards specific cell types, research to exploit exosome organotropism for drug targeting applications has been initiated. Herein we try to apply liposome engineering technologies to overcome particular problems encountered when trying to use exosomes for drug delivery and targeting applications. In this context, we have initiated studies to produce cellular vesicles (due to the low yield of exosome production from cells), loaded with fluorescent labels, and evaluate their physicochemical properties, stability and capability to taken up by autologous and other cell types.

After confirming that it is possible to use liposome-engineering methodologies to produce cellular vesicles as DDSs, we have studied the brain targeting capability of three different types of vesicles produced from different cell types. The hcMEC/D3 cellular model of the BBB was used and the uptake-by and permeability-across cells or monolayers, respectively, was measured. Interestingly, the BBB targeting capability of some of the cellular vesicle DDSs is similar or even better compared to recently developed targeted nanoliposomes, which were evaluated under identical experimental conditions.[1-3]

Concluding the use of exosomes for development of DDS seems to be an interesting concept that should be further explored in the near future.

1. E Markoutsa, et al. *Eur J Pharm Biopharm*. **2011,** *77*, 265.
2. E. Markoutsa, et al. *Pharm Res*. **2014,** *31*, 1275.
3. K. Papadia, et al. unpublished results

15 years of commercializing nanomedicine into real medical products

Thomas J. Webster

Art Zafiropoulo Professor, Chair, Department of Chemical Engineering, Northeastern University, Boston, MA 029115 USA.

Email of presenting author: th.webster@neu.edu

There is an acute shortage of organs due to disease, trauma, congenital defects, and most importantly, age related maladies. The synthetic materials used in tissue engineering applications today are typically composed of millimeter or micron sized particles and/or fiber dimensions. Although human cells are on the micron scale, their individual components, e.g. proteins, are composed of nanometer features. By modifying only the nanofeatures on material surfaces without changing surface chemistry, it is possible to increase tissue growth of any human tissue by controlling the endogenous adsorption of adhesive proteins onto the material surface. In addition, our group has shown that these same nanofeatures and nano-modifications can reduce bacterial growth without using antibiotics, which may further accelerate the growth of antibiotic resistant microbes. Inflammation can also be decreased through the use of nanomaterials. Finally, nanomedicine has been shown to stimulate the growth and differentiation of stem cells, which may someday be used to treat incurable disorders, such as neural damage. This strategy also accelerates USA FDA approval and commercialization efforts since new chemistries are not proposed, rather chemistries already approved by the FDA with altered nanoscale features. This invited talk will highlight some of the advancements and emphasize current nanomaterials approved by the USA FDA for human implantation.

Selected Lectures

Communicating with fluorescent molecular sensors in biological environments

Leila Motiei, David Margulies

Department of Organic Chemistry, Weizmann Institute of Science, 7610001, Rehovot, Israel.

Email of presenting author: leila.motiei@weizmann.ac.il

Fluorescent molecular sensors have become valuable tools in the analytical biosciences owing to their high sensitivity and their ability to track proteins in their native environment. A major limitation in using these probes, however, is the lack of a general and easily applicable method for providing them with high selectivity and high signal-to-noise ratios. In addition, many of these sensors are designed according to the "lock and key" paradigm; therefore, they cannot be used to analyze biomolecule combinations.
In our group, we aim to address these problems by developing novel classes of fluorescent molecular sensors.[1-3] I present our recent development of fluorescent switches that light up in the presence of specific protein biomarkers, as well as probes that can shed light on analyte combinations in biofluids. In addition, the design of a sensory system that utilizes both specific and non-specific interactions for distinguishing between protein isoforms will be discussed.

1. L. Motiei, Z. Pode, A. Koganitsky, D. Margulies, *Angew. Chem. Int. Ed.* **2014**, 53, 9289.
2. L. Unger-Angel, B. Rout, T. Ilani, M. Eisenstein, L. Motiei, D. Margulies, *Chem. Sci.* **2015**, 6, 5419.
3. B. Rout, L. Motiei, D. Margulies, *Synlett*, **2014**, 25, 1050.

Acknowledgments: This research was supported by the European Research Council Starting Grant 338265, the Israel Science Foundation (ISF), and the German-Israeli Foundation (GIF).

Dual therapeutic agents to improve the effect of Boron Neutron Capture Therapy on cancer cells

Diego Alberti,[a] Simonetta Geninatti Crich,[a] Nicoletta Protti,[b] Morgane Franck,[a] Rachele Stefania,[a] Silvia Bortolussi,[b] Saverio Altieri,[b] Paolo Boggio,[c] Annamaria Deagostino,[c] Silvio Aime [a]

[a] *Department of Molecular Biotechnology and Health Sciences, University of Torino, via Nizza 52, 10126, Torino, Italy;* [b] *Department of Physics, University of Pavia, via Bassi 6, 27100, Pavia, Italy;* [c] *Department of Chemistry, University of Torino,via P. Giuria 7, 10125, Torino, Italy.*

Email of presenting author: diego.alberti@unito.it

Boron Neutron Capture Therapy (BNCT) is a non-conventional radiotherapy that combines low energy neutron irradiation with the presence of boron-containing compound at the targeted cells. Neutrons are captured by nonradioactive isotope ^{10}B that disintegrates into alpha particles and lithium nuclei that cause non reparable damage to the cell where they were generated. Unfortunately, a large majority of chemotherapeutic protocols and radiotherapies can considerably reduce tumour masses, they often fail in causing their complete regression as shown by a high number of tumour recurrence cases. Moreover, the time-dependent development of chemoresistance and radioresistance by a minor cell population within the tumour and the nonspecific toxicity toward normal cells are the other major limitations of standard therapies. For these reason, in recent years, much attention has been devoted to the use of combinations of different therapeutic modalities to treat cancer. Exploiting this approach, BNCT has been used as therapy strategy combined with 1) the anti-cancer activity of curcumin and 2) the inhibition of carbonic anhidrase IX (CAIX) enzyme. In the first study the curcumin boron complex Rubrucurcumin[1] was loaded in folate-targeted Polylactic-co-glycolic acid nanoparticles with an amphiphilic Gd based MRI contrast agent (Gd-DOTAMA). The co-incorporation of Gd-DOTAMA allowed to measure indirectly the boron concentration at the target site by MRI. The measurement of local boron concentration is crucial to determine the optimal neutron irradiation time, to calculate the delivered radiation dose and to evaluate the toxicity of the treatment by determining differences in boron concentration between tumor and healthy tissues. PLGA nanoparticles are targeted to ovarian cancer cells (IGROV-1), by including in the formulation a pegylated phospholipid functionalized with the folate moiety. NCT is performed on IGROV-1 cells internalizing 6.4 and 160 ppm of ^{10}B and ^{157}Gd, respectively. In the second study, carboranes (10 boron atoms) functionalized with sulfonamides[2] have been used as CAIX inhibitors on breast and mesothelioma tumor cell lines. Carbonic anhydrase IX (CAIX) is a hypoxia-inducible enzyme that is overexpressed by cancer cells and plays an important role in tumour acid-base homeostasis by promoting cancer cell survival in hypoxic microenvironment. Recently, a relationship between CA overexpression and tumour cells resistance to chemio or radio therapy has been evidenced and novel antitumour therapies based on the use of CAIX inhibitors derived from acetazolamide, ethoxzolamide, sulfanilamide are under study in clinical. In this study, the CAIX inhibition properties were combined with BNCT and their synergic effect has been evaluated with respect to BNCT used alone.

1. Z. Sui, R. Salto, J. Li, C. Craik, P.R. Ortiz de Montellano, *Bioorg. Med. Chem.* **1993**, *1*, 415.
2. J. Brynda, P. Mader, V. Šícha, M. Fábry, K. Poncová, M. Bakardiev, B. Grüner, P. Cígler, P. Řezáčová, *Angew Chem Int Ed Engl.* **2013**, *52*, 13760.

The capture of PEG and POZ NPs by monocytes/macrophages in serum is mediated by complement activation

Regina Tavano,[a] Silvia Visentin,[a] Alessandra Geffner-Smith,[a] Giorgio Arrigoni,[a] E. Benetti,[c] Dmitri Simberg,[d] S.M. Moghimi,[e] Elisa Lubian,[b] Fabrizio Mancin,[b] Emanuele Papini [a]

[a] *Department of Biomedical Sciences, University of Padua,via Colombo 3, 35127 Padua, Italy;*
[b] *Department of Chemical Sciences, University of Padua, Padua, Italy;* [c] *Department of Materials, ETH, Zurich, CH;* [d] *Department of Pharmaceutical Sciences, University of Colorado, Aurora, USA;* [e] *School of Medicine Pharmacy and Health, University of Durham, UK.*

Email of presenting author: Emanuele.papini@unipd.it

Rapid clearance by RES and pro-inflammatory effects are major problems in nanotheranostics developments. Interacting host proteins in plasma (or *protein corona*) may favor or hamper macrophages capture of nanoparticles (NPs) [1]. NP-mediated complement activation may also contribute to proinflammatory and infusion-related reactions [2]. NPs coating of poly(ethylene glycol) (PEG) to minimize proteins adsorption may trigger complement, thereby influencing phagocytic cell capture and infusion-related reactions. Poly(2-mehyl-2-oxazoline) (POZ) has been proposed as alternative polymer for surface modification of NPs, due to its improved chemical and physicochemical features compared with PEG [3]. With the double goal of characterizing POZ performance and of comparing the importance of protein corona formation vs complement activation, we tested the effect of human serum (HS) on the efficacy of human monocytes and macrophages in capturing 100 nm ORMOSIL-NPs coated with PEG or POZ (prepared by ammonia-catalyzed co-polymerization of vinyltriethoxysilane and suitable PEG or POZ trimethoxysilane derivatives in Brij 35). In no proteins monocytes/macrophage capture was reduced for PEG and POZ-modified NPs with respect to naked NPs. However, in HS, polymer-coated NPs were captured more efficiently compared with the naked NPs counterparts. PEG, but especially POZ coating, increased complement activation and monocytes/macrophages capture through complement opsonization. Importantly, the intensity of such effects were also tested using HS from different human subjects and mouse and pig sera and found to be both subject- and species-dependent. Biochemical functional ad shot-gun proteomic approaches show that, in condition of complement inhibition, the specific presence of defined non-complement proteins and their relative amount in the corona is poorly relevant in determining the stealth property of coated NPs. Complement activation is a major factor affecting the stealthing efficacy of PEG and POZ coats on NPs. POZ, although a promising alternative to PEG, was proved to be much more effective in activating the complement on NPs. This demands the search for complement-inert nanoparticle coating.

1. Fedeli et al. *Nanomedicine*, **2015**, *7*, 17710.
2. S. M. Moghimi, Z. S. Farhangrazi, *Nanomedicine*, **2013**, *9*, 458.
3. Sedlacek O. et al. *Macromol. Rapid Commun*., **2012**, *33*, 1648.

Acknowledgments: This work was supported by the University of Padova (Ex 60%, 2010–2015, PRAT 2011, Progetto Strategico di NAMECA) and by the Italian Ministry of Research (FIRB 2011, RBAP114AMK-RINAME) and is in partial fulfilment of the PhD degree of A. G-S.

Nanoparticles for an Innovative Administration of Statins

Adryana Clementino,[a,b] Michele Pozzoli,[a] Nkemjika Abiakam,[a] Rugero Bettini,[a] Fabio Sonvico [a]

[a] *Department of Pharmacy., University of Parma, Parco Area delle Scienze, 43124, Parma, Italy;*
[b] *National Counsel of Technological and Scientific Development – CNPq – Science without Borders Program- CsF, SHIS QI 1, Brasilia, Brazil.*

Email of presenting author: adryanarc@gmail.com

The blood brain barrier (BBB) represents the major obstacle to the delivery of therapeutics for Central Nervous System (CNS) disease treatments. Intranasal (IN) route has emerged as a potential way to provide a non-invasive method of bypassing the BBB and to potentially deliver drugs to the CNS.[1] Despite its potential, nasal delivery system faces some distinctive limitations that restrict its application: for instance, the mucociliary clearance reduces the permanence of active molecules in the nasal cavity, the delivery of hydrophobic molecules can be hampered by their poor aqueous solubility and high concentrations of drugs need to be present in the formulation. Mucoadhesive nanoparticles have been suggested to overcome these limitations, promoting an increase in the residence time, permeation enhancement and a prolonged release of the drug into nasal cavity.[2] Simvastatin (SVT) is a poorly soluble pro-drug that recently has been proposed for the treatment of several diseases, such has ischemia and neurodegenerative diseases due to its pleiotropic properties. These properties require high levels of SVT at the target organ and it is well known that statins oral bioavailability is suboptimal as a consequence of the hepatic first pass effect. In this context the purpose of this study is to develop and characterize a novel formulation based on lecithin-chitosan nanoparticles (NLC) with the addition of different oils able to encapsulate this drug, exploiting, so far, an approach that allows high drug loading, mucoadhesion and a quick release of the drug, in contrast to previously adopted polymer nanoparticles.
NLC are formed by the electrostatic interaction between chitosan and lecithin. The nanoparticles were prepared injecting of an ethanol solution of 2.5% soybean lecithin containing SVT 12.5 mg/mL and selected oils into a 0.01% chitosan aqueous solution. Nanoparticles were characterized for the zeta potential (ZP), mean diameter, polydispersity index, drug load content and number of nanoparticles for mL of the nanoemulsion by Nanoparticles Tracking Analysis (NTA). The release of the system was evaluated in simulated nasal fluid (SNF). In order to improve the SVT-NLC drug loading capacity, different oils were tested. The binary combination between Maisine™ and Labrafac® oils showed the best results in terms of diametric size (200±18 nm), polydispersity index (0.098±0.04), surface charge (+49±5,48 mV), drug loading capacity (96.87±2.0%) and excellent stability up to 3 months. The NTA display a concentration of 1.57 10^{14} nanoparticles/mL. The *in vitro* drug release was measured via the dialysis bag diffusion method for 8 hours period in SNF pH 6.5 in the presence of Bovine Serum Albumin (BSA) according to the sink conditions. The experiment highlighted that SVT-NLC displayed a considerably faster release than the SVT-Suspension, with the cumulative amount released around 40±2.4% released by SVT-NLC formulation compare to 21±0.5% by the drug suspension.
In conclusion, it could be ascribe that SVT-NLC have demonstrated to possess the required characteristics necessary, at this first stage, to be testing for nasal drug delivery, showing a very good potential to overcome the main limitations of the IN route.

1. J. Lochhead, R. Throne, *Advanced Drug Delivery Reviews*, **2012**, *64*, 614.
2. L. Illum, *Journal of Pharmaceutical Sciences*, **2007**, *96*, 473.

"Bionanoparticles": versatile platforms for nanomedicine

Jakub W. Trzcinski,[a] Regina Tavano,[b] Emanuele Papini,[b] Polo Scrimin,[a] Fabrizio Mancin [a]

[a] *Dipartimento di Scienze Chimiche, Università di Padova, Via Marzolo 1,35131 Padova, Italy;*
[b] *Dipartimento di Scienze Biomediche, Università di Padova, Viale Giuseppe Colombo 3, 35131 Padova, Italy.*

Email of presenting author: jakubwaldemar.trzcinski@unipd.it

One of the major problems in nanoparticle-based biomedical applications is related to the fate of the nanosystems after their administration. Biodegradable carriers are highly desirable. However, they should be able to conjugate such feature with a prolonged stability in aqueous solution, to prevent unwanted and premature degradation. In this communication we report on our recent advances in the study on "bionanoparticles" which demonstrate prominent potential in the field of nanomedicine. In particular we focused our interest on the lipoic acid molecule, which has antioxidant properties and is an enzymes cofactor. Taking advantage for the well-known thiol induced polymerization of lipoic acid[1], we were able to prepare a universal and non-toxic nano-platform constituted by a solid crosslinked polymeric matrix of lipoic acid monomers stabilized by the poloxamer surfactant (Figure 1). The presence of carboxyl moiety in lipoic acid provides easy monomer functionalization aimed at obtaining a library of adaptable precursors for a nano-carriers formation. The nanoparticles are stable in water for months and are prepared by facile and fast nanoprecipitation methodology in aqueous environment, ensuring control over the size and chemical composition (Figure 2). By dint of hydrophobic effect the polymeric core can be loaded with a wide range of active molecules: fluorophores - for tracking and theragnostic, likewise drugs, that can be crosslinked together with lipoic acid monomers, or entrapped between polymer strands. The preliminary biological studies demonstrate an high stability in the biological media together with relevant "stealth" features giving opportunity to selectively "address" nanoparticles to the interesting tissue. Consequently, polymeric nanoparticles, might offer novel opportunities for the design of new classes of highly biocompatible nano-carriers for therapeutic use.

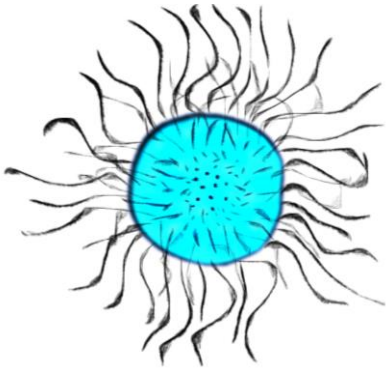


Figure 1

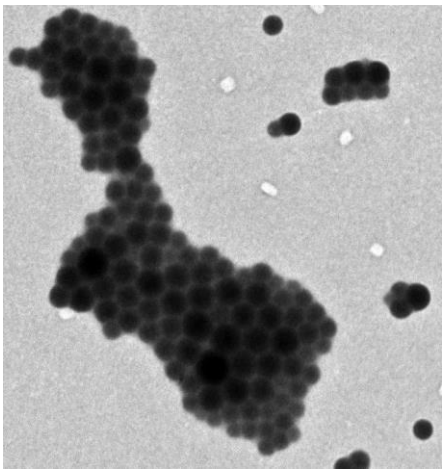


Figure 2

[1] G. Gasparini et al., *J. Am. Chem. Soc*. **2014**, *136*, 6069.

Safety and uptake of solid lipid nanoparticles by airway epithelial cells: an *in vitro* study

Stefano Castellani,[a] Adriana Trapani, [b] Delia Mandracchia, [b] Sante Di Gioia,[a] Miriana d'Alessandro,[a] Giuseppe Trapani,[b] Massimo Conese [a]

[a] *Department of Medical and Surgical Sciences, University of Foggia, Viale Luigi Pinto 1, 71122, Foggia, Italy;* [b] *Department of Pharmacy-Drug Sciences, University of Bari "Aldo Moro", Via E. Orabona 4, 70125 Bari, Italy.*

Email of presenting author: stefano.castellani@unifg.it

Objectives: In the last decade, it has been demonstrated that solid lipid nanoparticles (SLN) are pharmaceutical nanocarriers capable to incorporate biologically active substances. The aim of the present study is to test the safety and the internalization efficiency of SLN in airway epithelial cells in vitro, in order to use these nanoparticles as carriers for anti-oxidant natural substances such as resveratrol or lycopene in future studies.

Methods: Fluorescently labelled SLN were formulated according to the melt-emulsification method, by using Gelucire® 50/13 as lipid and green fluorophore 6-coumarin (6-COUM) as dye[1]. Starting by an initial dose of 6 mg of 6-COUM, the resulting particles were found around 235 nm in mean size and quite spherical in morphology as shown by TEM microphotograph (Fig. 1). Moreover, a core-shell structure is well evidenced according to that reported for these nanocarriers[2]. Thus, the 4 nm wide dark circular line around the SLN can be reasonably ascribed to the surfactant monolayer shell surrounding the SLN.

To study the uptake of the particles, airway epithelial cells H441 were incubated with three doses (0.2, 1, 10 μg/ml) of freshly prepared SLN carrying 6-COUM for 4, 8 or 24 h; then cells were treated or not with trypan blue (0.04%) to quench the fluorescence of the membrane-associated but not internalized nanoparticles, and then analyzed by flow cytometry. We also evaluated the cytotoxicity of the SLN by incubating cells with the same doses and for the same times used in the uptake experiments and performing MTT assay. The viability of the treated cells was also studied by propidium iodide exclusion assay by flow-cytometry.

Results: Flow cytometry analysis demonstrated that even at the lowest dose (0.2 μg/ml) and after only 4 h of incubation, the cells were highly positive for the 6-COUM fluorescent signal (~80%) and the percentage increased to 100% with the other doses. We also observed the persistence of 6-COUM signal in cells up to 10 days, 14 days and 16 days with 0.2, 1, and 10 μg/ml respectively, after 4 h of incubation. Both MTT assay and propidium iodide exclusion assay demonstrated a very low and not significant cytotoxicity at the three tested doses.

Conclusions: We have found optimal and safe doses of SLN to obtain a high rate of internalization in an airway epithelial cell line; in the next future we will load these SLN with anti-oxidant natural substances to asses their efficacy *in vitro* as a potential therapy for airway chronic inflammatory diseases such as asthma or COPD.

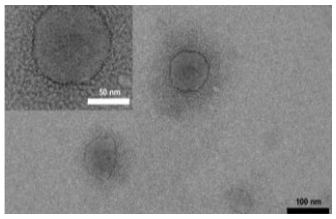


Figure 1. TEM observations of 6-COUM loaded SLN.

1. Trapani, D. Mandracchia, C. Di Franco, H. Cordero, P. Morcillo, R. Comparelli, A. Cuesta, M.A. Esteban. *Biointerfaces*, **2015**, *127*, 79.
2. N. Matougui, L. Boge, A.C. Groo, A. Umerska, L. Ringstad, H. Bysell, P. Saulnier. *Int J Pharm*, **2016**, *502*, 80.

Acknowledgments: Stefano Castellani is a researcher funded by the project "Future in research" of the Regione Puglia.

Liposome-based steroidal nano-drugs for the treatment of inflammatory diseases: from basic to the clinics

Keren Turjeman, Yechezkel Barenholz

Department of Biochemistry and Molecular Biology, Institute for Medical Research Israel-Canada (IMRIC), The Hebrew University-Hadassah Medical School, Jerusalem, Israel.

Email of presenting author: Kerenbarhum@gmail.com

During the last 50 years different strategies have been used for formulating liposomal glucocorticosteroids (GCs). The attractiveness of liposomal GCs lies in the well-known and extensive use of GCs in many diseases. However, in many cases their use is limited by severe side effects and toxicities. These are related to the drugs' unfavorable pharmacokinetics and biodistribution which require high and frequent administrations to achieve sufficient therapeutic levels and therapeutic efficacy.

Various liposomal formulations were tried in order to overcome these major obstacles. But now it is clear that for systemic use only GCs in PEGylated nano-liposomes may serve as good candidates. Such nano-liposomes show steric stabilization, high stability in plasma, and long circulation time. This enables them to "take advantage" of the inflamed (and cancerous) tissues' unique micro-anatomical vascular abnormality, the enhanced permeability and retention (EPR) effect, achieving passive targeting to the inflamed tissue.

We formulated such 80nm PEGylated nano-liposomes (NSSL), remotely loaded via a trans-membrane acetate gradient, with "water-soluble" amphipathic weak acids, steroid prodrugs as the active pharmaceutical ingredient. This remote loading is further stabilized by the precipitation of the amphipathic weak acid with the selected counterion (Ca^{+2}), contributing to the control of drug release at various temperatures. The use of the high T_m (53^0C) hydrogenated soy phosphatidylcholine (HSPC) as the "liposome-forming lipid" together with high mole% cholesterol (38 mole%) result in a zero-order, slow, drug release at the targeted inflamed tissue. The resulted nano-drug demonstrates high drug-to-lipid mole ratio, high loading efficiency (>95%), and high stability (>1.5 years upon storage). A GMP scaled-up production procedure was developed resulting in liters scale sterile pyrogen free product.

Our results using animal models of rheumatic arthritis, multiple sclerosis, cerebral malaria, lupus, Duchenne muscular dystrophy, carrageenan- and zymosan-induced inflammation show that these nano-drugs therapeutic efficacies are much superior to the same steroid administered "as is" and to other drugs and/or biologicals currently used to treat these diseases. The superior therapeutic effect was accompanied with improving the pathology of the inflamed tissue, reduction in the secreted inflammatory cytokines' levels as well as reduced toxicity following long-term treatment.

The much improved tolerability to these nano-drugs, together with their highly efficacious therapeutic features support our current clinical development of steroidal NSSL as potential therapeutic agents for inflammatory diseases.

Nanoassemblies based on folate-tailored amphiphilic cyclodextrin / pheophorbide complexes for targeted PDT

Giuseppe Sortino,[a] Anna Piperno,[b] Angela Scala,[b] Roberto Zagami,[a] Valentina Rapozzi,[c] Luigi Monsù Scolaro,[b,d] Antonino Mazzaglia [a]

[a] *CNR-ISMN c/o Dept. of Chemical, Biological, Pharmaceutical and Environmental Sciences of the University of Messina, Viale F. Stagno D'Alcontres 31, 98166, Messina, Italy;* [b]*Dept. of Chemical, Biological, Pharmaceutical and Environmental Sciences, University of Messina, Viale F. Stagno D'Alcontres 31, 98166, Messina, Italy;* [c]*Dept. of Medical and Biological Sciences, University of Udine, P.le Kolbe 4, 33100 Udine, Italy;* [d] *C.I.R.C.M.S.B. Unità di Messina, Italy.*

Email of presenting author: antonino.mazzaglia@ismn.cnr.it

Nowadays, active targeting of a drug in tumor sites is generally accomplished by tailoring a nanosystem with receptor targeting groups such as folate, antibodies, saccharides and peptides. Targeted PDT treatment relies on the selective accomodation of a photosensitiser (PS) in tumor sites following irradiation, thus generating the cytotoxic singlet oxygen ($^1O_2$).[1] Here we report a nanoassembly based on amphiphilic cyclodextrin carrier (aCD, SC6OH), entrapping pheophorbide (Pheo) as PS and tailored with folate–adamantanyl (Ada-Fol) as folate receptor (FOLR1) targeting group. SC6OH@Ada-Fol /Pheo nanoassemblies (**1**) (see Figure 1), prepared by hydration of organic film and sonication, were studied by complementary techniques such as UV-Vis, steady-state and time-resolved fluorescence. The nanosystem **1** showed an hydrodynamic radius of ~ 300 nm , Z-potential of ~ -45 mV, Pheo loading and entrapment efficiency of ~ 4% and ~66%, respectively. Pheo was retained for ≅ 2 weeks from **1** in PBS (pH=7.4) at 37°C. In order to verify the targeting properties, we evaluated in vitro the effectiveness of **1** on cell growth for different cancer cell lines over-expressing FOLR1 (MCF-7, MD-MBA and A549) and very low expressing FOLR1 (PC3). Our data indicate that the nanoassembly **1**, upon red-light irradiation, inhibits cell proliferation depending on FOLR1 expression.

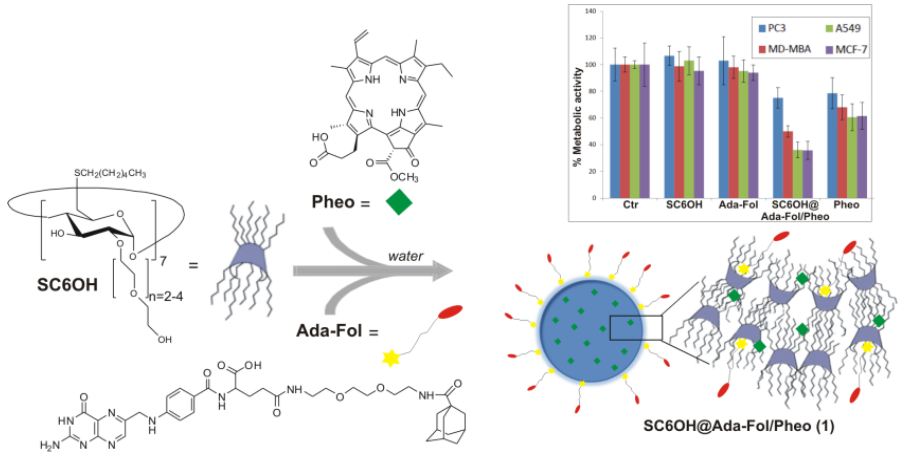


Figure 1. Sketched depiction of **1** formation. In the inset: metabolic activity on MCF-7, MD-MB A549 and PC3 treated with **1** ([Pheo] = 500 nM, 3 h, Resazurin assay) ad upon red-light irradiation.

1. C. Conte, F. Ungaro, A. Mazzaglia, F. Quaglia, in Nano-Oncologicals: New Targeting and Delivery Approaches, Advances in Delivery Science and Technology, eds. M. J. Alonso and M. Garcia-Fuentes, 2014, CRS Springer p. 123 and ref therein.

Near-infrared photoactivable poly-methylmethacrylate core-shell fluorescent nanoparticles

Claudia Ferroni,[a] Sara Ramos-Romero,[c] Barbara Dozza,[b] Marta Guerra-Rebollo,[c] Luca Cattini,[d] Marco Ballestri,[a] Paolo Dambruoso,[a] Andrea Guerrini,[a] Giovanna Sotgiu,[a] Greta Varchi,[a] Enrico Lucarelli,[b] Elisa Martella,[b] Jeronimo Blanco,[c] Serena Duchi [b]

[a] *Institute of Organic Synthesis and Photoreactivity – Italian National Research Council, Via Gobetti, 101 40129 Bologna, Italy;* [b] *Osteoarticular Regeneration Laboratory, Rizzoli Orthopaedic Institute, Via di Barbiano 1/10 40136 Bologna, Italy;* [c] *Cell Therapy Group, Institute for Advanced Chemistry of Catalonia (IQAC), CSIC, Barcelona, Spain;* [d]*Laboratory of Immunorheumatology and Tissue Regeneration/RAMSES, Rizzoli Orthopaedic Institute, Via di Barbiano 1/10 40136 Bologna, Italy.*

Email of presenting author: claudia.ferroni@isof.cnr.it

Conventional photodynamic therapy (PDT) has shown to be beneficial in the treatment of different tumours. However, one of its major limitations is the use of photosensitizers (PS) photoactivable in the visible light range that restricts its efficacy towards superficial types of cancer.[1] In order to overcome this constraint, in the present work we describe the preparation of 80 nm poly-methylmethacrylate core-shell fluorescent nanoparticles (FNP) loaded with the photosensitizer tetrasulfonated aluminum phtalocynine (Ptl)[2] that can be activated by near-infrared light (Ptl@FNP). To demonstrate the efficacy of our Ptl@FNP we performed *in vitro* and *in vivo* studies using a human prostate tumor model. Our data revealed that Ptl@FNP are internalized by tumor cells and efficiently trigger cell death through the generation of ROS upon irradiation with 680 nm light. When directly injected into PC3 tumor xenografts intramuscularly induced in SCID mice, Ptl@FNP upon irradiation significantly reduced tumor growth with higher efficiency than the bare Ptl. With the aim of improving selectivity of Ptl@FNP, future investigation with Mesenchymal Stromal Cells as a cell-delivery vehicle will be performed.[3]

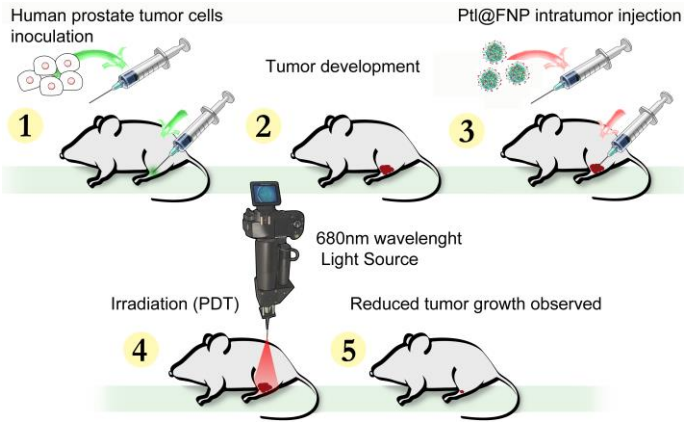


Figure 1. The efficacy of Ptl@FNP was tested in a human prostate tumor animal model. Near-infrared light irradiation of Ptl@FNP intratumorally injected significantly reduces tumor growth.

1. X. Zhang, T. Liu, Z. Li, X. Zhang, *Oncol. Lett.*, **2014**, 1403.
2. I. Mfouo-Tynga, H. Abrahamse, *Int. J. Mol. Sci.* **2015**, *16*, 10228.
3. S. Duchi, G. Sotgiu, E. Lucarelli, M. Ballestri, B. Dozza, S. Santi, A. Guerrini, P. Dambruso, S. Giannini, D. Donati, C. Ferroni, and G. Varchi, *J. Control. Release*, **2013**, *168*, 225.

Nano-heaters for cancer therapy: a comparison between gold nanorods and magnesium Nanoparticles

Mauro Comes Franchini, Erica Locatelli, Ilaria Monaco

Department of Industrial Chemistry "Toso Montanari", University of Bologna, Viale Risorgimento 4, 40136 Bologna, Italy.

Email of presenting author: mauro.comesfranchini@unibo.it

Photothermal therapy in nanomedicine is emerging as a very important strategy for cancer treatment but despite the many advantages of application of nanomaterials in the field of nanomedicine, increasing concerns have been expressed on their potential adverse effects on human health. There is urgency for novel green strategies toward novel materials with enhanced biocompatibility using safe reagents.
Herein, we report the synthesis of Mg NPs and their surface functionalization for the obtainment of a stable and biocompatible nanomaterial suitable for photothermal ablation therapy against cancer. Magnesium nanoparticles (20-30 nm) with a salt reduction methodology and their coating with organic ligand, while GNRs were synthetized using the so-called "seed-mediated growth method".
These novel system based on Mg proved the possibility of generating a temperature rise of a few to several degrees once nanoparticles batches containing 5.3 mM of Mg were illuminated with a 810 nm diode laser operating in continuous wave mode and we have investigated whether the photo-thermal response could vary as a function of Mg concentration

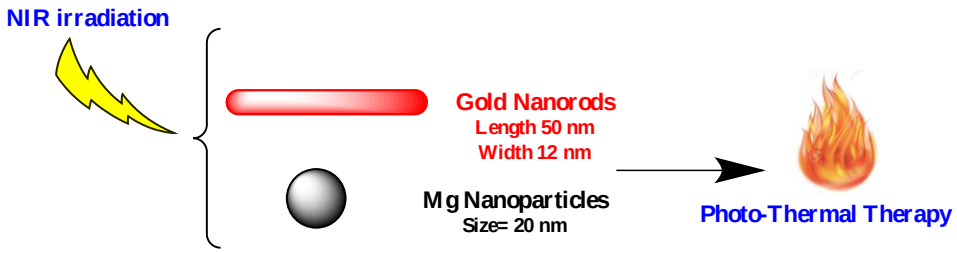


Figure 1. Near-Infrared Irradiation (NIR) of Mg nanoparticles and gold nanorods for photothermal therapy.

Following these protocols therefore, novel highly biocompatible nano/heater are characterized and tested as safe and non-toxic biomaterials for clinical applications.
A comparison among our well-established approach using gold nanorods[2] and Mg nanoparticles will be discussed.

1. E. Locatelli, P. Matteini, F. Sasdelli, A. Pucci, M. Chiariello, V. Molinari, R. Pini, M. Comes Franchini**.** *Chem. Commun*. **2014**, *50*, 7783.
2. R. C. Martin, Erica Locatelli, Y. Li, Paolo Matteini, Ilaria Monaco, G. Cui, S. Li, Martina Banchelli, Roberto Pini and M. Comes Franchini. *J. Mat. Chem. B.* **2016**, *4*, 207.
3. R. C. G. Martin II, E. Locatelli, Y. Li, W. Zhang, S. Li, I. Monaco, M. Comes-Franchini. *Nanomedicine*, **2015**, *10*, 1723.

Acknowledgments: University of Bologna is gratefully acknowledged

Keratin-based nanoparticles: novel tools for drug delivery

Greta Varchi,[a] Annalisa Aluigi,[a] Giovanna Sotgiu,[a] Claudia Ferroni,[a] Serena Duchi,[b] Andrea Guerrini,[a] Cecilia Martini,[a] Marco Ballestri,[a] Enrico Lucarelli [b]

[a] *Institute of Organic Synthesis and Photoreactivity – Italian National Research Council, Via Gobetti, 101 40129 Bologna, Italy;* [b] *Osteoarticular Regeneration Laboratory, Rizzoli Orthopaedic Institute, Via di Barbiano 1/10 40136 Bologna, Italy.*

Email of presenting author: greta.varchi@isof.cnr.it

Keratin it is the most abundant non-food natural protein, being the major component of wool, feathers, hair, horns and nails. It is a cysteine-rich structural protein, which possesses excellent bio-compatibility and low toxicity to cells, thus representing a very promising material in the nanoparticles-mediated drug delivery field.[1,2]

In the present work we describe the preparation of keratin and chlorin e6-conjugated keratin nanoparticles (KNPs@Ce6), and we provide an in vitro proof-of-concept of their ability to function as nanocarriers for cancer photodynamic therapy. Nanoparticles were synthesized by both self-assembling and desolvation methodologies and characterized in terms of yield, size, morphology, Ce6 loading ratio and ability to produce reactive oxygen species (ROS) upon irradiation with white light. In vitro internalization and photo-toxicity studies were performed on osteosarcoma (U2OS) and glioblastoma (U87) cells lines. Importantly, no dark toxicity was detected, while the amount of Ce6 inside the cells significantly increased when loaded onto nanoparticles. The irradiation of tumour cells loaded with KNPs@Ce6 resulted in an greater cell death percentage as compared to free Ce6 in both cell types and at all the considered concentrations.

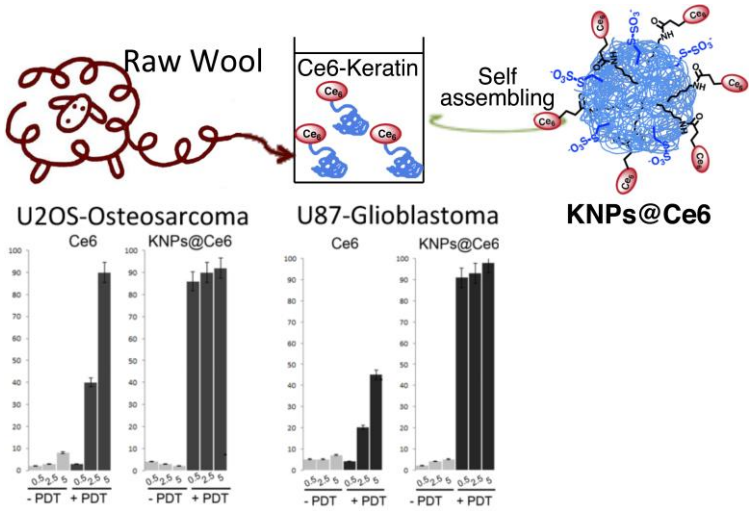


Figure 1. Keratin nanoparticles synthesis and phototoxicity on osteosarcoma and glioblastoma cell lines.

1. H. Xu, Z. Shi, N. Reddy, and Y. Yang, *J. Agric. Food Chem.*, **2014**, *62*, 9145.
2. X. Zhi, Y. Wang, P. Li, J. Yuan, and J. Shen, *RSC Adv.*, **2015**, *5*, 82334.

In vitro and *in vivo* characterization of new nano-sized diagnostic probes for Photoacoustic Imaging

Martina Capozza, Luisa Poggi, Simona Ghiani, Paolo Oliva, Claudia Cabella, Alessandro Maiocchi

Centro Ricerche Bracco, Bracco Imaging S.p.A., Colleretto Giacosa, Torino, Italy.

Email of presenting author: Martina.Capozza@bracco.com

Nano-sized agents with long circulation times accumulate preferentially into tumor tissue through a leaky tumor vasculature and retention in the tumor environment due to reduced lymphatic drainage. However it is known that the enhanced permeability and retention (EPR) effect is heterogeneous among tumor types and within individual tumors. Therefore, the availability of an imaging tool that can report on the extent of the EPR effect could in principle improve the effectiveness of the administered therapy particularly in case of nanomedicines providing additional insights on the response monitoring[1,2]. The technique explored in the present work is Photoacoustic Imaging (PAI), a new hybrid modality based on the detection of acoustic waves generated by the absorption of short laser pulses in biological tissues. The diagnostic value of this emerging imaging modality has been demonstrated in several recent preclinical and clinical studies[3].

In this research work, we report the *in vitro* characterization and *in vivo* application of some nano-sized probes for Photoacoustic Imaging. We focused on different nano-sized systems with different dimensions for a further evaluation of their extravasation properties in tumor tissues. The nano-sized systems tested were as follows: (i) a fluorescent small molecule with albumin binding properties, characterized by and average diameter of 5 nm; (ii) dye loaded Solid Lipid Nanoparticles (SLNs, with a mean diameter of 45 nm) (iii) dye loaded liposomes (100 nm); (iv) Gold Nanorods (40 nm x 10 nm)[4]. The main properties that influence the generation of photoacoustic signal, such as molar extinction coefficient, fluorescent quantum yield and albumin binding were investigated *in vitro*. Moreover, the photoacoustic signal was also measured by means of an agar phantom in different media such as phosphate buffer and serum. Based on the above parameters, a subset of nano-sized probes having enhanced photoacoustic signal was identified and their investigation was extended to healthy animals to confirm their *in vivo* efficiency. When possible also Near Infrared optical imaging was employed to characterize the biodistribution of the probes in the main organs and tumors.

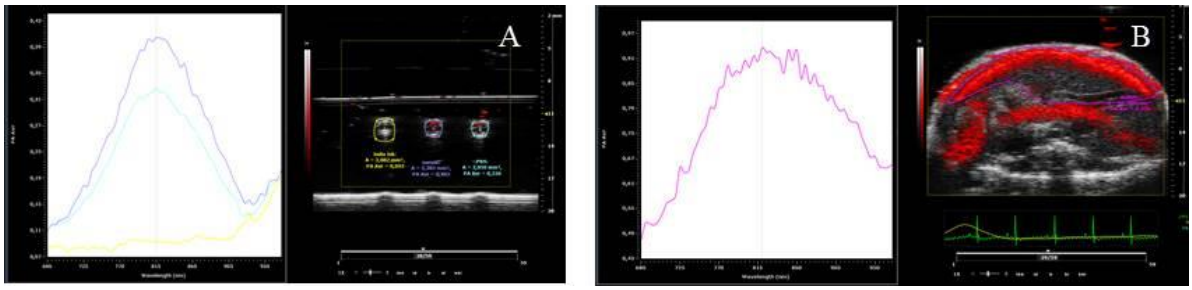


Figure 1. PAI spectra of Gold Nanorods (a) *in vitro* and (b) *in vivo* in liver.

1. Y. Matsumura, H. Maeda, *Cancer Res.*, **1986**, *46*, 6387.
2. F. Kiessling, ME Mertens, J. Grimm, T. Lammers, *Radiology.*, **2014,** *273*, 10.
3. S. Zackrisson, SM van de Ven, SS. Gambhir, *Cancer Res.*, **2014**, *74*, 979.
4. W. Li, X. Chen, *Nanomedicine (Lond).*, **2015**, *10*, 299.

ICG-loaded Mesoporous Silica Nanoparticles as photoacoustic contrast agents

Lorenzo Tei,[a] Giuseppe Ferrauto,[b] Fabio Carniato,[a] Enza Di Gregorio,[b] Mauro Botta,[a] Silvio Aime [b]

[a] *Dipartimento di Scienze e Innovazione Tecnologica, Università del Piemonte Orientale "Amedeo Avogadro", Viale T. Michel 11, 10121 Alessandria, Italy;* [b] *Department of Molecular Biotechnology and Health Sciences, University of Turin, Via Nizza 52, 10126 Turin, Italy.*

Email of presenting author: lorenzo.tei@uniupo.it

Indocyanine Green(ICG) has been extensively used in medical diagnostic, in preclinical optical studies and recently in photoacoustic imaging (PAI). However, the in vivo application of ICG is hampered by its intrinsic low stability and solubility. Herein, ICG dyes have been loaded inside MCM-41 Mesoporous Silica Nanoparticles (MSNs) and their optical/ photoacoustic properties have been tested and compared with those of free ICG.
MCM-41 were prepared through a sol-gel procedure and functionalized with aminoproyl groups. In a second step, MCM-41 NPs were suspended in a methanol solution of ICG for 8h at room temperature. Three different ICG loading were tested. Finally, their surface was functionalized with PEG5000 to increase the suspendibility and characterized by UV-Visible and Photoluminescence spectroscopy. PAI analysis was carried out by using a VisualSonics Vevo 2100 LAZR Instrument. For in vitro PAI, ICG-MSNs suspensions were loaded onto plastic capillaries sorrounded by agarose gel. For in vivo PAI, mice bearing subcutaneous tumors were injected with ICG-MSNs and PA-images of tumor region were acquired. Cell experiments have been carried out by using murine macrophages to evaluate toxicity of ICG-MSNs and their cellular uptake.
ICG-MSNs are 50nm-spherical particles with surface PEG and NH_2/NH_3^+ groups and containing different amounts (0.80-3.7 μmol/g) of ICG molecules inside the channels (2.5 nm size). ICG-MSNs have an UV-vis absorption at 810nm and emit at 820-840 nm. The photodegradation (Bleaching tests) of ICG inside silica is completely prevented after 1h of light. In opposite, the free dye showed a degradation of ca.60%. These particles are not toxic; cell viability of macrophages remains ca.100% up to a ICG-MSNs concentration of 5 mg/mL. Finally, the internalization efficiency of free ICG is higher than that one of ICG-MSNs. The acquisition of PAI spectra shows that that these nanoparticles display two PA peaks (725 and 810 nm). When ICG is inside MSNs, the PAI efficiency is one order of magnitude higher than that displayed by free ICG. As proof of concept, upon i.v. administration of ICG-MSNs in mice, a PAI signal enhancement of ca. 25% has been observed in a ROI drawn inside tumor region.

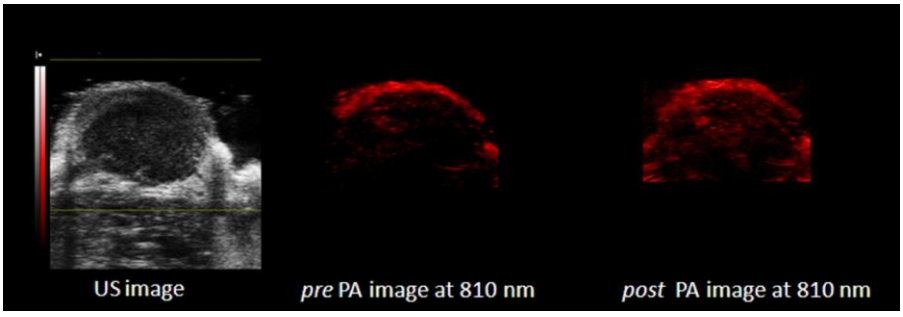


Figure 1. *left*) US image of subcutaneous tumor; (*middle*) PA image of subcutaneous tumor at 810 nm *pre* injection of ICG-MSNs; (*right*) PA image of subcutaneous tumor at 810 nm *post* injection of ICG-MSNs.

Cyclic RGD functionalized PEGylated gold nanoparticles for tumor targeting

Vito Maggi,[a,c] Francesca Bianchini,[b] Roberta Del Sole,[c] Elisabetta Portioli,[d] Andrea Sartori,[d] Roberto Fiammengo [a]

[a] *Center for Biomolecular Nanotechnologies@UniLe, Istituto Italiano di Tecnologia ,Via Barsanti, 73010, Arnesano(Lecce), Italy;* [b] *Dipartimento di Scienze Biomediche Sperimentali e Cliniche "Mario Serio" - Centro Interdipartimentale per lo Sviluppo Preclinico dell'Imaging Molecolare (CISPIM)-Università degli Studi di Firenze, Viale GB Morgagni 50 50134 Firenze;* [c]*Department of Engineering for Innovation, University of Salento, via per Monteroni Km 1, 73100 Lecce, Italy;*
[d] *Dipartimento di Farmacia, Università degli Studi di Parma,Viale Parco Area delle Scienze 27/a, 43124 Parma, Italy.*

Email of presenting author: vito.maggi@iit.it

Integrin $\alpha_v\beta_3$ is a cell-adhesion molecule involved in angiogenesis, tumor invasion and metastasis which is overexpressed by tumor cells as well as by the endothelial cells of tumor neovasculature. The most potent $\alpha_v\beta_3$ ligands are cyclic peptides (or peptide analogues) containing the RGD sequence. Ligand affinity can be further enhanced by utilizing multivalent scaffolds. Gold nanoparticles (AuNPs) are ideal platforms for the multivalent presentation of RGD ligands targeting $\alpha_v\beta_3$ integrins and could be exploited for tumor diagnosis and for therapeutic purposes in theranostic formulations.
We have developed novel PEGylated AuNPs[1] functionalized with multiple copies of cyclic-aminoproline RGD peptides (cAmpRGD)[2] which show high affinity and selectivity for $\alpha_v\beta_3$ integrins. In this contribution we will discuss the molecular design, the preparation and characterization of these nanoparticles. Furthermore we will show their excellent targeting properties from data collected using the human melanoma cell line M21 which overexpress integrin $\alpha_v\beta_3$. In fact, cAmpRGD-AuNPs target M21 cells 4 times more efficiently than control AuNPs as demonstrated by ICP-OES, selectively inhibit cellular adhesion to vitronectin (the natural, RGD containing ligand) by 50% at 1 nM concentration, and do not show any significant toxicity at concentrations as high as 10 nM after 24 h as demonstrated by Annexin V/ PI staining assays and no significant alterations of the cell cycle. In view of these characteristics we are working on developing cAmpRGD-AuNPs as novel microSPECT/CT tracers for diagnostic imaging.[3]

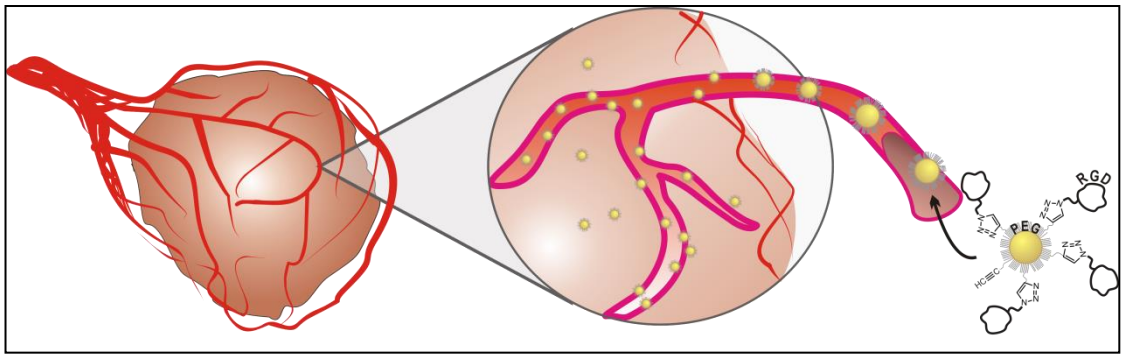


Figure 1. Schematic representation of tumor targeting by PEGylated RGD-AuNPs.

1. L. Maus, O.Dick, H. Bading, J. P.Spatz, R. Fiammengo, *ACS Nano* **2010**, *4*, 6617.
2. A. Sartori, F. Bianchini, S. Migliari, *et al. MedChemComm* **2015**, *6*, 2175.
3. Y.-H. Kim, J. Jeon, S. H. Hong, *et al. Small* **2011**, *7*, 2052.

Organic-inorganic coated Gold Nanorods for biomedical applications

Sarah Lai, Sonia Centi, Claudia Borri, Fulvio Ratto, Lucia Cavigli, Filippo Micheletti, Francesca Tatini, Roberto Pini

Institute of Applied Physyics IFAC-CNR, Florence, Italy.

Email of presenting author: s.lai@ifac.cnr.it

In this work Gold nanorods (GNRs) with two different coatings are presented. In the first, GNRs are coated with a rigid shells of silica starting from an organo-silica precursor. After PEGylation, coated-GNRs are conjugated with a cell penetrating peptides and incorporated into tumor-tropic bio-vehicles. This is an emerging approach as an innovative drug delivery system for photothermal and photoacoustic applications. In the present work we evaluate the thickness of the organosilica shell (from none to ~ 30 nm), in order to understand its effect on the uptake and cell viability. In particular, hybrid SiO_2-GNRs are uploaded into murine macrophages, and their kinetics of endocytic uptake and cytotoxicity are subject to a systematic survey, by standard methods and digital holographic microscopy.
In the second, GNRs are coated with a rigid shell of TiO_2 or a mixed TO_2-SiO_2 shell. A new procedure is used to coat GNRs with this rigid shell and the photostability is compared with GNRs with a silica shell.
Both of particle models are characterized step by step in terms of size, shape, electrokinetic potential, photostability and for SiO_2-GNRs, principal biological profiles are evaluated.
The perspective to engineer the shell at the lengthscale that is enabled by our synthetic protocol is a powerful asset to reach a good control of the properties in the material.

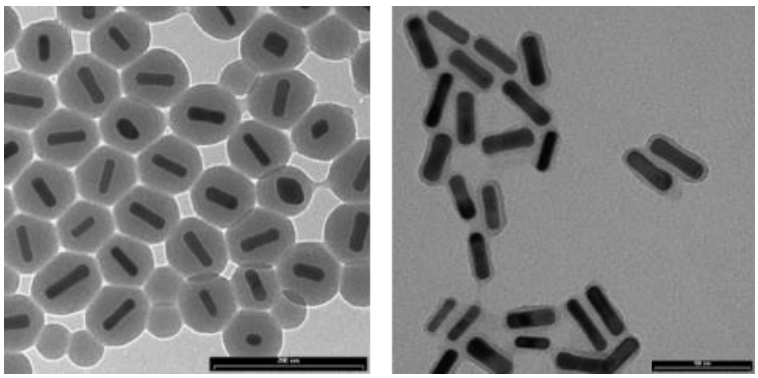


Figure 1. TEM images of GNRs coated with a 30 nm of SiO_2 shell (left) and GNRs coated with a 10 nm of mixed oxide SiO_2-TiO_2 (right).

Smart Hybrid Nanocontainers for Theranostic Applications

Ana S. Rodrigues,[a] Marie-Thérèse Charreyre,[b] Carlos Baleizão,[a] José Paulo S. Farinha [a]

[a] *CFMQ – Centro de Química-Física Molecular and IN – Intitute of Nanoscience and Nanotecnholog, Instituto Superior Técnico, University of Lisbon, Lisbon, Portugal, Av. Rovisco Pais 1040-001 Lisbon, Portugal;* [b] *Laboratory of Engineering of Polymer Materials (IMP), INSA de Lyon and Laboratory Jolio-Curie, ENS de Lyon, 46 Allée d'Italie, 69364 cedex 07 Lyon, France.*

Email of presenting author: anagrodrigues@tecnico.ulisboa.pt

The main objective of our work is to develop hybrid Mesoporous Silica Nanoparticles (MSNs) with theranostic capability, carrying fluorescent beacons for traceability and imaging, featuring a smart release control mechanism, and able to accommodate large drug-loads to be delivered on demand to a desired location. [1] By combining therapeutic and diagnostic (theranostic) functionalities with targeting capabilities and large surface areas, nanoparticles provide an ideal vehicle for personalized medicine. MSNs can be prepared with pore volumes above 1mL/g and particle size from 20 nm to several hundred nanometers. Fluorescent hybrid MSNs, containing fluorescent molecules embedded in the inorganic network, are impervious to self-quenching effects. Their external surface can be selectively functionalized to immobilize polymers or (bio)molecules for possible targeting or sensing, and the pore is available for solvent diffusion, allowing the incorporation of different molecules. [2]
The use of stimuli-responsive polymers to coat MSNs is particularly interesting, offering the possibility of controlling the polymer expanded/collapsed conformation in water by using an external stimulus, such as temperature or pH (Figure 1). This smart shell can act as a gate to control the release from the pore system, thus offering excellent prospects for application in drug delivery and biosensing. [3]
In this work we describe the preparation of fluorescent mesoporous silica nanoparticles with a diameter of ca. 90 nm, incorporating a fluorescent perylenediimide derivative (PDI) in the pore wall structure. The surface of this MSNs is functionalized with amino groups, which are further modified with a RAFT chain transfer agent and then used to polymerize a polymer shell. This polymer shell consists of a new thermoresponsive acrylate-based copolymer obtained by reversible addition-fragmentation chain transfer (RAFT) polymerization. The final goal is to study the copolymer expand/collapse conformation at different temperatures and pH. This vehicle could be used as a new drug delivery system, featuring a smart release mechanism to deliver the drug on demand and in a desired location

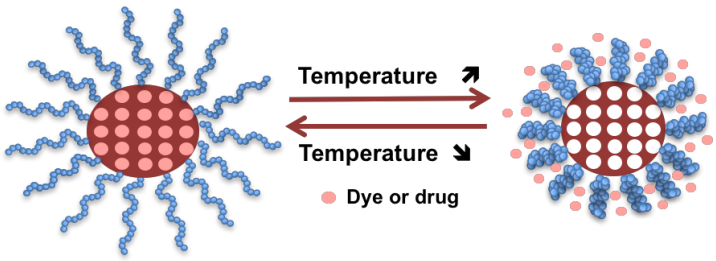


Figure 1. Development of hybrid MSNs as a platform to drug delivery.

1. S.S. Kelkar, T. M. Reineke, *Bioconjugate Chem.* **2011**, *22*, 1879.
2. G. J. A. A Soler-Illia, O. Azzaroni, *Chem. Soc. Rev.*, **2011**, *40*, 1107.
3. A. S. Rodrigues, et al, *Microsc. Microanal.*, **2013**, *19*, 1216.

Acknowledgments: This work was partially supported by Fundação para a Ciência e a Tecnologia (FCT-Portugal) and COMPETE (FEDER), projects RECI/CTM-POL/0342/2012, UID/NAN/50024/2013, PTDC/CTM-POL/3698/2014 and UltraFluoPol (FCT-Portugal / CNRS-France, PICS nº 6411). Ana S. Rodrigues also thanks FCT for PhD (SFRH/BD/89615/2012) grant.

Biosynthesis of injectable gelling peptides for applications in bone tissue regeneration

Laura Chronopoulou,[a] Giuseppina Nocca,[b] Adriana Amalfitano,[b] Alessandro Arcovito,[b] Simona Sennato,[c] Federico Bordi,[c] Ilaria Cacciotti,[d] Cleofe Palocci [a]

[a] *Chemistry Department, University La Sapienza, P.le A. Moro 5, 00185, Rome, Italy;* [b] *Biochemistry and Clinical Biochemistry Institute, Catholic University Sacro Cuore, largo F. Vito 1, 00168, Rome, Italy;* [c] *Physics Department, University La Sapienza, P.le A. Moro 5, 00185, Rome, Italy;* [d] *Engineering Department, Niccolò Cusano University, via Don C. Gnocchi 3, 00166, Rome, Italy.*

Email of presenting author: laura.chronopoulou@uniroma1.it

Tissue engineering and regenerative medicine are part of an emerging and interdisciplinary field that applies the principles of engineering and life sciences towards the development of biological substitutes. This innovative strategy may help identify suitable alternatives to current clinical treatments for a variety of pathologies, such as bone injuries and diseases, whose growing incidence is increasing the demand of medical and healthcare resources. Peptide hydrogels may be used in this context as biocompatible and biodegradable materials suitable for cell encapsulation and for the controlled spatial and temporal delivery of biomolecules (e.g. growth factors) able to direct cell differentiation.
Recently, we developed an enzymatic approach for the preparation of injectable, self-assembling materials based on Fmoc-oligopeptides[1]. The reaction products spontaneously self-assemble in water to form a 3D structure of entangled nanofibers. These materials can be used as controlled drug delivery systems for bioactive molecules[2] and may enhance cell production of growth factors[3]. We employed such hydrogels for the preparation of composite materials specifically designed for bone tissue regeneration. These tailor-made hydrogel systems contain biopolymeric spheres delivering bioactive molecules, as well as pure and substituted calcium phosphate (CaP) nanoparticles to provide bioactivity, osteoconductivity and improved mechanical properties. Ongoing work is aimed at investigating the biological properties of the composite hydrogel systems, in terms of adhesion, growth and differentiation of human mesenchymal stem cells.

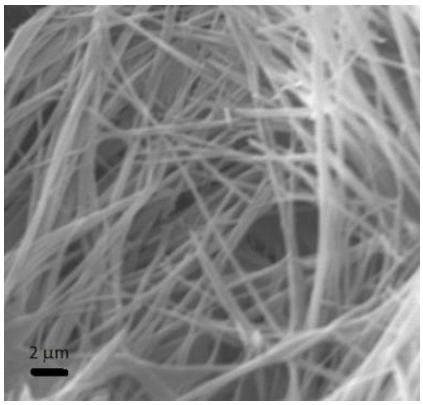


Figure 1. SEM micrograph of FmocFGFF hydrogel.

1. L. Chronopoulou et al., *Soft Matter*, **2010**, *6*, 2525.
2. L. Chronopoulou et al., *Soft Matter*, **2014**, *10*, 1944.
3. L. Chronopoulou et al., *Soft Matter*, **2012**, *8*, 5784.

Promising applications of multifunctional magnetic nanoparticles in biomedicine

Silvia Villa,[a] Chiara Lambruschini,[a] Luca Banfi,[a] Paola Riani,[a] Fabio Canepa,[a] Annalisa Relini,[b] Fabio Morana,[a] Fulvio Silvetti,[a] Renata Riva [a]

[a] *Department of Chemistry and Industrial Chemistry, University of Genoa, Via Dodecaneso 31, 16146, Genoa, Italy;* [b] *Department of Physics, University of Genoa, Via Dodecaneso 33, 16146, Genoa, Italy.*

Email of presenting author: silvia.villa@chimica.unige.it

Multifunctional nanoprobes combining magnetic nanoparticles (MNPs) with organic dyes have attracted great interest due to their promising applications in biomedical field. Among the wide selection of different nanoprobes, iron oxide nanoparticles (SPIONs),[1] loaded with different functionalities, have a very promising application in the drug delivery therapy.
In cancer treatment a possible drug delivery approach is the well known prodrug monotherapy (PMT), in which the drug is released by enzymes naturally overexpressed in tumor tissues. A recognized enzyme suitable for this method is plasmin, a serine protease.
Our project aimed at combining those approaches planning a superparamagnetic probe based on SPIONs, conjugated to a fluorescent tag through a tripeptide linker.[2,3] This peculiar system has been designed to be cleaved by plasmin with the resulting release of the fluorescent tag. Therefore, this original system could find applications either in the imaging diagnostic or in the drug delivery fields (figure 1).

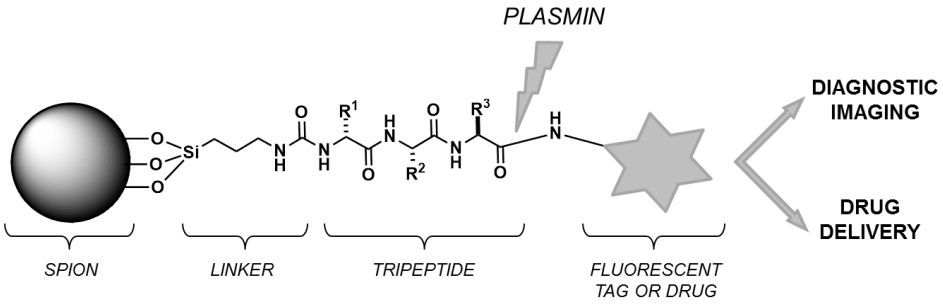


Figure 1. Superparamagnetic iron oxide nanoparticles (SPIONs) conjugated to a fluorescent tag which can be released by plasmin for application in the biomedical field.

Herein, we present the synthesis and the characterization of the two components (SPIONs and the tripeptide linked to a fluorescent tag) and the study of their conjugation. Furthermore, we present the preliminary results of the enzymatic cleavage, as proof of concept of our project.

1. V. V. Mody et al., *Appl Nanosci.*, **2014**, *4*, 385.
2. L. Banfi et al., *Eur. J. Org. Chem.*, **2003**, 1319.
3. G. Eisenbrand et al., *Synthesis*, **1996**, 1246.

Self-assembling octapeptide-polymer conjugate to be used as drug carriers

Federica Novelli,[a] Serena De Santis,[a] Cesare Giordano,[b] Pasqualina Punzi,[a] Valerio Viali,[a] Giancarlo Masci,[a] Anita Scipioni [a]

[a] *Department of Chemistry, Sapienza University of Rome, P.le A. Moro, 00185, Rome, Italy;*
[b] *Institute of Molecular Biology and Pathology, CNR, Sapienza University of Rome, P.le A. Moro, 00185, Rome, Italy.*

Email of presenting author: federica.novelli@uniroma1.it

Soft biomaterials based on engineered peptide nanotubes have attracted huge interest for applications in the fields of drug delivery.[1] Among different kinds of peptide nanotubes, those based on peptides characterized by regularly alternating enantiomeric sequences are particularly attractive, since they provide low-pitch helices that self-assemble in stacks directed and stabilized by hydrogen bonds.[2] When such peptides are conjugated with the suitable polymer in order to achieve the proper balance of hydrophobic/hydrophilic regions, these conjugates are able to self-assemble in water in stable nanoparticles (NPs) with core/shell morphology and enhanced resistance to phagocytosis due to the enantiomeric sequences. Herein, the synthesis of the conjugate For-(D-Phe-L-Cys(Acm))$_4$-NH-(CH$_2$-CH$_2$-O)$_{45}$-CH$_3$ (PEP8-Phe-PEG) and investigations on its self-assembling properties through NMR, CD and fluorescence spectroscopies, DLS, TEM and SEM microscopies were reported. NPs with a rod-like structure were obtained (Figure 1A) proving that the self-assembling of the conjugate is ruled by the hydrophobic octapeptide that prevalently assume a flat *β*-like conformation able to self-assemble through a hydrogen bond network.[2] Based on TEM and DLS results, a structural model (Figures 1B and C) is proposed for PEP8-Phe-PEG where the hydrophobic octapeptides form the tubular core whilst PEG in the outer shell confers stability to the system. The ability of the NPs to act as an efficient drug delivery system was investigated using curcumin as a probe to determine the uptake efficiency and pharmacokinetics. A good DL of 6 wt% and a sustained drug release over 48 hours was obtained. The assessed thermodynamic stability and uptake efficiency along with biocompatibility make these NPs promising candidates for drug delivery applications.

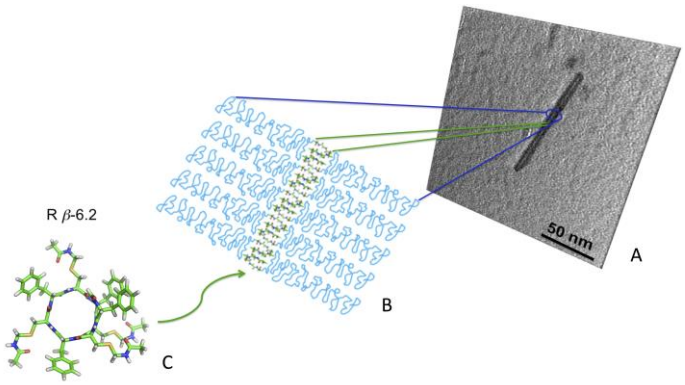


Figure 1. A) TEM micrographs of PEP8-Phe-PEG NPs; B) Structural model proposed for the corresponding NPs; C) ß-like conformation with a 6.2 residues per turn preferred by the linear DL-octapeptides.

1. W. Hamley, *Angew. Chem. Int Ed*., **2014**, *53*, 6866.
2. P. De Santis, S. Morosetti, R. Rizzo, *Macromolecules*, 1974, *7*, 52.

Small Mesoporous Silica Nanoparticles for Nanomedicine

Tânia Ribeiro, Ana Sofia Rodrigues, Carlos Baleizão, José Paulo S. Farinha

CQFM-Centro de Química-Física Molecular and IN- Institute of Nanoscience and Nanotechnology, Instituto Superior Técnico, Universidade de Lisboa, Av. Rovisco Pais, 1049-001 Lisboa, Portugal.

Email of presenting author: tania.ribeiro@tecnico.ulisboa.pt

Since the emergence of nanotechnology in the past decades, the development and design of nanomaterials has become an important field of research. An emerging aspect of this field is nanomedicine, wherein nanoparticles are being developed for use as imaging agents or for drug delivery applications. Particle diameters bellow a few tens of nm are desirable, especially to apply in organelles inside cells or to pass the blood brain barrier. Among these nanoparticles, mesoporous silica nanoparticles (MSNs) have gained special attention for biomedical applications due to their excellent biocompatibility, high surface areas, large pore volumes, high loading capacity, uniform and tunable pore sizes, and versatile surface functionalization.

In the literature, only a few cases describe particles below 100 nm diameter, by changing the silica source or the temperature. [1] The most common processes to synthesize MSNs use ammonium bases and co-solvents or hydroxide base in aqueous media, leading to diameters that are usually higher than 100 nm.

Here, we describe the preparation of MSNs with controlled diameters below 100 nm, under mild synthesis conditions. [2] The synthesis is performed using a sol-gel method, in an aqueous medium, with tetraethyl ortosilicate as silica source, an ionic surfactant, without ammonium bases or co-solvents. We are capable of controlling the diameter of MSNs, as also the dimension and shape of their pores by varying the pH or the ionic strength of the reactional mixture under constant temperature conditions. The diameter was tuned from 20 to 100 nm, with low size dispersity (Figure 1). Additionally, during the synthesis we can covalently incorporate a fluorescent hydrophobic perylenediimide dye in the silica network. This way, it is possible to synthesize fluorescent MSNs, with sizes below 100 nm, controlling the pore diameter and morphology, for applications in bioimaging and drug delivery.

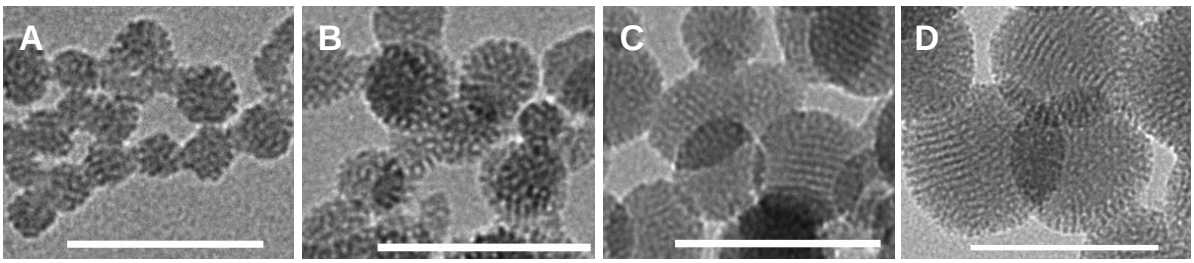


Figure 1. TEM images (scale bar 100 nm) of MSNs (average diameters: A – 25 nm; B – 38 nm; C – 55 nm; D – 70 nm).

1. S.-H. Wu, C.-Y. Moua, H.-P. Lin, *Chem. Soc. Rev.* **2013**, *42*, 3862.
2. T. Ribeiro, A.S. Rodrigues, C. Baleizão, J.P.S. Farinha, **2016**. *Process for the production of mesoporous silica nanoparticles with diameters under 100 nanometers and precise control of the particle diameter and the pore geometry*. Portuguese Invention Patent nº 109110.

Acknowledgments: This work was partially supported by Fundação para a Ciência e a Tecnologia (FCT-Portugal) and COMPETE (FEDER), projects RECI/CTM-POL/0342/2012, UID/NAN/50024/2013 and PTDC/CTM-POL/3698/2014. T.R. and A.S.R. also thank FCT for Post-Doc (SFRH/BPD/96707/2013) and Ph.D. (SFRH/BD/89615/2012) grants.

3D Cell Cultures in Porous Scaffolds with Oriented Microtubules Designed for Dental Regeneration

Samuele M. Dozio,[a,b] Silvia Panseri,[a] Monica Montesi,[a] Elisabetta Campodoni,[a] Monica Sandri,[a] Anna Tampieri [a]

[a] *Institute of Science and Technology for Ceramics National Research Council, Via Granarolo 64, 48018, Faenza, Italy;* [b] *Departement of Medical, Oral and Biotechnological Sciences, University of "G. d'Annunzio" School of Advanced Studies of Chieti-Pescara, Via dei Vestini 31, 66100, Chieti, Italy.*

Email of presenting author: samuele.dozio@istec.cnr.it

Tooth loss is a common result of a variety of oral diseases due to physiological causes, trauma, genetic disorders and aging, and can lead to physical and mental suffering that markedly lower the individual's quality of life [1-3]. Tooth is composed of mineralized tissues and soft connective tissues. Dentin is the most voluminous tissue of the tooth and its formation (dentinogenesis) is a highly regulated process displaying several similarities with osteogenesis. In this study a low-cost scaffold made by gelatin biomineralized with magnesium-doped hydroxyapatite and blended with chitosan, was developed for hard tissue engineering. We synthetized a dentin-like scaffolds using a controlled freeze-drying process permitting the formation of microscopic channels comparable to dentin tubules and appropriate for cell penetration and matrix deposition.
Mesenchymal stem cells (MSCs) and dental pulp steam cells (DPSCs) were seeded in direct contact with the scaffolds and cultured with medium supplemented with osteogenic factors. Cell viability and cell morphology were analysed up to 14 days of cultured. Moreover gene and protein quantification were investigated.
Results and Discussion: The scaffolds show an aligned porosity suitable for the colonization of its inner part by the seeded cells (Figure 1). The scaffold had no cytotoxicity, cells morphology was accordant to a non-stress cell condition and a good adhesion to the scaffold. 3D cell culture with MSCs and DPSCs showed the promising properties of the new scaffolds for tooth regeneration. In detail, the chemical composition of the biomineralized gelatin facilitate the cell adhesion, the aligned porosity is suitable for cell colonization and fine cell/material interactions together with mineral component permit the cells differentiation and matrix deposition.

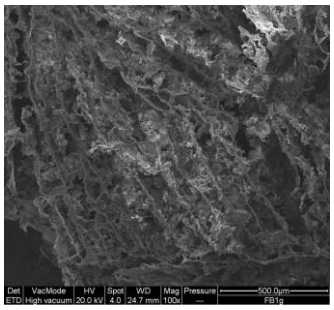


Figure 1: SEM image of biomineralized gelatin-based scaffold with an aligned channel-like porosity.

1. Y. Chai, H.C. Slavkin, *Microscopy Research and Technique*, **2003,** *60*, 469.
2. S.P. Ho, B. Yu, W. Yun, et al., *Acta Biomaterialia*, **2009**, *5*, 707.
3. A. Linde, M. Goldberg, *Critical Reviews in Oral Biology & Medicine*, **1993**, *4*, 679.

Acknowledgements**:** The authors would like to thank the European Project SMILEY (NMP4-SL-2012-310637) for providing financial support to this project.

Ultrastructural investigation of different collagen-based scaffolds and their *in vitro* interactions.

Valentina Laghezza Masci,[a] Anna Rita Taddei,[b] Franco Giorgi,[c] Gabriella Gambellini,[b] Anna Maria Fausto [a]

[a] *Department for Innovation in Biological, Agro-food and Forest systems (DIBAF), University of Tuscia, Largo dell'Università, snc, Viterbo, Italy;* [b] *Center of Great Equipment-section of Electron Microscopy, University of Tuscia, Largo dell'Università, snc, Viterbo, Italy;* [c] *University of Pisa, Italy.*

Email of presenting author: laghezzamasci@unitus.it

The primary aim of tissue engineering is to develop bio-functional matrices for wound healing under normal or chronic situations. Recent advances in the fabrication of biomaterials have led to the development of a variety of collagen-based scaffolds simulating tissue healing *in vitro*. The aim of this work was to verify how these collagen-based scaffolds are structurally modified when combined with such active substances as sodium hyaluronate (CS&NaYal) or Oxidate Regenerated Cellulose (Coll&ORC). The overall morphology of these collagen matrices was therefore examined by light and electron microscopy. To verify how different collagen scaffoldings affect cell behaviour, aliquots of NIH3T3 fibroblasts were cultured *in vitro* for time intervals ranging from 4 hours to 14 days and cell proliferation was quantitatively determined by the MTT assay. This study demonstrates that equine collagen type I scaffold remains structurally stable and capable of sustaining cell migration and proliferation *in vitro* for the entire culture period. We interpret this observation as indicating that addition of NaYal does not interfere with collagen assembly, the matrix retaining its overall integrity and the collagen fibers under native conditions. Apparently, the Coll&ORC scaffold exhibited similar architectural organization with larger pores and the matrix laminae organized as fibrillar bundles. However, no periodic banding could be envisioned on this scaffold. CS&NaYal and Coll&ORC scaffolds differ also in their capacity to sustain cell interaction. While cells assumed a typical migratory behaviors in the CS&NaYal scaffold by spreading into larger portions of the collagen matrix, they maintained a rather roundish shape and did not migrate significantly well in the Coll&ORC scaffold. In spite of the complexity of wound healing *in vivo*, and the variety of factors actually involved in the process, it is conceivable that the type of cell-to-matrix interactions envisioned in this study may represent the first step in a cascade of events leading to tissue remodelling. Given this possibility, the experimental conditions worked out in this study may constitute a first pivotal attempt to test additional cell parameters under conditions that could not be adequately controlled *in vivo*.

Acknowledgments: Euroresearch srl, Milan, Italy.

Development of cellular vehicles for delivery of gold nanorods to tumors

S. Centi,[a] C. Borri,[a] S. Lai,[a] G. Comito,[b] P. Chiarugi,[b] F. Ratto,[a] R. Pini [a]

[a] *Institute of Applied Physics, National Research Council of Italy (CNR), Via Madonna del Piano 10, 50019 Sesto Fiorentino, Italy;* [b] *Department of Biomedical, Sperimental and Clinical Sciences "Mario Serio", University of Florence, Viale Morgagni 50, 50134 Florence, Italy.*

Email of presenting author: s.centi@ifac.cnr.it

Over recent years, gold nanorods (GNRs) have emerged as a promising material in biomedical optics and have been proposed as contrast agents for the photothermal therapy and the photoacoustic imaging of tumors[1,2]. A pioneering approach to target tumors is the use of cellular vehicles, i.e. cells of the immune system that exhibit an innate tropism to tumors and that can be serve as Trojan horses[3,4]. This strategy relies on cell types, such as tumor-associated macrophages, mesenchymal stem cells or T cells, that are recruited by or naturally traffic to the microenvironment of tumors and that can be isolated from a patient and loaded with plasmonic particles in vitro. In this work, GNRs were synthesized and designed to combine high optical and photo-stability and the ability to accumulate into cells of the immuno system. Particles were PEGylated and conjugated with cationic moieties. Different cationic compounds were tested and the cell viability and uptake of the particles were studied on different cell types. The cytotoxicity test was based on a colorimetric WST-8 assay while the intracellular amount of gold and the optical absorbance of the cells were quantified by spectrophotometry. Moreover, we investigated the effect of GNRs on the cell migration and the production of cytokines in the presence of pro-inflammatory stimuli, which provide a functional overview on the feasibility of this approach to target tumors.
These experiments allowed us to establish the best conditions to prepare multifunctional cellular vehicles displaying plasmonic features for photothermal and photoacoustic applications.

1. R. K. Kannadorai, G. G. Ying Chiew, K. Qian Luo, Q. Liu. *Cancer Letters*, **2015**, *357*, 152.
2. J. Zhong, L. Wen, S. Yang, L. Xiang, Q. Chen, D. Xing. *Nanomedicine: Nanotechnology, Biology, and Medicine*, **2015**, *11*, 1499.
3. S.H. Thorne, C.H. Contag. *Gene Therapy*, **2008**, *15*, 753.
4. F. Ratto, S. Centi, C. Avigo, C. Borri, F. Tatini, L. Cavigli, C. Kusmic, B. Lelli, S. Lai, S. Colagrande, F. Faita, L. Menichetti, R. Pini. *Advanced Functional Materials*, **2016**, in press.

Acknowledgments: This work has been partially supported by the European Eranet Plus project LUS BUBBLE.

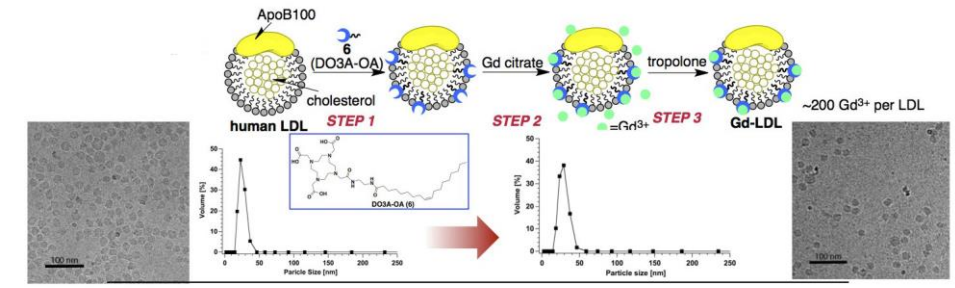
LDL for targeted delivery of MRI-CA to atherosclerosis

Yoko Yamakoshi,[a] Rong Zhou [b]

[a] *Laboratorium für Organische Chemie, ETH Zürich, Switzerland;* [b] *Department of Radiology, University of Pennsylvania, USA.*

Email of presenting author: yamakoshi@org.chem.ethz.ch

Low-density lipoprotein (LDL) isolated from the healthy human donors was modified with a GdDO3A derivative to create an MR-active nanoparticles. A DO3A derivative with an oleic acid anchor was synthesized and subjected to the intercalation to LDL lipid layer. Subsequent chelation reaction with Gd^{3+} and careful removal of non-specifically-bound toxic free Gd^{3+} successfully provided the modified LDL with a high payload (> 200 Gd^{3+} per LDL particle). Obtained MR-active LDL was characterized by ICP-MS, DLS, cryoTEM, and relaxivity measurements to show that individual LDL particles were dispersed well and with much higher r_1 relaxivity per Gd^{3+} (20.2 $mM^{-1}s^{-1}$) compared to the clinically used Dadoteridol (small molecule GdDOTA, 3.8 $mM^{-1}s^{-1}$). The LDL particle was stable shown by DLS (for the aggregation) and ICP-MS analysis (for the free Gd^{3+} leakage). Finally, the Gd^{3+}-LDL was injected to the atherosclerosis mouse model (*ApoE*$^{-/-}$, fed with western diet) *via* tail vain and subjected to the MR imaging (24-48 hours post injection). The visibly clear bright enhancement was observed 48 hours after injection in the brachiocephalic artery of the mouse model which was corresponding to the atheroplaques taken together with the results of *ex vivo* study.



[mM^{-1} s^{-1}]	Contrast agent		
	Gd^{3+}-LDL (40:1)	Gd^{3+}-LDL (200:1)	Gadoteridol
r_1 relaxivity per Gd^{3+}	12.1	20.2	3
r_1 relaxivity per LDL	48.4	4040	–

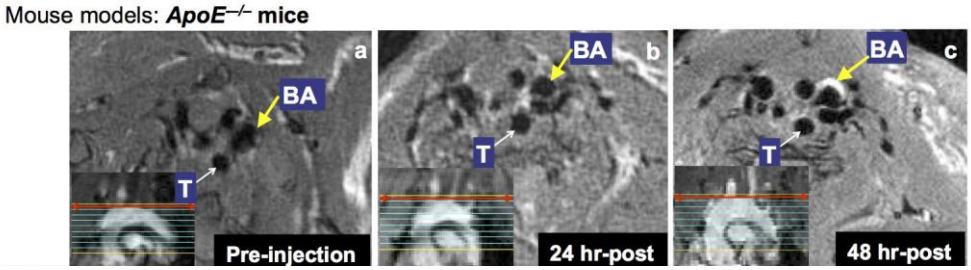


Figure. Scheme of LDL modification, DLS and cryoTEM image of modified LDL, relaxivity of Gd^{3+}-LDL and GdDOTA, and *in vivo* image of atherosclerosis.

1. Y. Yamakoshi, H. Qiao, A. N. Lowell, M. Woods, B. Paulose, Y. Nakao, H. Zhang, T. Liu, S. Lund-Katz, R. Zhou, *Chem. Comm.* **2011**, *47*, 8835.
2. A. N. Lowell, H. Qiao, T. Liu, T. Ishikawa, H. Zhang, S. Oriana, M. Wang, E. Ricciotti, G. A. Fitzgerald, R. Zhou, Y. Yamakoshi, *Bioconjugate Chem.* **2012**, *23*, 2313.

Ex vivo plaque permeability evaluation in ApoE$^{(-/-)}$ mice using fluorescent blood pool agents.

Paolo Oliva,[a] Alessia Cordaro,[a] Erika Reitano,[a] Luigi Miragoli,[a] Giovanni Valbusa,[b] Chiara Brioschi,[a] Claudia Cabella [a]

[a] *Centro Ricerche Bracco, Bracco Imaging S.p.A., Colleretto Giacosa (TO), Italy;* [b]*Ephoran Multi-Imaging Solutions, Colleretto Giacosa (TO), Italy.*

Email of presenting author: paolo.oliva@bracco.com

Introduction. Atherosclerotic cardiovascular disease (ACD) is an artery degenerative disease resulting in plaques leading to stenosis, embolization and thrombosis; its origin derives from complex interactions among multiple factors including the genes encoding apolipoprotein E[1]. Currently, most imaging techniques are not able to clearly define plaque composition as a predictor of an acute event; a diagnostic tool aimed to stratify plaques with respect to different permeability (i.e. different risk of rupture) could help clinicians to predict the response to a drug-loaded nanosystem based therapy. Optical Imaging (OI) was adopted to study the endothelial local permeability in aortic-tree atherosclerotic plaques in ApoE$^{(-/-)}$ mice. This model develops severe hypercholesterolemia and atherosclerosis, with characteristic and distribution similar to those observed in humans[2,3]. Two agents were compared: human serum albumin conjugated with a fluorescent probe (HSA-Cy5) and the albumin-binder aminodeoxycholic acid conjugated with IRDye800CW (B26170). They were administered to ApoE$^{(-/-)}$ mice and fluorescence signal was analyzed *ex vivo* in the excised aortic trees.
Methods. ApoE$^{(-/-)}$ mice were fed with a high-cholesterol diet from 6 weeks of age. *Ex vivo* OI images of aortic tree were acquired after intravenous administration of fluorescent probes and subsequent myocardial perfusion. Fluorescent signal was calculated as the ratio between regions of interest corresponding to atherosclerotic plaques and regions without lesions along the aortic tree. The selected tracts of aortic tree were then subjected to histological processing: serial plaque sections were stained either with hematoxylin-eosin (H&E) to evaluate plaque morphology and composition or by specific immune-staining in order to assess the content of macrophages and foam cells and endogenous mouse serum albumin.
Results. OI and histological results were compared to find a correlation between the results of the two fluorescent probes and the plaque features. Using both HSA-Cy5 and B26170, the fluorescence ratio decreases with increasing plaque grade, in correlation with an advanced stage of development and subsequently loss of plaque permeability. Moreover: 1- the higher the fluorescent ratio, the higher the presence of macrophages and foam cells that characterizes the early stage of plaque development and the inflammatory status; 2- the higher the fluorescent ratio, the higher the content of endogenous serum albumin found in the plaques.
Conclusions and Perspectives. The proof of concept obtained both with a fluorescent conjugated albumin (HSA-Cy5) and with an albumin-binder fluorescent probe (B26170) demonstrates the potentiality of albumin to stratify atherosclerotic lesions basing on different morphology and composition. Thus, translating to a clinical MRI application, the method could be proposed as a diagnostic tool, based on an analogue gadolinium-based albumin-binder contrast agent. This molecule, namely B22956/1, contains the same aminodeoxycholic residue present in the fluorescent B26170 probe. In this sense, a stratification of the pathology performed by MRI and based on permeability of B22956/1 could allow clinicians to classify the plaque and to define a proper therapeutic regimen based on drug-loaded nanoparticles.

1. S.H. Zang et al., *Science*, **1992**, *258*, 468.
2. Y. Nakashima et al., *Arterioscler. and Thromb.*, **1994**, *14*, 133.
3. J.L. Breslow, *Curr. Opin. Lipidol.*, **1994**, *5*, 175.

Acknowledgments: This work is supported by FP7 funded project Call NMP 2012.1.2-2 – NanoAthero.

Industry Report

Microcalorimetry of Nanoparticles: a ground-breaking approach for drug delivery platforms design

Andrea Pigozzo

Alfatest Srl, via Giulio Pittarelli 97, 00166, Rome, Italy.

Email of presenting author: andrea.pigozzo@alfatest.it

Microcalorimetry is the universal detector of heat. Since heat is generated or absorbed in every chemical process, microcalorimetry is a super-versatile technique for characterize interactions. Of all the techniques that are currently available to measure binding, isotherm titration calorimetry (ITC) is the only one capable of measuring not only the magnitude of the binding affinity but also the magnitude of the two thermodynamic terms that define it (Enthalpy and Entropy).
Nanoparticles offer several advantages as drug delivery systems. Efficient nanoparticles formulations should combine a number of different properties such as efficient drug loading, controlled drug release, stealthiness and drug targeting. This can be achieved by the design of nanoparticles bearing different functionalities at their surface.
Thanks to the universality of the technique, ITC provides a widely applicable method for monitoring nanoparticle drug delivery systems development in a label-free environment and so it represent a powerful technology for use in drug discovery, drug delivery, pharmaceutical formulations as well as the study of NPs interaction with living system. Applications of this innovative approach will be presented.

Poster

Novel physically stable nanocarriers displaying a pseudo-polymeric micellar structure by the conjugation of amphiphilic diblocks to multifunctional nanodiamonds as molecular anchors

Doaa Abu Saleh,[a] Olga Shimoni,[b] Alejandro Sosnik [a,c]

[a] *Department of Materials Science and Engineering, Technion-Israel Institute of Technology, Haifa, Israel;* [b] *School of Physics and Advanced Materials, University of Technology, Sydney, Australia;* [c] *Russell Berrie Nanotechnology Institute (RBNI), Technion-Israel Institute of Technology, Haifa, Israel.*

E-mail of presenting author: sdoaaas@gmail.com

More than 50% of the approved drugs are poorly water soluble according to the Biopharmaceutic Classification System (BCS). Polymeric micelles (PMs) represent a nanotechnology platform largely used to solubilize and stabilize drugs and increase their oral bioavailability (1). However, a main challenge is preventing the disassembly of PMs under dilution in the body fluids, which leads to uncontrolled release of the encapsulated cargo. This study investigates a new amphiphile architecture, namely core-anchored PMs, to improve the physical stability and performance of these nanocarriers. In this framework, carboxylated nanodiamonds (cNDs) and carboxylated red fluorescent nanodiamonds (rFNDs) were used as molecular anchor for the conjugation of amphipathic methoxy-poly(ethylene glycol)-*b*-poly(epsilon-caprolactone) diblocks (MPEG-PCL). Characterization conducted by TGA, FTIR and ^{1}H-NMR confirmed the conjugation of amphiphiles to the surface of both inorganic nanoparticles. In addition, TEM revealed the presence of a thick polymeric layer (Figure 1). Dynamic light scattering and nanoparticle tracking analysis were used to measure the size and the stability of the nanoparticles in water. Cell viability was evaluated in Caco2 cell line, an *in vitro* model of the intestinal epithelium. Encapsulation of anti-helmintic drug nitazoxanide (NTZ) increased from 11 to 500 µg/mL in a 0.4% w/v system, reaching 12.5% w/w drug loading. Results support the feasibility of this strategy for the development of more robust nanotechnology platforms for drug delivery.

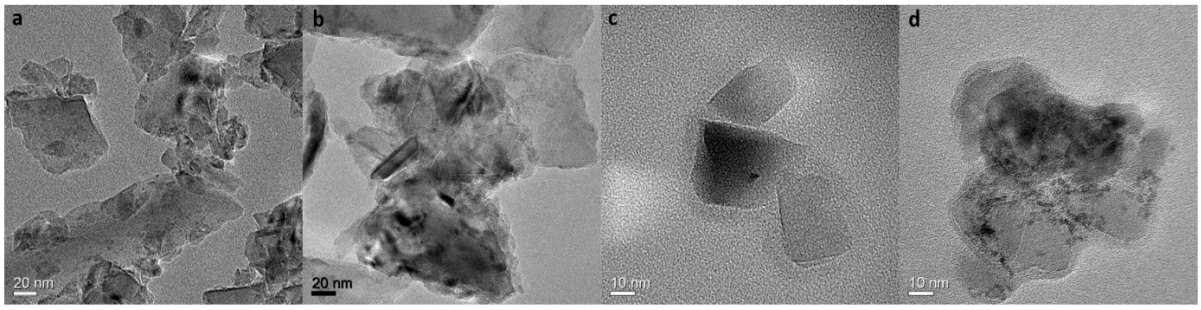


Figure 1. TEM micrograph of pristine (a) cNDs, (b) MPEG-PCL-modified cNDs, (c) pristine rFNDs and (d) MPEG-PCL-modified rFNDs. Scale bar: (a,b) = 20 nm; (c,d) = 10 nm.

1. A. Sosnik**,** Royal Society of Chemistry, **2013**, Chapter 5, pp. 115.

Acknowledgments: This work was supported by the European Union`s - Seventh Framework Programme under grant agreement #612765-MC-NANOTAR and Technion (internal grant #2019300).

PLGA-*g*-PVP -based nanocapsules for the controlled delivery of antimalarials

Jenny Alongi,[a] Giovanna Capuano,[a] Amedea Manfredi,[a] Roberta Cavalli,[b] Xavier Fernàndez-Busquets,[c] Elisabet Marti,[c] Paolo Ferruti,[a] Elisabetta Ranucci [a]

[a]*Dipartimento di Chimica, Università degli Studi di Milano, via Golgi 19, 20133, Milano, Italy;* [b]*Department of Drug Science and Technology, University of Turin, via Pietro Giuria 9, 10125, Torino, Italy;* [c] *Nanomalaria Group, Institute for Bioengineering of Catalonia (IBEC), Baldiri Reixac 10-12, ES-08028, Barcelona, Spain.*

E-mail of presenting author: jenny.alongi@unimi.it

Amphiphilic PLGA-*g*-PVP copolymers with different PLGA and PVP content were recently obtained by the radical polymerization of 1-vinylpyrrolidin-2-one in liquid poly(lactic-*co*-glycolic acid) (PLGA) (50:50) at 100°C [1]. Saponification of the PLGA portion allowed isolating the PVP side chains and measuring their molecular weight, which turned to be lower than the threshold for glomerular filtration. The orthogonal solvent pair ethyl acetate-methanol gave PLGA-*g*-PVP fractions with different PLGA and PVP content. Following the same procedure, PLGA/PVP blends gave the two homopolymers. PLGA-*g*-PVP and PLGA/PLGA-*g*-PVP blends, but not PLGA/PVP blends, gave long-term stable nanodispersions in water [1].

In this work, PLGA-*g*-PVP copolymers were employed to obtain novel artemisinin and curcumin formulations. Both drugs are endowed with potential and pitfalls for malaria treatment. Artemisinin is a potent *Plasmodium falciparum* malaria parasite inhibitor (IC_{50} = 10^{-8} - 10^{-7} M) but with low bioavailability, poor pharmacokinetic properties and high cost [2]. Curcumin inhibits the growth of *P. falciparum* with a dose dependent trend and IC_{50} = 5 μM. Despite the absence of secondary effects in humans, the use of curcumin is limited by the low solubility in water, the high chemical instability and photosensitivity, resulting in low bioavailability[3,4]. To increase bioavailability, artemisinin and curcumin were loaded into nanocapsules consisting of a biocompatible oily core acting as drug solvents, and a PLGA-*g*-PVP shell. Loaded nanocapsules were characterized in terms of morphology, physico-chemical properties and release tests. In particular, transmission electron microscopy (TEM) showed spherical morphologies and dynamic scattering measurements (DLS) revealed size in the range 50 - 100 nm. The encapsulation efficiency was very high with both drugs and in the case of artemisinin it approached 100%. All formulations showed long-term shelf stability in aqueous solution. In vitro activity tests as *P. falciparum* inhibitors are currently in progress.

1. E. Ranucci, G. Capuano, A. Manfredi, P. Ferruti, *J. Polym. Sci., Part A: Polym. Chem.*, **2016**, *54*, 1919.
2. N. J. White, *Antimicrob. Agents Chemother.*, **1997**, *41*, 1413.
3. R.C. Reddy, P.G. Vatsala, V.G. Keshamouni, G. Padmanaban, P.N. Rangarajan, *Biochem. Biophysic. Res. Com.*, **2005**, *326*, 472.
4. R.K. Maheshwari, A.K. Singh, J. Gaddipati, R.C. Srimal, *Life Sci.*, **2006**, *78*, 2081.

Acknowledgments: Financial support by Fondazione Cariplo (Italy), grant no. 2013-0583, is gratefully acknowledged.

Inclusion of usnic acid in glucosylated liposomes

Barbara Altieri,[a,b] Iolanda Francolini,[c] Luisa Giansanti,[a,b] Antonella Piozzi,[c] Alessandro Mauceri,[b,c] Giovanna Mancini [c]

[a] *Dipartimento di Scienze Fisiche e Chimiche, Università degli Studi dell'Aquila, Via Vetoio, 67100 Coppito (Aq), Italy;* [b]*CNR – Istituto di Metodologie Chimiche, Via Salaria km 29.300, 00016 Monterotondo Scalo, Rome, Italy;* [c] *Dipartimento di Chimica, Università degli Studi di Roma "Sapienza", P.le Aldo Moro 5, 00185 Rome, Italy*

Email of presenting author: barbaraltieri@gmail.com

(+)-Usnic acid (UA) is a natural compound that exerts a potent antibacterial and antitumoural activity.[1] However, its clinical application is limited by its scarce solubility in water.[2] UA was included in glucosylated liposomes. The investigated liposome formulations were composed of an unsaturated natural phospholipid, 1,2-dioleoyl-*sn*-glycero-3-phospholcholine (DOPC), cholesterol and one of the two glucosylated amphiphiles **1** and **2** reported in Figure 1 that differs for the length of the hydrophilic spacer linking the glucose residue to the quaternary nitrogen. The size, the size distribution and the surface potential of the formulations were determined by DLS measurements and fluorescence measurements, respectively.
The activity of UA-loaded liposomes versus sessile growing bacteria was evaluated on a 24 h-old *S. epidermidis* biofilm grown on glass slides (10x10 mm). UA loaded in liposomes showed a stronger antimicrobial activity against biofilms of *Staphylococcus* with respect to free UA.

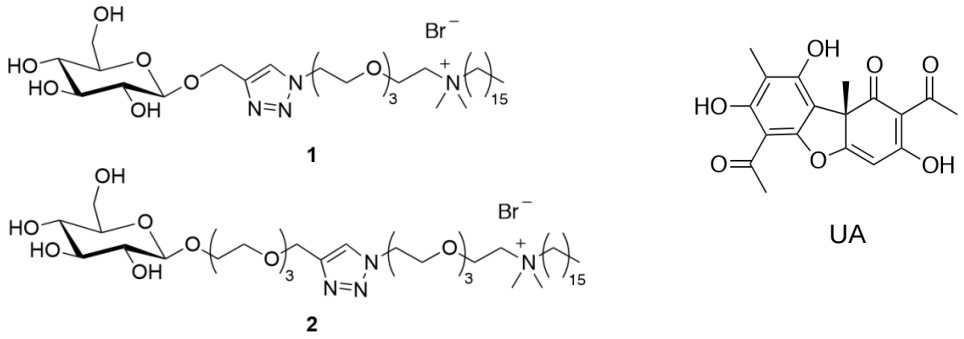


Figure 1. Molecular structure of **1**, **2** and (+)-usnic acid

1. O. K. Ingólfsdóttir, *Phytochemistry*, **2002**, *61*, 729
2. I. Francolini, P. Norris, A. Piozzi, G. Donelli, P. Stoodley, *Antimicrob. Agents Chemother.*, **2004**, 4360.

Acknowledgments: The authors thank the FIRB Project 2012 RBFR12BGHO for the financial support

Evaluation of the antiproliferative effect of 18β -Glycyrrhetinic acid-loaded nanoparticles for treatment of drug-induced gingival overgrowth

Adriana Amalfitano,[a] Alessandro Arcovito,[a,b] Cinzia Callà,[a] Laura Chronopoulou,[c] Massimo Cordaro,[d] Monica Cantiani,[d] Giuseppina Nocca,[a,e] Cleofe Palocci [c]

[a] *Inst. Biochemistry and Clinical Biochemistry, UCSC, L.go F. Vito 1, 00168 Rome, Italy;* [b] *Centro di ricerca sulle Biotecnologie applicate alla Cosmetologia (CRBA), UCSC, L.go F. Vito 1, 00168 Rome, Italy;* [c] *Dep. Chemistry, Sapienza University of Rome, P.le A. Moro 5, 00185 Rome, Italy;* [d] *Institute Odontoiatric Clinic, UCSC, L.go F. Vito 1, 00168 Rome, Italy;* [e] *Institute of Chemistry of Molecular Recognition, CNR, c/o L.go F. Vito 1, 00168 Rome, Italy*.

Email of presenting author: adry_amalfitano@libero.it

Introduction. Drug-induced gingival overgrowth (DIGO) is an oral disease that is detrimental to oral function and facial appearance; among them antiepileptics (*i.e* valproic acid) can increase the number of fibroblasts within gingival tissues (1). 18-β Glycyrrhetinic acid (GA) (a compound derived from liquorice) inhibits cell proliferation *via* cell cycle arrest and induction of apoptosis (2), and it has been demonstrated that could be used to treat Human Gingival Fibroblasts (HGFs) derived from DIGO patients (3). The aim of our study is to develop a delivery system able to provide a controlled release of GA in HGFs *in vitro*. For this purpose, we have selected polymeric nanoparticles (NPs) to enhance in situ release of GA, overcoming its poor solubility.

Materials and Methods. Preparation of drug-loaded NPs: GA-loaded Poly-d,l-lactide-co-glycolide (PLGA) NPs were produced by using a one-step osmosis based methodology (patent Sapienza University n° RM2004A000555) and were successively coated with chitosane (CS) (4). Drug loading evaluation: The drug content of GA-loaded NPs was measured using a spectrophotometric method. NPs were dissolved in chloroform and the drug concentration was determined by measuring the absorbance of the solution at λ= 246 nm. Dynamic Light Scattering (DLS) Analysis: DLS experiments were carried out with a Zetasizer Nano S (Malvern Instruments, Malvern, U.K.) equipped with a 4 mW He-Ne laser (633 nm). Peak intensity analysis was used to determine the average hydrodynamic radius of the scattering particles. All samples were prepared at 0.1 mg/mL in filtered PBS. Isolation and culture of HGFs: Cells were obtained (with informed consent) from patients subjected to gingivectomy of the molar region. The specimens were plated in tissue culture flasks with complete DMEM, at 37°C, 5% CO_2 atmosphere. The HGFs were used before the fifth passage. Cytotoxic Assay: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) test was used to determine the GA concentration value able to provoke cytotoxicity. Statistical Analysis: Data were expressed as mean ± SD. Analysis was performed by ANOVA, $p<0.05$ was assumed significant.

Results. The mean diameters of NPs was 200 nm with a GA content value of 26 µg/mg PLGA. No cytotoxic effect was observed in the presence of NPs. The toxic effect of GA was observed at concentration values higher than 100 µmol/L.

Conclusions. Such formulations seem very promising for reducing cell proliferation *in vitro*, even if further studies will be needed to determine the concentrations of GA able to reduce the proliferation of HGFs derived from patients with DIGO pathology.

1. Y. Shimizu, et al, *J. Periodontol.*, **2002**, *73*, 861.
2. Y. Satomi, et al, *Anticancer Res.*, **2005,** *25*, 4043.
3. R. Takeuchi, et al, *Br. J. Pharmacol*., **2016,** *173*, 913.
4. L. Chronopoulou, et al, *Colloids Surf. B. Biointerfaces*, **2012**, *97*, 117.

Acknowledgments. We would like to thank Indena Company for providing 18-β GA.

Functionalized virus-like particles with antimicrobial peptides

Francesca Bugli,[a] Adriana Amalfitano,[b] Francesco Paroni Sterbini,[a] Cecilia Martini,[a] Maurizio Sanguinetti,[a] Massimiliano Papi,[c] Valentina Palmieri,[c] Marco De Spirito,[c] Alberto Vitali,[d] Alessandro Arcovito [b e]

[a] *Institute of Microbiology, School of Medicine, Catholic University of Sacred Heart, largo F. Vito 1, 00168, Rome, Italy;* [b] *Institute of Biochemistry and Clinical Biochemistry, School of Medicine, Catholic University of Sacred Heart, largo F. Vito 1, 00168, Rome, Italy;* [c] *Institute of Physics, School of Medicine, Catholic University of Sacred Heart, largo F. Vito 1, 00168, Rome, Italy;* [d]*Institute of Chemistry of Molecular Recognition, CNR, c/o L.go F. Vito 1, 00168 Rome, Italy.* [e]*Centro di ricerca sulle Biotecnologie applicate alla Cosmetologia (CRBA), UCSC, L.go F. Vito 1, 00168 Rome, Italy.*

Email of presenting author: alessandro.arcovito@unicatt.it

The search for new antimicrobial drugs to replace the presently dominating drug products and integrate with traditional chemotherapy, minimizing antibiotic use and bacterial resistance, is becoming imperative. One possible strategy to defeat microbial resistant strains is to explore the efficacy of a large class of biomolecules that are already available in nature: the antimicrobial peptides (AMPs). In this respect, we selected a natural AMP, eumenitin (E) from wasp venom, as its supposed mechanism of action is related to the disruption of the bacterial cell membrane. On the other hand, the efficacy of antimicrobial therapeutics is not only merely due to the mechanism of molecular interaction between drug and its target, but also to the effectiveness of drug delivery. This is particularly true for an approach mediated by intrinsically short-lived biomolecules such as AMPs. Moreover, considering that the most threatening form of microbial life is represented by the biofilm, a polymeric complex matrix that confers a strong resistance towards antibiotic drugs, a prerequisite for a successful approach is the ability to create a long-lived scaffold, able to present the desired AMPs. Virus like particles (VLPs), that are obtained by the self-assembly of viral selected proteins (in our case the VP6 from human rotavirus [1]), are in this sense a new and potent technology to be pursued. Indeed it is possible to spatially co-localize active peptidic epitopes realizing recombinant VP6 mutants exposing the desired AMPs at the C-terminus, and let them rearrange as VLP-AMP units. Finally, this intrinsically powerful feature may be enhanced by the contemporary administration of selected mucolytic substances (N-acetyl cysteine), using the interior cavity of the VLP as a nanocarrier, in order to favour a specific release as a consequence of an enhanced proteolysis activity in the proximity of the infection. The antimicrobial activity against specific pathogens of these functionalized VLPs-E in the presence or not of N-acetyl cysteine is shown.

1. F. Bugli, V. Caprettini, M. Cacaci, C. Martini, F. Paroni Sterbini, R. Torelli, S. Della Longa, M. Papi, V. Palmieri, B. Giardina, B. Posteraro, M. Sanguinetti, A. Arcovito, *Int J Nanomedicine*, **2014**, *9*, 2727.

Polydiacetylenic liposomes as sensors for the detection of biomarker enzymes

Sara Battista,[a] Barbara Altieri,[a,b] Serena Ponte,[a] Giorgio Cerichelli,[a] Luisa Giansanti [a,b]

[a] *Department of Physical and Chemical Science, University of L'Aquila, Via Vetoio, 67100, Coppito, Aquila, Italy;* [b] *CNR-Istituto di Metodologie Chimiche, Via Salaria km 29.300, 00016 Monterotondo Scalo, Rome, Italy.*

Email of presenting author: sara.battista@student.univaq.it

Thymidylate synthase, thymidine phosphorylase (TP) and dihydropyrimidine dehydrogenase are three proteins involved in the metabolism of pyrimidines and are the target of a potent chemotherapeutic agent, 5-fluorouracil (5-FU), widely employed in the treatment of some of the most frequently occurring malignant tumors (breast, colon, and skin cancer).[1] Their presence or their absence in biological fluids is indeed related to a specific state of health and their easy and fast detection is a problem of major concern. In fact, there is an urgent the need for a rapid and accurate method for dosing of the activity of these enzymes before and during 5-FU treatment to reduce its sever side effects and increase its efficacy.

Recently, there is a growing interest in polydiacetylene (PDA)-based materials for sensing applications because of their optical properties that make them sensitive to external stimuli.[2] Among them, PDA liposomes are one of the most investigated systems.[3] Here we report the results obtained investigating the capability of the different specific PDA liposomes to give a colorimetric response upon the interaction with TP, one of the target enzymes of 5-FU. Liposomes were formulated with 10,12-pentacosadiynoic acid in the presence or in the absence of 1,2-dioleoyl-*sn*-glycero-3-phosphocholine or 1,2-dimirystoyl-*sn*-glycero-3-phosphocholine. To make them specific for TP, one of the two non-ionic surfactants **1** and **2** reported in Chart 1 were included in the formulation: in these molecules 5-FU is linked to an hydrophobic chain containing the diacetylene moiety by polyoxyethylenic spacers of different length; they can be easily embedded in liposome bilayers, polymerize upon irradiation and, thanks to the presence of 5-FU, promote the change of color of the solution upon the specific interaction with TP.

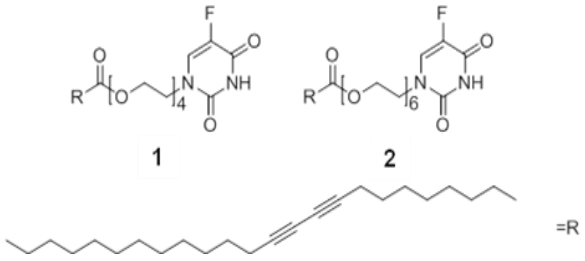


Chart 1. Molecular structure of non-ionic diacetylenic surfactant **1** and **2**.

1. J. L. Arias, *Molecules*, **2008**, *13*, 2340.
2. J. Lee, H. Jun, J. Kim, *Adv. Mater.*, **2009**, *21*, 3674.
3. O. Yarimaga, J. Jaworski, B. Yoon, J.-M. Kim, *Chem. Commun.*, **2012**, *48*, 2469.

Acknowledgments: The authors thank the FIRB Project 2012 RBFR12BGHO for the financial support

Neuronal commitment of human circulating multipotent cells by carbon nanotube-polymer scaffolds and biomimetic peptides.

Thomas Bertalot,[a #] Giorgia Scapin,[b #] Nicola Vicentini,[c] Teresa Gatti,[c] Simone Tescari,[a] Vincenzo De Filippis,[a] Carla Marega,[c] Enzo Menna,[c] Marco Gasparella,[d] Pier Paolo Parnigotto,[e] Rosa Di Liddo,[a *] Francesco Filippini [b *]

[a] Department of Pharmaceutical and Pharmacological Sciences, [b] Department of Biology and [c] Department of Chemical Sciences, University of Padua, 35131 Padua, Italy; [d] Department of Woman and Child Health, University of Padua, 35128 Padua, Italy; [e]TES-Tissue Engineering and Signalling-Onlus, Caselle di Selvazzano Dentro, Padova, Italy. [#] equal contributions [*] co-last authors.

Email of presenting author: thomas.bertalot@unipd.it

We recently set up freestanding nanocomposite scaffolds combining the biocompatibility of a poly-L-lactic (PLLA) matrix with the peculiar electrical, mechanical and chemical properties of carbon nanotubes (CNTs) and such scaffolds, together with biomimetic peptides derived from the extracellular domains of Cell Adhesion Molecule (CAM) and Extracellular Matrix (ECM) proteins, proved functional in boosting neuronal differentiation of SH-SY5Y cells (derived from human neuroblastoma)[1]. Then, neuronal differentiation could be further improved by using electrospun scaffolds[2]. In this work, we isolated and characterized a population of human circulating multipotent cells (hCMCs) presenting a high degree of stemness and a multidifferentiative potential. Such peripheral blood stem cells represent an attractive option in regenerative medicine applications as they are free from ethical restrictions, allow safe autologous transplant and are easily accessible. In order to recapitulate natural tissue features and cues providing hCMCs with information for controlling their neuronal differentiation, we used CNT-based nanocomposite scaffolds, which mimic the electrical/nanotopographical features of the neural environment, and biomimetic peptides reproducing axon guidance cues from neural proteins. Such combined stimuli could induce and boost differentiation of hCMCs towards neuronal lineage despite the absence of exogenously added, specific growth factors. This study suggests the scaffold-peptide system combined with autologous stem cells such as hCMCs could be a functional biomimetic and self standing prototype for neural regenerative medicine applications.

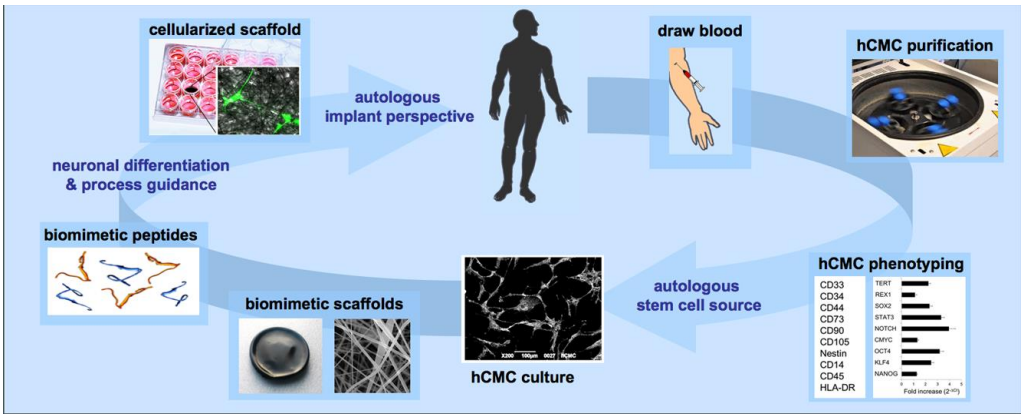


Figure 1. Circulating multipotent cells (hCMCs) are isolated by density gradient separation from human peripheral blood collected from healthy volunteer donors; phenotyping and cell differentiation assays demonstrate the stemness degree and multidifferentiative potential for hCMCs. When seeded onto nanocomposite scaffolds (see abstract from Vicentini *et al.* for details on the scaffolds), hCMCs are committed to the neuronal lineage, and biomimetic peptides can boost their neuronal differentiation.

1. G. Scapin, P. Salice, S. Tescari, E. Menna, V. De Filippis, F. Filippini, *Nanomedicine*, **2015**, *11*, 621.
2. N. Vicentini, T. Gatti, P. Salice, G. Scapin, C. Marega, F. Filippini, E. Menna, *Carbon*, **2015**, *95*, 725.

Determination of free ICG in fluorescent SLNs formulation

Mariangela Boccalon,[a] Zsolt Baranyai,[a] Simona Ghiani,[b] Alessandro Maiocchi [b]

[a] *CRB/Discovery, Bracco Imaging SpA, S.S.14 Km 163.5, 34149, Basovizza, Trieste, Italy;*
[b] *CRB/Discovery, Bracco Imaging SpA, via Ribes 5, 10010, Colleretto Giacosa, Torino, Italy.*

Email of presenting author: mariangela.boccalon@bracco.com

Indocyanine Green (ICG) is an FDA-approved fluorescent probe which has been characterized by the fast metabolic clearance via the liver. Because of the efficient hepatobiliary excretion, which prevents the selective accumulation of ICG in specific pathological site, ICG has been mainly used for the examination of the hepatic function and the blood flow with optical imaging in ocular angiography.
By taking into account the fluorescent properties of the proposed optical imaging probes, ICG is one of the most promising candidate for the detection of different tumors and lymph nodes with the high sensitivity in the clinical fluorescence imaging applications. However, ICG molecules in aqueous solutions can take place in aggregation processes via the formation of dimers, trimers and tetramers which have been characterized by different optical properties.[1]
In order to avoid the aggregation processes and the fast clearance from the blood stream, ICG has been encapsulated by Solid Lipid Nanoparticles (SLNs) which are characterized by the longer residence time in the living system.[2] SLNs have a supramolecular structure based on the tripalmitin solid core covered by phospholipid shell which can incorporate the ICG molecules. However, it could be possible that the incorporation of ICG molecules is not optimal during the formulation: it is likely that small amount of ICG remains outside of SLNs, which can also release ICG molecules during the elapsing time. Thus, in order to optimized the preparation process and quantify the release of ICG during the time, an analytical method for the quantification of ICG in the external phase of SLNs formulation is required.
Herein we report the analytical methods that have been developed for the separation and quantification of the ICG by CE (Capillary Electrophoresis) and SEC (Size Exclusion Chromatography).

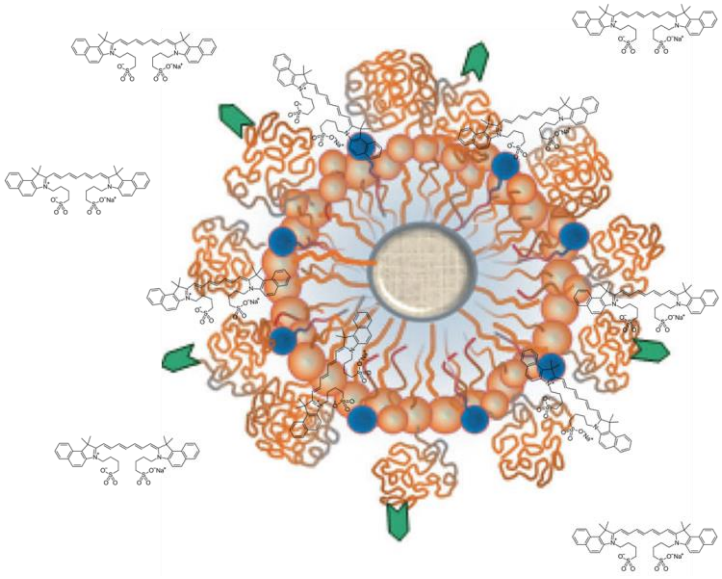


Figure 1. Schematic representation of ICG-SLN in presence of ICG free.

1. M. L. Landsman, G. Kwant, G. A. Mook, W. G. Zijlstra, *J. Appl. Physiol.* **1976**, *40*, 575.
2. S. Ghiani, A. Maiocchi, L. Caminiti, L. Miragoli, Patent WO2014/191467.

Mucoadhesive Solid Lipid Microparticles (SLM) for sustained release of corticosteroids to the lungs

Maria Luisa Bondì,[a] Erika Amore,[b] Mary Ferraro,[c] Marco Santonocito,[b] Elisabetta Martorana,[b] Francesca Di Gaudio,[d] Serena Fanara,[e] Mark Gjomarkaj,[c] Gaetano Giammona,[b] Elisabetta Pace [c]

[a] *Istituto per lo Studio dei Materiali Nanostrutturati (ISMN), U.O.S. Palermo, CNR, Via Ugo La Malfa, 153, 90146 Palermo, Italy;* [b] *Dipartimento di Scienze e Tecnologie Biologiche, Chimiche e Farmaceutiche (STEBICEF), Università degli Studi di Palermo, Via Archirafi 32, 90123 Palermo, Italy;* [c] *Istituto di Biomedicina e Immunologia Molecolare (IBIM), U.O.S. Palermo, CNR, Via Ugo La Malfa, 153, 90146 Palermo, Italy;* [d] *Dipartimento di Biopatologia e Biotecnologie Mediche e Forensi (DiBiMeF), Università degli Studi di Palermo, Via del Vespro 133, 90127 Palermo (Italia) Palermo, Italy;* [e] *Azienda Ospedaliera Universitaria Paolo Giaccone, Settore CQRC (Controllo Qualità, Laboratori e Rischio Chimico), Via del Vespro 133, 90127 Palermo, Italy.*

Email of presenting author: marialuisa.bondi@ismn.cnr.it

Pulmonary delivery is the preferred route of drug administration in the treatment of many respiratory disease, such as asthma and chronic obstructive pulmonary disease (COPD).
Over the years, several kinds of carriers have been studied for sustained release of corticosteroids and bronchodilators to the lungs. Solid Lipid Microparticles (SLM) due to their biocompatibility and size (3-5 µm) can reach the bronchial epithelium directly, circumvent first pass metabolism and avoid systemic toxicity [1,2].
In this work we describe the preparation and the characterization of two different systems subjected to chitosan and alginate coating for sustained release of fluticasone propionate (FP) into the lungs. The presence of mucoadhesive polymers allows SLMs to adhere better to the mucous layer on the respiratory epithelium as compared with conventional carriers. The obtained systems are characterized in terms of size, polydispersity index (PDI), zeta potential and morphology. We also evaluated the loading capacity (LC) as well as the kinetics release.
Afterwards, we evaluated the cytotoxicity in vitro of the free FP or entrapped into SLMs and empty microparticles by MTS viability assays on 16-HBE (human bronchial epithelial cells) cell lines. Neither FP-loaded SLMs nor empty SLMs at the different tested concentrations showed cytotoxicity compared to the free FP. Finally, we tested the effect of CSE (cigarette smoke extract) in ROS production by bronchial epithelial cells evaluating the expression of survivin and p-erk/tot-erk ratio. FP-loaded SLM significantly reduced survivin expression and p-erk/tot-erk expression ratio.

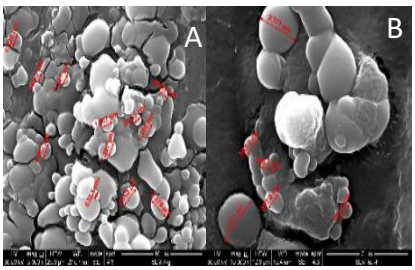


Figure 1. SEM images of FP loaded alginate SLM (A) and FP loaded chitosan SLM (B)

1. M.L. Bondì, M. Ferraro, S. Di Vincenzo, S. Gerbino, G. Cavallaro, G. Giammona, C. Botto, M. Gjomarkaj, E. Pace, *Journal of Nanobiotechnology*, **2014**, *12*, 46.
2. Z. Liang, R. Ni, J. Zhou, S. Mao, *Drug Discovery Today*, **2015**, *20*, 380.

Cationic Solid Lipid Nanoparticles (cSLNs) for shNUPR1 plasmid delivery in the treatment of hepatocellular carcinoma (HCC)

Maria Luisa Bondì,[a] Chiara Botto,[b] Erika Amore,[b] Marco Santonocito,[b] Elisabetta Martorana,[b] Giuseppa Augello,[c] Maria Rita Emma,[c] Gennara Cavallaro,[d] Melchiorre Cervello [c]

[a] *Istituto per lo Studio dei Materiali Nanostrutturati (ISMN), U.O.S. Palermo, CNR, Via Ugo La Malfa, 153, 90146 Palermo, Italy;* [b] *Dipartimento di Scienze e Tecnologie Biologiche, Chimiche e Farmaceutiche (STEBICEF), Università degli Studi di Palermo, Via Archirafi 32, 90123 Palermo, Italy;* [c] *Istituto di Biomedicina ed Immunologia Molecolare (IBIM), "A. Monroy", CNR, Via Ugo La Malfa, 90146 Palermo, Italy;* [d] *Dipartimento di Scienze e Tecnologie Biologiche, Chimiche e Farmaceutiche (STEBICEF), Università degli Studi di Palermo, Via Archirafi 32, 90123 Palermo, Italy.*

Email of presenting author: marialuisa.bondi@ismn.cnr.it

Over the last decade, gene therapy has gained enormous attention as a therapeutic strategy for a large number of pathologies including genetic, neoplastic and infectious diseases. Gene therapy consists in any procedure intended to treat or alleviate a disease by genetically modifying the cell of a patient. In particular, fragments of DNA or RNA are delivered into specific cells in order to modulate the expression or suppression of specific altered proteins involved in the onset of the disease [1].

The aim of this study was to investigate the potential of cationic solid lipid nanoparticles (cSLNs) to deliver and transfect shNUPR1 plasmid into cancer liver cells for application in hepatocellular carcinoma (HCC) therapy. NUPR1 is a small multifunctional protein whose expression is increased in HCC tissues and has been shown to control HCC cell growth, proliferation, migration, invasion and response to chemotherapy. For all these reasons NUPR1 might be a protein whose blockade would prevent HCC progression and metastasis development. In the present study, different positively charged nanocarriers were prepared, characterized and complexed with a plasmid DNA. The biocompatibility of the particles and their complexes were confirmed by hemolysis assay (Figure 1) and the cytotoxicity studies were carried out on the human hepatocellular carcinoma cell line Hep3B. Finally, the nanoparticles showed to protect shNUPR1 plasmid from degradation by DNase I and to transfect it into Hep3B cells.

These findings suggest that these systems can be considered quite biocompatible and this feature opens new scenarios for the use of this class of biocompatible cationic nanomaterials for biomedical applications, such as gene delivery.

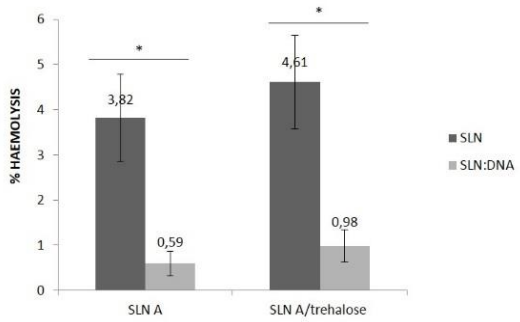


Figure 1. Haemolysis test results after incubation of the cSLNs samples (empty or complexed with the shNUPR1 plasmid) with red blood cells for 1 hour at 37 °C.

1. M.L. Bondì, E.F. Craparo, *Expert Opinion in Drug Delivery*, **2010,** *7*, 7.

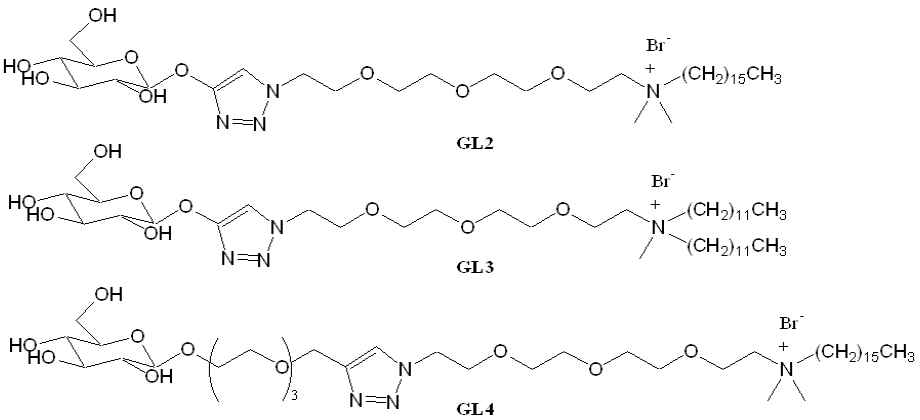
Glycosilated liposomes for targeted drug delivery to breast cancer cells

Giuseppina Bozzuto,[a] Maria Condello,[a] Luisa Giansanti,[b,c] Giovanna Mancini,[c] Alessandro Mauceri,[c,d] Agnese Molinari [a]

[a] *Dipartimento di Tecnologie e Salute, Istituto Superiore di Sanità, Viale Regina Elena 299, 00161 Rome, Italy;* [b] *Dipartimento di Scienze Fisiche e Chimiche, Università degli Studi dell'Aquila, Via Vetoio, 67100 Coppito (Aq), Italy;* [c] *CNR – Istituto di Metodologie Chimiche, Via Salaria km 29.300, 00016 Monterotondo Scalo, Rome, Italy;* [d.] *Dipartimento di Chimica, Università degli Studi di Roma "La Sapienza", P.le A. Moro 5, 00185 Rome, Italy.*

Email of presenting author: giuseppina.bozzuto@iss.it

The functionalization of liposomes with glycolipids might ascribe them specificity toward cancer cells overexpressing GLUT family members correlated with invasive potential, survival and uncontrolled proliferation.[1]. Liposomes composed of 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC) were functionalized with the glucosylated lipids reported below, and evaluated on three human breast cancer cell lines expressing GLUT1 and GLUT 4 receptors. The uptake and intracellular distribution of the different liposomes, labeled by a fluorescent lipid, were analyzed by flow cytometry and laser scanning confocal microscopy (LSCM), respectively. Flow cytometry analysis showed that uptake efficacy depends on cell type and that DMPC/GL3 7:3 formulation was more efficiently uptaken by all three breast cancer cell lines. The observations performed by LSCM showed different intracellular localizations of liposomes (Figure 1).



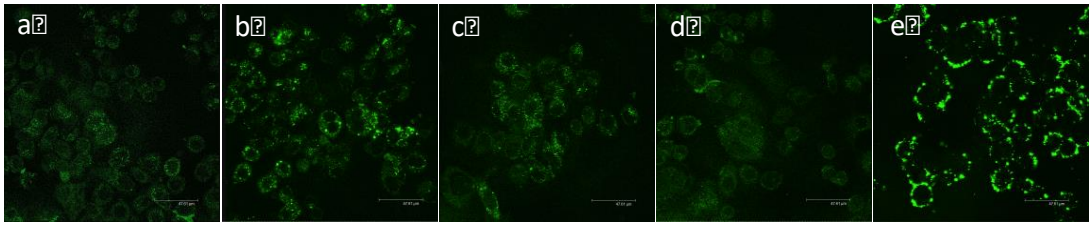


Figure 1. Observations by laser scanning confocal microscopy of SKBR3 breast cancer cells treated with different liposome formulations for 18 h. (a) DMPC; (b) DMPC/GL2 95:5; (c) DMPC/GL4 95:5; (d) DMPC/GL3 95:5; (e) DMPC/GL3 7:3.

1. L. Szablewski, *BBA*, **2013**, *1835*, 164.

Sugar-modified polymeric micelles for physical stabilization and improved performance of hydrophobic drugs

Alexandra Bukchin,[a] Guillem Pascual-Pasto,[b] Carles Monterrubio,[b] Nagore G. Olaciregui,[b] Ángel Carcaboso,[b] Alejandro Sosnik [a]

[a] *Laboratory of Pharmaceutical Nanomaterials Science, Department of Materials Science and Engineering, Technion-Israel Institute of Technology, Haifa, Israel;* [b] *Department of Pediatric Hematology and Oncology, Hospital Sant Joan de Déu Barcelona, Spain.*

Email of presenting author: alexbukchin@gmail.com

Polymeric micelles (PMs) were introduced to address the poor water solubility of about 50-70% of the marketed drugs as well as the ones that are still being developed. In this context, the amphiphilic poly(ethylene oxide)-poly(propylene oxide) (PEO-PPO) block copolymers emerged as one of the most promising families. However, their relatively low physical stability is jeopardized under extreme dilution. Moreover, they are incapable of actively targeting specific cell types. Aiming to improve the micellization and to increase the uptake by cells expressing sugar receptors, we investigated the synthesis and characterization of PMs surface-decorated with sugar residues of growing size employing two different chemical pathways. Microwave-assisted ring-opening conjugation of gluconolactone and modification with lactobionic acid using the conventional Steglich esterification reaction [1,2]. Also, a combination of the two pathways was investigated to obtain a hybrid product. The improved self-assembly was verified by the decrease in the critical micellar concentration. First, the binding of the micelles to sugar receptors was demonstrated *in vitro* by the concanavalin A agglutination assay. Drug encapsulation in pristine and modified PMs was studied with two hydrophobic drugs with similar structure and different melting temperature and intrinsic aqueous solubility. The solubility of the drugs in water increased by several hundred-folds. Then, the interaction of the micelles with the clinically relevant sugar-binding cells was assessed. Features such as cell compatibility and uptake mechanism in the presence of the pristine and modified PMs with and without the drug were evaluated.

1. R. J. Glisoni, A. Sosnik, *Macromol. Biosci.*, **2014**, *14*, 1639.
2. M. L. Cuestas, R. J. Glisoni, V. L. Mathet, A. Sosnik, *J. Nanoparticle Res.*, **2013**, 15, 1389.

Acknowledgments: This work was supported by the European Union's - Seventh Framework Programme under grant agreement #612765-MC-NANOTAR and Technion (internal grant #2019300).

Effect of citrate stabilized gold nanoparticles on β-2microglobulin fibrillation process

Cristina Cantarutti,[a] Alessandra Corazza,[a] Sara Raimondi,[b] Sofia Giorgetti,[b] Vittorio Bellotti,[b] Federico Fogolari,[a] Gennaro Esposito [a,c]

[a] *Department of Medical and Biological Sciences, University of Udine, p.le M. Kolbe, 33100, Udine, Italy;* [b] *Department of Molecular Medicine, Biochemistry Unit, University of Pavia, via Taramelli, 27100, Pavia, Italy;* [c] *Science & Math Division, New York University of Abu Dhabi, Abu Dhabi, UAE.*

Email of presenting author: cantarutti.cristina@spes.uniud.it

The relevance of nanoparticle-protein interaction extends nowadays over different research fields. We have investigated the behaviour of citrate stabilized gold nanoparticles (AuNP) with β-2microglobulin (β2m) which is a paradigmatic system of amyloidogenic proteins. β2m itself is the protein responsible for dialysis related amyloidosis (DRA) affecting long-term hemodialysed individuals by aggregating in insoluble fibers that deposit on tissues.[1]
NMR experiments have shown that the overall globular structure of the protein is conserved in the presence of AuNP and that there is a region of β2m that is more involved in the interaction with the particle surface.[2]
To investigate the influence of AuNP on the fibrillation process of β2m, we used a naturally occurring mutant, namely D76N, which is responsible for an aggressive systemic amyloidosis.[3] We analyzed the fibril formation by three different methods: thioflavin T fluorescence, native agarose gel electrophoresis and transmission electron miscroscopy. Our results indicate that AuNP are able to hamper D76N fibrillogenesis through an effective interaction that competes with protofibril recruitment.
These findings open the perspective for applications of functionalized gold nanoparticles as highly specialized chemical surfaces that can be employed for multiple purposes including therapeutic and diagnostic applications.

1. F. Gejyo, T. Yamada, S. Odani, Y. Nakagawa, M. Arakawa, T. Kunitomo, H. Kataoka, M. Suzuki, Y. Hirasawa, T. Shirahama, A.S. Cohen, K. Schmid, *Biochem Biophys Res Commun*, **1985**, *129*, 701.
2. G. Brancolini, A. Corazza, M. Vuano, F. Fogolari, M.C. Mimmi, V. Bellotti, M. Stoppini, S. Corni, G. Esposito, *ACS Nano*, **2015**, *9*, 2600.
3. S. Valleix, J.D. Gillmore, F. Bridoux, P.P. Mangione, A. Dogan, B. Nedelec, M. Boimard, G. Touchard, J.M. Goujon, C. Lacombe, P. Lozeron, D. Adams, C. Lacroix, T. Maisonobe, V. Planté-Bordeneuve, J.A. Vrana, J.D. Theis, S. Giorgetti, R. Porcari, S. Ricagno, M. Bolognesi, M. Stoppini, M. Delpech, M.B. Pepys, P.N. Hawkins, V. Bellotti, *N. Engl. J. Med.*, **2012**, *366*, 2276.

Acknowledgments: We thank Paolo Bertoncin (Department of Life Sciences, University of Trieste, via Weiss, 34128, Trieste, Italy) for TEM measurements.

Inclusion of antioxidants in mitochondriotropic liposome-based formulations

Fabrizio Ciranna,[a] Francesca Ceccacci,[b] Simona Sennato,[c] Stefano Gallina,[a] Alessia Ciogli,[d] Rocchina Sabia,[d] Cecilia Bombelli [b]

[a] *Sapienza University, Chemistry Department, P.le A. Moro 5, Rome, Italy;* [b] *CNR-IMC-Sezione Meccanismi di reazione c/o Chemistry Department, Sapienza University, P.le A. Moro 5, Rome, Italy;*
[c] *CNR-ISC- UOS Sapienza c/o Physics Department, Sapienza University, P.le A. Moro 5, Rome, Italy;*
[d] *Sapienza University, Department of Chemistry and Technology of Drug, P.le A. Moro 5, Rome, Italy.*

Email of presenting author: fabrizio.ciranna@gmail.com

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. With the final goal of delivering resveratrol and trolox, two well-known antioxidants (AO), we developed a mitochondriotropic liposome-based drug delivery system with a core-shell structure, composed of

i) mixed liposomes, formulated with a natural phospholipid (1,2-dioleoyl-*sn*-glycero-3-phosphocholine, DOPC, or 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine, DPPC), the cationic triarilphosphonium bolaamphiphile **1** and cholesterol, if need be. Because of the extended delocalization of the positive charge on the headgroups, **1** should favor the crossing of the mitochondrial membranes guided by the negative potential of the inner membrane;
ii) a biocompatible polymer coating (BC), decorating the liposome. The BC, composed of polymers such as chitosan and dextran, is able to tune the surface properties, as charge, hydrophilicity and, more interestingly, it opens the possibility to specific functionalize liposome surface with proper moieties for the targeting of specific cells. Such a coating is also supposed to help liposomes to reach undamaged the cytosol and, finally, mitochondria.

Two different protocols, *i.e* passive loading in the lipid bilayer and active loading in the internal aqueous phase by the acetate gradient method,[1] were explored to load the antioxidant into liposomes. The entrapment efficiency (EE) of the antioxidants was determined by HPLC (resveratrol) or UV spectroscopy methods.

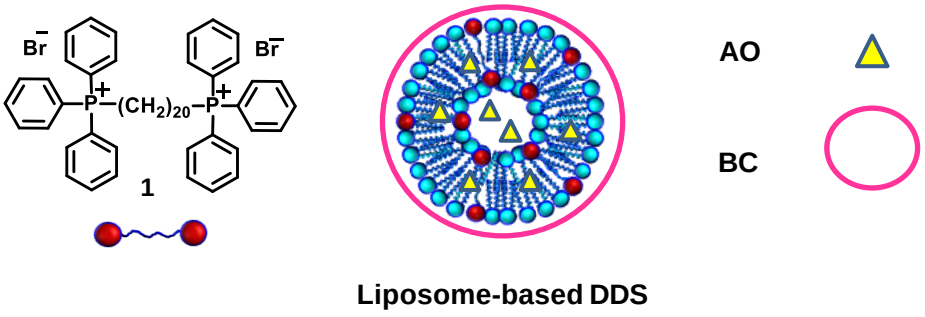


Figure 1. Representation of the liposome-based drug delivery system and chemical structure of the bolaamphiphile **1**

1. S. Clerc, Y. Barenholz, *Biochim. Biophys. Acta*, **1995**, *1240*, 257.

Acknoledgments: three of the authors (Ceccacci, Bombelli, Sennato) thanks the FIRB project RBFR12BUMH funded by MIUR for financial support.

Drug Delivery Systems and Breast Cancer Cells

Marisa Colone,[a] Annarica Calcabrini,[a] Subramanian Kaliappan,[b] Mariarosaria Tortora,[b] Francesca Cavalieri,[b] Annarita Stringaro [a,b]

[a] *Department of Technology and Health, Istituto Superiore di Sanità, 00161 Rome, Italy;* [b] *Department of Chemical Science and Technology, University of Tor Vergata, 00133 Rome, Italy*.

Email of presenting author: marisa.colone@iss.it

Micro/nanoparticles are being increasingly used in drug delivery systems and can be designed to have a chemically and biologically active surface with functional groups for conjugation of tumor targeting ligands and therapeutic agents. The use of micro/nanoparticles offers many advantages. First of all, their size and surface characteristics can be easily manipulated and this could be used for both passive and active drug targeting. Moreover, they can be designed to control and sustain release of the drug during the transportation as well as the location of the release. In addition, polymeric nanoparticles have emerged as a versatile nanotechnology platform for controlled, sustained and targeted delivery of anticancer agents including small molecular weight drugs and macromolecules such as genes and proteins. They offer the advantage of prolonged circulation in the blood stream, targeting to specific sites, and reduced side effects.

To trigger and control the delivery of the drug at a specific tumor site, different stimuli can be applied such as ultrasound, magnetic or electric fields. To this aim, in this study, ultrasound-responsive micro/nanoparticles have been developed to release drug content in response to an acoustic stimulus. Recently, polymer-shelled microbubbles (LSMBs) and microcapsules (LSMCs) have been synthesized by high-intensity ultrasound-induced emulsification and self-cross-linking of lysozyme and thiolated poly methacrylate in an aqueous solution polymer-shelled (1, 2). LSMBs and LSMCs may provide drug payload capacity and a large surface for conjugation of targeting ligands.

As well known, biological reducing agents (such as glutathione, GSH) are present at higher concentration in tumor cytosol than in normal tissues. Based on this finding, redox shell-sheddable polymeric capsules possess great prospective for anticancer drug release because they are stable in the extracellular environment but able to rapidly respond to the intracellular GSH, releasing drugs into the cytosol and nuclei. For this purpose we have developed a delivery system for breast cancer cells using a redox-active microcapsules based on thiolated polymethacrylic acid (PMA_{SH}) (3). The interaction of LSMBs, LSMCs and PMA_{SH} with human breast adenocarcinoma cells (SKBR3), as well as their biocompatibility, kinetics of uptake and intracellular degradation, have been evaluated.

Data obtained in our study strongly suggest that LSMBs, LSMCs exhibit a strong adhesion on SKBR3 surface and an efficient internalization without inducing cytotoxic effects. PMA_{SH} capsules loaded with doxorubicin are efficiently internalized by the cells and could inhibit their viability through the release of drug at long incubation times, suggesting that antitumoral drug maintained its activity even if conjugated to a polymer by a non-hydrolyzable linkage. These results highlight the potential uses of these responsive platforms suited for biomedical and pharmaceutical applications.

1. F. Cavalieri, M. Ashokkumar, F. Grieser, F. Caruso, *Langmuir*, **2008**, *24*, 10078.
2. F. Cavalieri, M. Colone, A. Stringaro, *et al*., *Part. Part. Syst. Charact.*, **2013,** *30*, 695.
3. M. Colone, S. Kaliappan, A. Calcabrini, *et al*., *J. Mol. Genet. Med*., **2016**, *10*, 1.

The improvement of 5-Fluorouracil antitumor efficacy by cationic liposomes delivery

Manuela Petaccia,[a,#] Maria Condello,[b,#] Luisa Giansanti,[a] Angela La Bella,[c] Francesca Leonelli,[d] Stefania Meschini,*[,b] Denise Gradella Villalva,[c] Evelin Pellegrini,[b] Francesca Ceccacci,[e] Giovanna Mancini *[,f]

[a] *Department of Physical and Chemical Sciences, University of L'Aquila, Via Giovanni Falcone 25, 67100 L'Aquila, Italy;* [b]*Department of Technology and Health, National Institute of Health, Viale Regina Elena 299, 00161, Rome, Italy;* [c] *Department of Chemistry, University of Rome "Sapienza", P.le Aldo Moro 5, 00185 Rome, Italy;* [d] *Department of Plant Biology and Animal, University of Rome "Sapienza";* [e] *Institute of Chemical Methodologies, National Research Council, c/o Department of Chemistry, University of Rome "Sapienza;* [f] *Institute of Chemical Methodologies, National Research Council, Via Salaria km 29.300, 00016 Monterotondo Scalo, Rome, Italy.*

[#] *These two authors contributed equally to this work.*

Email of presenting author: maria.condello@iss.it

5-Fluorouracil (5-FU), is widely employed as chemotherapic agent in the treatment of malignant tumors such as head, neck, breast, ovarian, and colon cancer. The main toxicities of 5-FU are stomatitis, diarrhea, dermatitis, and myelosuppression. Cancer patients who are receiving 5-FU treatment can develop severe side effect as neurologic toxicity that very often requires admission to intensive care unit. A strategy to reduce the therapeutic dose and improving the anticancer action of 5-FU concerns the use of drug delivery systems based on biodegradable colloidal particles, such as polymeric particles and liposomes.

The synthesis, the physicochemical characterization and the biological evaluation of three new non ionic amphiphilic derivatives of 5-FU **1-3** (Figure 1), designed to be easily embedded in liposomal bilayers, are here reported. The novel 5-FU derivatives were included in liposomes composed of a natural phospholipid, dioleoyl-snglycero-phosphocholine (**DOPC**, Figure 1), and of gemini cationic amphiphile **4**, (Figure 1) that had been shown to attribute to phospholipid formulations high efficacy in drug delivery (1). The cytotoxicity of the formulations was evaluated by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay on colorectal carcinoma HCT116 cell line after 48 h of treatment. The formulations were found active against HCT116 tumor cells, and it was shown that the efficacy of the treatment increases as a function of the length of the polyoxyethilenic segment of the 5-FU derivatives, while the polyoxyethilenic chain itself has no influence on their biological activity.

Ongoing studies by flow cytometry and confocal microscopy on the mechanism of interaction with cells and internalization will clarify the mechanism of action of the new formulations.

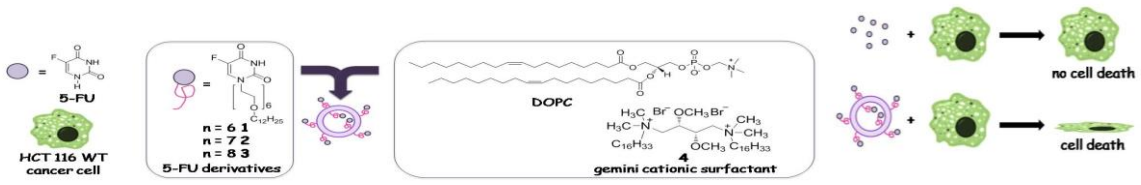


Figure 1. Schematic representation of results.

1. C. Bombelli, F. Faggioli, P. Luciani, G. Mancini, M.G. Sacco, *J. Med. Chem.*, **2005**, *48*, 5378.

A spoonful of sugar helps the medicine go down: glyconanoparticles for nanomedicine

Carina Crucho,[a,b] Maria Teresa Barros,[b] Carlos Baleizão,[a] José Paulo Farinha [a]

[a] *CQFM – Centro de Química-Física Molecular and IN – Institute of Nanoscience and Nanotechnology, Instituto Superior Técnico, Lisboa, Portugal;* [b] *LAQV, REQUIMTE, Departamento de Química, Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa, 2829-516 Caparica, Portugal.*

Email of presenting author: carina.crucho@tecnico.ulisboa.pt

In a 1964 Disney musical classic, Mary Poppins sang that "A spoonful of sugar helps the medicine down in a most delightful way", which was not far from the truth. Sugars are ubiquitous in nature and intervene in a wide range of biological activities.[1] From a pharmaceutical point of view, they exhibit interesting properties such as low toxicity, biocompatibility, stability, low cost, hydrophilic nature, and availability of reactive sites for chemical modification. One area with great potential is the use of glycopolymers as vehicles for therapeutics or as therapeutics themselves.[2]

Recent advances and progress in nanoscience have demonstrated the great potential of nanomaterials for application in healthcare.[3] Essentially, if a material is divided into very small particles, these will cast a much larger shadow. In the field of drug delivery, nanomedicines have been used *in vivo* to protect drugs from being metabolized by the body´s natural defenses, and to enhance its bioavailability in the bloodstream. In particular smart nanoparticles have received remarkable attention due to their responsiveness to specific stimuli, making them promising candidates for more accurate and programmable drug delivery.[4]

During the last decade, there have been many attempts to integrate sugars in nanomaterials.[5] Glyconanoparticles were used as carriers with high affinity and binding specificity due to the multivalent interactions between surface sugar ligands and targeted receptors.

On this light, novel sucrose-based polymeric nanoparticles [6] and mesoporous silica nanoparticles with a shell of stimuli-responsive glycopolymers were designed and synthesized to tune their targeting and blood circulation behavior, and provide controlled drug release. It is expected that our new materials will provide a platform for further developments in the area of precision drug delivery.

1. C.I.C. Crucho, P. Correia-da-Silva, K.T. Petrova, M.T. Barros, *Carbohydrate Research*, **2015**, *402*, 124.
2. S. G. Spain, N. R. Cameron, *Polym. Chem*. **2011**, *2*, 60.
3. M. L. Etheridge, S. A. Campbell, A. G. Erdman, C. L. Haynes, S. M. Wolf, J. McCullough, *Nanomedicine: NBM*, **2013**, 1.
4. a) C.I.C. Crucho, *ChemMedChem*, **2015**, *10*, 24. b) C. Baleizão, J. P. Farinha, *Nanomedicine*, **2015**, *10*, 2311
5. G. Yilmaz, C. R. Becer, *Polym. Chem*. **2015**, *6*, 5503.
6. a) C.I.C. Crucho, M.T. Barros, *J. Mater. Chem. B*, **2014**, *2*, 3946; b) C.I.C. Crucho, M.T. Barros, *Polymer*, **2015**, *68*, 41.

Acknowledgments: This work was partially supported by Fundação para a Ciência e a Tecnologia (FCT-Portugal) and COMPETE (FEDER), projects RECI/CTM-POL/0342/2012, UID/NAN/50024/2013 and PTDC/CTM-POL/3698/2014.

2-Hydroxyiminoaldehydes: Synthesis and characterization of novel, highly versatile organic molecules for applications in nanomedicine

Patrizia Gentili,[a] Francesca D'Acunzo,[b] Paolo Cambise,[a] Mariangela Clemente,[a] Martina Nardi,[a] Alessandro Pinna,[a] Giampiero Proietti [a]

[a]*Department of Chemistry, Sapienza University of Rome, P.le Aldo Moro 5, 00185 Rome,Italy;* [b]*Institute of Chemical Methodologies, CNR, Via Salaria km 29.300, 00015 Monterotondo, Rome, Italy.*

Email of presenting author: Francesca.dacunzo@cnr.it

2-Hydroxyiminoaldehydes (HIA, Figure 1) are a class of organic molecules bearing an oxime group next to an aldehyde moiety. These two adjacent groups and their mutual interaction endow HIAs with peculiar physico-chemical properties of interest for biomedical applications, particularly in the field of nanomedicine. These properties are an unusually low pKa for an oxime; accessible oxidation potentials for their conjugated bases; inter- and intramolecular hydrogen bonding, together with a thermally-reversible photoisomerization behavior driven by the oxime group. By varying the substituent R, HIAs can be designed as adjuvants in drug release systems, as self-assembling amphiphiles for stimuli-responsive liposomal formulations, as monomers for multi-stimuli responsive polymeric micelles and polymerosomes, or as ligands for metal cations. In this work, a convenient aldehyde α-oximation method, developed in our laboratory (1), was applied to the synthesis of HIAs with different R groups (Figure 1). Their pKa, redox potential, UV-Vis spectra were then investigated. pKa values were in the 7.7-9.9 range, i.e. about 3 units lower than the corresponding oxime, devoid of the α-aldehyde group. Oxidation potentials for the oximate ion of HIAs were *quasi*-reversible and lower than 1V *vs* NHE. A bathochromic shift and a hyperchromic effect were observed in the UV-Vis spectra of the conjugated bases of HIAs. As for their photochemistry, we confirmed that the relative abundance of different configurational isomers is altered upon exposure to light (350 nm), and we observed that this phenomenon, and the rate at which it is thermally reversed, are a function of the R group. In the case of a long-chain, branched HIA we observed its liposome-forming quality, both as a stand-alone amphiphile, and in combination with a surfactant. We also explored the ability of a methacrylic-containg HIA to withstand conditions for a radical polymerization, and found AIBN to be a compatible initiator.

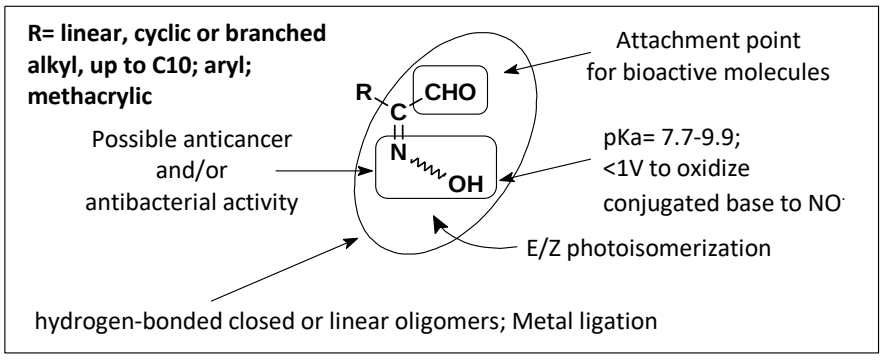


Figure 1. 2-hydroxyiminoaldehydes (HIAs) and their properties

1. P. Gentili, S. Pedetti, *Chemical Communications*, **2012**, *48*, 5358.

Acknowledgments: We thank Dr. Simona Sennato of CNR – ISC UOS Sapienza, for Dynamic Light Scattering and Z-potential measurements.

Enhanced antimalarial efficacy of a controlled release in situ gel of arteether - lumefantrine

Shilpa Dawre,[a] Sulabha Pathak,[b] Shobhona Sharma,[b] Padma V. Devarajan [a]

[a] *Department of Pharmaceutical Sciences and Technology, Institute of Chemical Technology, Deemed University, Matunga, Mumbai- 400019;* [b] *Department of Biological Science, Tata Institute of Fundamental Research, Mumbai, India.*

Email of presenting author: shilpadawre@gmail.com

Artemisinin-based combination therapy (ACT) is the WHO recommended gold standard treatment for uncomplicated and severe malaria. Among the artemisinin derivatives Arteether (ART) exhibits high *in vitro* efficacy against plasmodium and is available as oily intramuscular injection to be administered in 3 doses for consecutively 3 days. In the present study we report a new ACT of Arteether with Lumefantrine (LUM). The objective of the present study was on one hand development of this new ACT as single shot therapy and also to establish the antimalarial efficacy *in vivo* in a preclinical mouse model. A stable i*n situ* gel of ART-LUM which exhibited rapid gelling following injection and controlled release of the drug over three days was developed as a patient friendly formulation. *In vivo* evaluation was carried out using two models namely modified Peter's 4 day suppressive test and the clinical simulation model in male swiss mice infected with lethal ANKA strain of *Plasmodium berghei*. The innovator formulation of Arteether was taken as reference administered at therapeutic dose of 3.2mg/Kg for consecutively 3 days. A single dose of the *in situ* gel of ART-LUM (1:6) was administered at different dose levels starting at Artether 3.2mg/Kg with LUM 19.2 mg/kg followed by dilutions up to 1/10 to 1/160. While the marketed formulation showed recrudescence and 100% mortality within 20 days, the *in situ* gel conferred complete protection at 1/40 therapeutic dose in the Peter's 4 day suppressive test ($P<0.01$). In the clinical simulation study the marketed formulation showed initial clearance followed by relapse after 5 days and 100% mortality within 15 days. The *in situ* gel of ART-LUM at 1/40 dose evident 92.65% inhibition of parasites and 62 % survival after 30 days ($P<0.05$). Although *in situ* gel of ART-LUM at therapeutic dose and also at lower dilutions 1/5-1/20 dose level caused rapid reduction in parasitemia with no recrudesce of the parasites till day 45. The clinical simulation study suggests that 1/20 dose of ART-LUM equivalent to ART 0.16 mg/Kg and LUM 0.96mg/Kg *in situ* gel given as a single dose was sufficient to cure the mice and allow 100% survival. The high antimalarial efficacy exhibited by the ART-LUM *in situ* gel proposes the same as a promising and superior drug delivery system for ACT.

1. R. Gudhka, et al., *Recent Patents on Nanomedicine*, **2015**, *5*, 38

Acknowledgment: Micro Orgo Chem for gift sample of Arteether and Lumefantrine University Grants Commission (Government of India) for funding and fellowship to Shilpa Dawre

Multimodal Photoresponsive Nanoassemblies of an amphiphilic Calix[4]arene for Antibacterial Applications

Ivana Di Bari,[a] Anna Rita Blanco,[b] Roberta Picciotto,[b] Grazia M. L. Consoli,[c] Salvatore Sortino[a]

[a] *Department of Drug Science, University of Catania, V.le A. Doria 6, 95125, Catania, Italy;* [b] *SIFI S.p.a., Via E. Patti 36, 95020, Lavinaio – Aci S. Antonio, Italy;* [c]*CNR-Institute of Biomolecular Chemistry, Via P. Gaifami 18, 95126, Catania, Italy.*

Email of presenting author: ivanadibari@hotmail.it

Photodynamic Therapy (PDT) is based on the administration of a photosensitizing agent (PS) that generates mainly the highly reactive singlet oxygen ($^1O_2$), leading to oxidative damage and cell death. However, many PSs have a hydrophobic nature, which favors their aggregation in aqueous medium strongly precluding their photochemical behavior.[1] The entrapment of PS in nanocarriers permits to overcome these drawbacks and ensures protection from degradation, site-specific delivery, enhanced bioavailability, increased local concentration. Combination of PDT with other photogenerated cytotoxic species such as nitric oxide (NO) is particularly attractive, as NO possesses excellent anticancer and antibacterial properties. $^1O_2$ and NO are able to react with different biological substrates, do not suffer multidrug resistance problems and, due to their short half-life in blood (<1s), lack of charge and small sizes, they can diffuse in the cellular environment over short distances (<200 μm), confining their region of action and reducing side effects. Moreover, since the NO photorelease from NO photodonors is independent from O_2 availability it can potentially very well complement the $^1O_2$ effects at the onset of hypoxic conditions typical of some tumors and infections by anaerobic bacteria.[2] Synthetic versatility and good biocompatibility are crucial requisites which make the calixarene macrocycles appealing in pharmaceutical and biomedical field.[3] In the search of novel antibacterial strategies designed to simultaneously address problems of antibiotic resistance and biofilm formation, we achieved the first example of calixarene (CA)-based nanocontainers incorporating multiple photoresponsive agents (Fig. 1). The capability of the obtained nanosystems to generate $^1O_2$ and/or NO upon to irradiation was investigated directly and in real time by means infrared luminescence spectroscopy and photoamperometric techniques. The light-stimulated bactericidal effect was successfully tested on Gram positive and Gram negative bacteria. Enhancement of NO photorelease, due to the action of the CA assembly as a nanoreactor, size, and the presence of targeting groups on the surface of CA the open new perspectives for antibacterial application in nanomedicine.

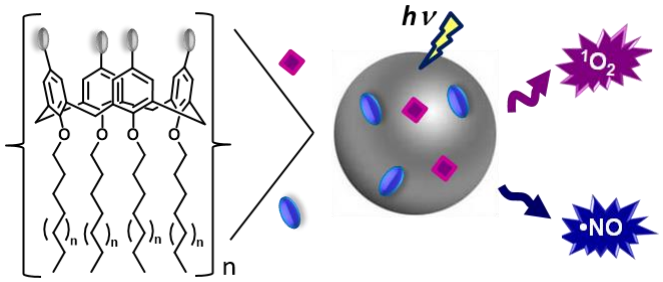


Figure 1. Schematic representation of a CA-based photoresponsive nanosystem.

1. K. Lang, J. Monsinger, D. M. Wagnerovà, *Coordin. Chem. Rev.*, **2004**, *248*, 321.
2. A. Fraix, N. Marino, S. Sortino, *Topics Curr. Chem.*, **2016**, *370*, 225.
3. S. B. Nimse, *T. Kim, Chem. Soc. Rev.*, **2013**, *42*, 366.

Antinociceptive effects of Curcumin-loaded PLGA vesicles

Rita Di Giovannandrea, Francesca Marzoli, Paola Minosi, Amalia Di Giannuario, Stefano Pieretti

Department of Pharmacology and Therapeutic Research, Istituto Superiore di Sanità, Viale Regina Elena 299, 00161, Rome, Italy.

Email of presenting author: amalia.digiannuario@iss.it

Curcumin is yellow polyphenol, extracted from the rhizomes of turmeric (*Curcuma longa*) and used in traditional medicine for many centuries in countries such as India and China (1). Curcumin demonstrated a wide range of pharmacological activities that include antitumor, anti-amyloid, antioxidant, anti-inflammatory properties and analgesia. At present, no data are reported in the literature on the antinociceptive effects induced by curcumin loaded in PLGA vesicles (PLGA-CURC) and we investigated first the effects of PLGA-CURC in acute models of pain after systemic and central administration. Male CD-1 mice (Harlan, Italy) weighing 25-30 g were used for all experiments. The research protocol was authorized by the Italian Ministry of Health, according to Legislative Decree 26/14. Subcutaneous injection of a dilute solution of formalin (1%, 20 µl/paw) into the mice hind paw evokes nociceptive behavioral responses, such as licking, biting the injected paw or both, which are considered indices of nociception (2). The nociceptive response shows a biphasic trend, consisting of an early phase occurring from 0 to 10 min after the formalin injection, due to the direct stimulation of peripheral nociceptors, followed by a late prolonged phase occurring from 15 to 40 min, that reflects the response to inflammatory pain. The total time (s) that the animal spent licking or biting its paw during the formalin-induced early and late phase of nociception was recorded. In the first series of experiments curcumin-vehicle, curcumin, blank-PLGA and curcumin-PLGA (0.045 mg curcumin/mg of nanoparticles, a generous gift of dr. A. Ranjan, University of North Texas Health Science Center, Fort Worth, TX, USA) were administered i.v. at the dose of 20 mg/kg, in curcumin. In the second series of experiments curcumin-vehicle, curcumin, blank-PLGA and curcumin-PLGA were administered i.t. at doses of 5 and 25 µg/mouse, in curcumin. The significance among the groups ($P<0.05$) was evaluated with ANOVA followed by Tukey's post-hoc comparisons using GraphPad Prism 6.03 software. After i.v. treatment, ANOVA revealed no difference between groups in the early phase of the formalin test. On the contrary, in the late phase of the test i.v. curcumin-PLGA was able to strongly reduced the nociceptive behavior induced by formalin. After i.t. administration at the dose of 5 µg/mouse, treatments did not change licking behavior induced by formalin neither in the early nor in the late phase of the test. After i.t. administration at the dose of 25 µg/mouse, curcumin-PLGA was able to reduce licking activity - in confront to curcumin-vehicle and blank-PLGA treated animals - both in the early and in the late phase of the test. These data suggest that curcumin-PLGA may be developed as a medicine to treat pain, by warranting further rigorously conducted studies to define the long-term efficacy and safety.

1. P. Anand, S.G. Thomas, A.B. Kunnumakkara, C. Sundaram, K.B. Harikumar, B.Sung, S.T.Tharakan, K. Misra, I.K. Priyadarsini, K.N. Rajasekharan, B.B. Aggarwal, *Biochem. Pharmacol*. **2008**, *76*, 1590.
2. M. Colucci, F. Maione, M.C. Bonito, A. Piscopo, A. Di Giannuario, S. Pieretti, *Pharmacol Res*., **2008**, *57*, 419.

Live monitoring of intracellular fate of novel superparamagnetic iron-doped hydroxyapatite nanoparticles

Monica Montesi, Silvia Panseri, Samuele M. Dozio, Alessio Adamiano, Michele Iafisco, Monica Sandri, Anna Tampieri

Institute of Science and Technology for Ceramics, National Research Council, Via Granarolo 64, 48018 Faenza, Ravenna, Italy.

Email of presenting author: samuele.dozio@istec.cnr.it

Strong coupling between nanotechnology and cell/molecular biology leaded to a breakthrough in medicine due to the exiting opportunities in designing and developing a tailored approach in response to different disease. Magnetic nanoparticles (NPs) have attracted the attention of scientific community for biological and medical purposes as promising materials for a broad range of application.[1-3] Here novel biomimetic, fully biodegradable and cytocompatible NPs fabricated by doping hydroxyapatite (HA) with Fe ions (FeHA), avoiding the presence of magnetic secondary phases and any coating, were biologically analysed in vitro.
In detail, FeHA NPs were prepared by a neutralization process using $FeCl_2$ and $FeCl_3$ as a source of Fe2+ and Fe3+ doping ions;[4] HA NPs and commercial fluidMag NPs (Chemicell) were used as a control. Mouse pre-osteoblast cells line (OBs), MC3T3-E1, and human Osteosarcoma cell line, MG63, were cultured with 100 μg/ml NPs up to 72 hours. The molecular pathways of cellular response (apoptosis/necrosis, ROS production and autophagy) to NPs were investigated. Moreover, the mechanism of internalization by Caveolae-mediated endocytosis was studied. In a pilot in vivo experiment the biodistribution of different concentrations of NPs (ranging from 0.5 mg/kg up to 50 mg/kg) was evaluated.
The in vitro live monitoring showed that FeHA NPs were rapidly and easily internalized by both cell lines without producing cell damages and death (Fig.1). OBs uptake of FeHA NPs seems to be mediated by Caveolae-mediated endocytosis, while a different endocytic mechanism is required by MG63 cell line. NPs seem to act as modulator of autophagy pathway. The in vivo study showed the absence of systemic toxicity even with the higher concentration. The data obtained on the cellular uptake of FeHA NPs lay the basis to clarify the intracellular fate of the FeHA NPs and open brilliant prospective for their use as innovative tools for nanomedicine.

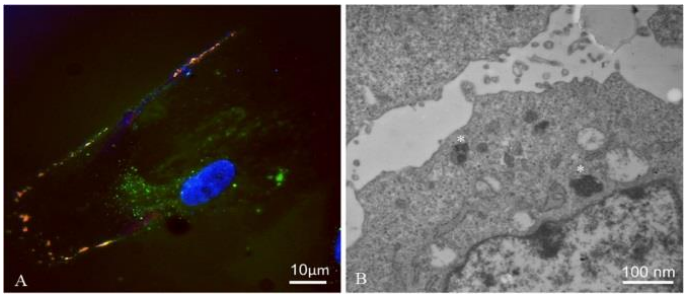


Fig.1. Intracellular localization of FeHA NPs: A. FITC-NPs and endosome (red); B. TEM image of internalized NPs (*).

1. M.I. Khan et al., *Biomaterials*, **2012**, *33* 1477.
2. A. Amirfazli et al., Nature Nanotech., **2007**, *2*, 46.
3. M. Arruebo et al., Nano Today, **2007**, *2*, 22.
4. A. Tampieri et al, Acta Biomater., **2012**, *8*, 843.

Acknowledgments: The authors thank the Eu Project SMILEY (NMP4-SL-2012-310637) and Flagship Project NanoMAX (PNR-CNR 2011-2013) for providing financial support.

Synthetic Low Density Lipoprotein (sLDL) for *in vivo* Imaging

Alessandro Fracassi, Sean Orian, Yoko Yamakoshi

Laboratory of Organic Chemistry, ETH Zürich, Vladimir-Prelog-Weg 3, 8093, Zürich, Switzerland.

Email of presenting author: afracassi@org.chem.ethz.ch

Lipoproteins are a powerful tool for targeted delivery of biological and chemical agents.[1] Their biocompatibility ensures a longer blood circulation allowing an efficient drug delivery without the addition of polymers such as PEG.[2] Since the use of native LDL has limitation in the production of large amount by isolating from donor's blood[3] with potential cause of infectious side effect and in sufficient loading of therapeutic agents,[4] synthetic LDL (sLDL) was developed[5] as a highly promising alternative vehicle that preserve the biocompatibility of the native lipoproteins with the possibility to load the imaging probes or drugs in order to obtain theranostic systems.[6-7]
Here, we report a versatile method for the preparation of chemically funcionalized LDL. All of the LDL components (triolein (TO), cholesteryl oleate (CO) and phosphatidylcholine (PC)) were dissolved in an organic solvent, which were evaporated to obtain a uniform lipidic film of a mixture of LDL components. The film was subsequently hydrated with a buffer, subjected to the sonication (two hours) to obtain a dispersion, which was extruded through a 100-nm and a 50-nm membranes. In addition to the three main components above, a fourth lipidic component was added as a scaffold for the chemical functionalization of the sLDL surface using a ligation reaction. The prepared sLDL will be subjected to the biological tests using *in vitro* cell culture and *in vivo* animal test using model mice.

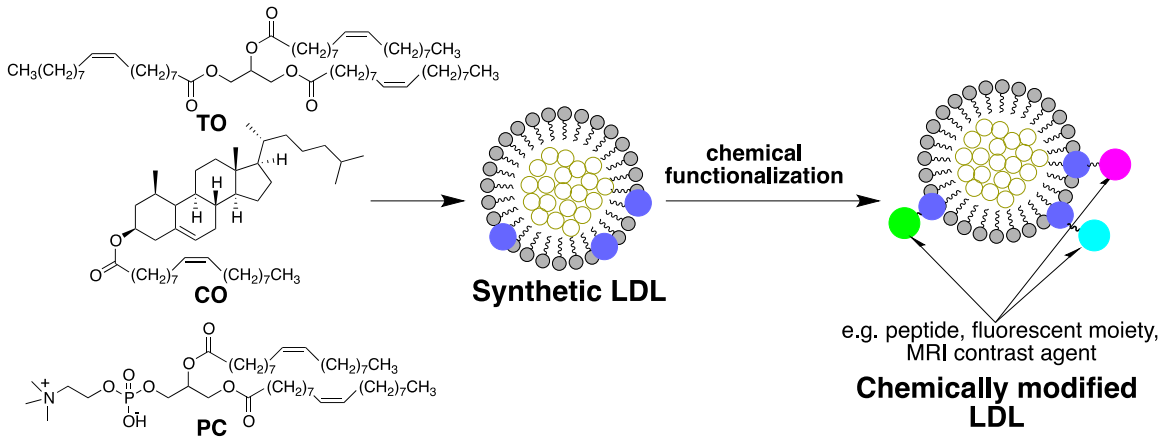


Figure 1. Schematic illustration of sLDL preparation and subsequent chemical modification.

1. H. Huang, et al., *WIREs Nanomed Nanobiotechnol* **2015**, *7*, 298.
2. Q. Lin, et al., *Small,* **2014**, *10*, 3072.
3. P.C. De Smidt, et al., *Cancer Res* **1990**, *50*, 7476.
4. M. Nikanjam, et al., *J. Control. Release*, **2007**, *124*, 163.
5. G. Baillie, et al., *J. Lipid Res.* **2002**, *43*, 69.
6. S. Geninatti, Crich *Neoplasia* **2007**, *9*, 1046.
7. Y.Yamakoshi, et al., *Chem. Commun.* **2011**, *47*, 8835.

A molecular dynamics investigation of penetration of antioxidant lipid-based nitroxides in lipid bilayer

Roberta Galeazzi,[a] Emiliano Laudadio,[a] L.Massaccesi,[a] M Cristina Minnelli,[a] Giovanna Mobbili,[a] Piero Stipa [b]

[a] *Department of Life and Environmental Science (DISVA);* [b] *Department SIMAU, Marche Polytechnic University , via Brecce Bianche, 60131, Ancona, Italy.*

Email of presenting author: r.galeazzi@univpm.it

Liposomes are artificially prepared vesicles made of phospholipid bilayers able to enhance the bioavailability of administered drugs. Nitroxide radicals are known to protect against oxidative processes in different media and under different stress conditions.[1] With the aim to improve the affinity of nitroxides to lipid particles and membrane and other oxidation-susceptible sites *in vivo*, a series of lipid-functionalized nitroxides having a pyrroline nitroxide moiety linked either to a glycerol or to a steroid unit has been synthesized, and their inclusion inside phospholipid bilayers has been investigated by Electron Paramagnetic Resonance (EPR) spectroscopy.[2] Here, we present the results of an *in silico* molecular dynamics study aimed to investigate their penetration within the bilayer. To this purpose, we extended the AMBER force-field to provide an accurate description of large and flexible nitroxide free-radicals. New atom types have been included, and relevant parameters have been fitted based on geometries, vibrational frequencies and potential energy surfaces computed at the DFT level.[3] Thus, we performed an atomistic MD simulation using GROMACS 5.0.starting with an equilibration time of 2 ns in NVT and carrying out an overall 100 ns in NPT at 310 K. At the end of the simulations, we reached a steady state as confirmed by RMSD analysis and by Area per lipid values. As a result, we pointed out the liponitroxide depth inside the.membrane Our aim is to find out a correlation between the nitroxide antioxidant activity with its positioning inside the liposomial nanovector in order to better design novel active compounds.

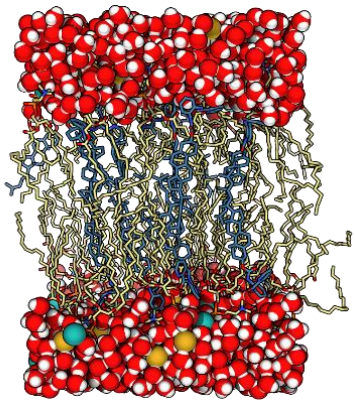


Figure 1. Lipid-functionalized nitroxide in a model bilayer.

1. L. Greci, E. Damiani, P. Carloni, P. Stipa P. in “Free Radicals in Biology and Environment”, F. Minisci Ed., Kluwer Academic Publisher, NL, **1997**.
2. G. Mobbili, E. Crucianelli, A. Barbon, M. Marcaccio, M. Pisani, A. Dalzini, E. Ussano, M. Bortolus, P. Stipa, Astolfi *RSC Advances*, **2015**, *5*, 98955.
3. E. Stendardo, A. Pedone, P. Cimino, M. Cristina Menziani, O. Crescenzi, V. Barone. *Phys Chem Chem Phys.* **2010**, *12*, 11697.

Permeation Kinetic studies of 5,7-dihydroxy-6-methyl-8-prenyl-4'-methoxy flavanone using nanoemulsion system

Valeri Domínguez Villegas,[a] Paola Salgado Bustos,[b] Beatriz Clares Naveros,[c] María Luisa Garduño-Ramírez,[a] Ana Calpena Campmany [b]

[a] *Centro de Investigaciones Químicas, Instituto de Investigación en Ciencias Básicas y Aplicadas, Universidad Autónoma del Estado de Morelos, Av. Universidad 1001. C.P. 62209 Cuernavaca, Morelos, México;* [b]*Departament of Pharmacy and Pharmaceutical Techonology, School of Pharmacy, University of Barcelona, Spain;* [c] *Departament of Pharmacy and Pharmaceutical Technology, School of Pharmacy, University of Granada, Spain.*

E-mail of presenting author: lgarduno@uaem.mx

Many of the inflammatory diseases are becoming common in aging society throughout the world.[1] Some of the clinically used anti-inflammatory drugs suffer from the disadvantage of side effects. Alternative to these drugs are traditional medicines and natural products, since ancient times traditional medicines are being used for the treatment of inflammatory.[2] Nanoemulsions are homogeneous, transparent, thermodynamically stable dispersions between immiscible phases, recently presented as pharmaceutical drug delivery systems. The 5,7-dihydroxy-6-methyl-8-prenyl-4'-methoxyflavanone (Figure 1) was isolated from alcoholic extracted of *Eysendhartia platycarpa*[3] and it showed anti-inflammatory properties. Gender *Eysenhardtia* (legume) comprises 14 species, is located in the northern and central Mexico, and some species have been used in traditional medicine as diuretics, antidiabetic, antiseptic and to treat kidney and liver infections. *E. platycarpa* is a small tree distributed in southern Mexico, where it is known as "taray", "palo dulce" and "palo azul." It has been used in the treatment of kidney diseases and liver as well as complications arising from diabetes mellitus.
The emulsion nanoestructurated of 5, 7-dihydroxy-6-methyl-8-prenyl-4'-methoxy flavanone was preparated with, labrasol, labrafac, plurol oleique and propyleneglycol as excipients. The particle size was measured by Zeta-Sizer, Malvern Instruments. Permeation studies (n = 3) were carried out with vertical Franz diffusion cell of 0.6 cm^2.

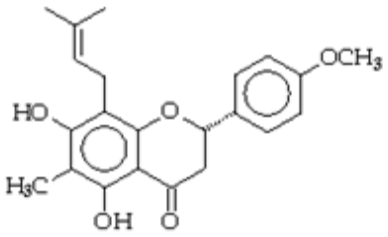


Figure 1. Structure of 5,7-dihydroxy-6-methyl-8-prenyl-4'-methoxy flavanone.

1. R. Gautam, S. Jachak, *Medicinal Research Reviews*, **2009**; *29*, 767.
2. H. Pyo Kim, K. Kun Ho Son, H. Wook Chang, S. Sik Kang, *Journal of Pharmacologycal Sciences*, **2004**, *96*, 229.
3. J.M Narváez-Mastache, M.L. Garduño-Ramírez, I. Álvarez, G. Delgado, *Journal of Natural Products*, **2006**, *69*, 1687.

Delivery study of nanoemulsion methylated derivate from natural flavanone

Berenice Andrade-Carrera,[a] Beatriz Clares,[b] Ana Calpena,[c] María Luisa Garduño-Ramírez [a]

[a] *Centro de Investigaciones Químicas, Instituto de Investigación en Ciencias Básicas y Aplicadas, Universidad Autónoma del Estado de Morelos, Av. Universidad 1001. C.P. 62209 Cuernavaca, Morelos, México;* [b] *Departament of Pharmacy and Pharmaceutical Technology, School of Pharmacy, University of Granada, Spain;* [c] *Departament of Pharmacy and Pharmaceutical Techonology, School of Pharmacy, University of Barcelona, Spain.*

E-mail of presenting author: lgarduno@uaem.mx

Introduction: *Eysenhardtia platycarpa* is a small tree distributed in Southern Mexico, where it is known as "taray", "palo dulce" (sweet wood), and "palo azul" (blue wood). Previous chemical analyses of *E. platycarpa* have allowed the isolation of flavonoids as 5,7-dihydroxy-6-methyl-8-prenylflavanone (**1**); 5,7-dihydroxy-6-methyl-8-prenyl-4'-methoxy-flavanone (**2**); 5,7-dihydroxy-6-prenylflavanone (**3**) and 5-hydroxy-7-methoxy-6-prenylflavanone (**4**) and others compounds.[1] This project is a basic investigation to be able to design and create some seed who helps to evaluated the permeation in the skin from a topical dosage form, considering the flavanone 5,7-dihydroxy-6-methyl-8-prenyl-4'-methoxy-flavanone (**1**) as a lead to generate new compound by lead modification strategy drug design (**1b**).

Materials and Methods: From the methanolic extract of the leaves of *Eysenhardtia platycarpa* was isolated the flavanone: (**1**), where the natural flavanone surrendered to structural change, synthetic reaction being carried out as: methylation (**1b**).[2] To characterizate natural flavanone and product of the reaction we used Spectra NMR 1D and 2D de ^{1}H and ^{13}C was obtained to 200 and 400 MHz.[3] The nano-structured emulsion preparation, using a surfactant, co-surfactant, emollient and moisturizer. Once prepared, the flavanone was incorporated, the final concentration was 5 %, the particle size was determinated by the equipment Z-Sizer.[4] The permeation test was carried out using human skin (n=3) from abdominal lipectomies, from three donors in a manual sampling system cells Co. Mod Franz Crown Glass. CDCF-9. The receptor phase was ethanol:water (7:3), under temperature of 32 ± 1 ºC. Samples were taken at fixed times during 24 hours. Each sample concentrations were obtained by High Performance Liquid Chromatography, The analyses were preformed with a Column C18 Macherey-Nagel. The mobil phase, consisting of ACN/Water. [4]

Results and Discussion: The nanoemulsion (**NE1b**) prepared has Z-Average of 65.89 nm with a PDI of 0.165.

Conclusions: The results obtained with this experiment demonstrated the small size of (**NE1b**). The transdermal permeation test shows that the constant of permeation and opens the possibility to investigate about the same flavanone and some other because this kind of natural products has an interesting biological and therapeutics proprieties.

1. V. Domínguez-Villegas, et al., *Nat., Prod. Comm.* **2013**, *8*, 177.
2. W. Ma, *et al*., *Phytochemistry*; **1995**, *39*, 049.
3. Studies using the equipment of Laboratorio Nacional de Estructuras de Macromoléculas de la Universidad Autónoma del Estado de Morelos (LANEM).
4. V. Domínguez-Villegas, *et al*., *Colloids and Surfaces B: Biointerface*, **2014,** *116*, 183.

Acknowledgments: The authors would like to thank to Gattefossé, CONACyT, Mexico scholarship 253949, the Ministry of Science, Innovation of Spain with the project MAT201459134R and Laboratorio Nacional de Estructuras de Macromoléculas de la Universidad Autónoma del Estado de Morelos (LANEM).

Mixed Chol/SPAN-Tween20 systems: the effect of chitosan on the thermodynamic properties

Patrizia Nadia Hanieh,[a,*] Mariachiara Acampora,[a] Monica Pianella,[a] Federica Rinaldi,[b] Carlotta Marianecci,[a] Maria Carafa,[a] Federico Bordi,[c] Simona Sennato [c]

[a] *Department of Drug Chemistry and Technologies, La Sapienza, University of Rome, P.le A. Moro 5, 00185, Rome, Italy;* [b] *Center for Life NanoScience – IIT@Sapienza, Istituto Italiano di Tecnologia Viale Regina Elena, 00185, Rome, Italy;* [c] *Institute of Complex Systems ISC-CNR and Physics Department La Sapienza University of Rome, P.le A. Moro 5, 00185, Rome, Italy.*

Email of presenting author: patrizianadia.hanieh@uniroma1.it

Chitosan is a natural polysaccharide derived from chitin, used for biomedical applications and in pharmaceutical and biotechnology industries, as fat reduced, bactericide agent and wound healing material and coating agent for stabilization of vesicular systems[1]. In all these uses, its interesting properties such as biocompatibility, biodegradability and non-toxicity are exploited. While the efficacy for some of them is proven, little is known about the molecular-level interactions involved in these applications. More interestingly,owing to its cationic character in acidic solutions, chitosan may interact with the negatively charged surface of biomembranes, and therefore understanding such interactions is important for the its use.

In this work we have employed Langmuir monolayers as membrane model to probe the interactions of chitosan with monolayers formed by Tween20 or Span20 non-ionic surfactants, mixed to Cholesterol and anionic DCP, to mimic the composition of niosomal vesicles whose efficacy as drug delivery systems has been widely explored [2].The aim of this investigation is to elucidate the controlling factors useful for a rational design of a chitosan-coated drug delivery system and understand in which conditions chitosan decoration of the vesicle can improve the niosomal structural properties and drug delivery efficiency.

The surface pressure–area isotherms of equimolar Span 20/Chol and Tween 20/Chol monolayer have been measured at 25°C. Chitosan was dissolved in acetate buffer solution (0.03M, pH 4.5) at different concentrations up to 0.45 mg/mL, where it is not surface-active. To understand the role of the different forces involved in the polycation-monolayer interaction, we also considered the effect of the addition of the anionic lipid Dicetylphosphate (DCP) to form charged Tween20/Chol/DCP films, with molar ratio 1/2/1.

Our study revealed that chitosan strongly adsorbed at monolayers surface inducing a film expansion both at low and at high surface pressure, where it interacts not only superficially but also inserted to a certain degree into the film. In certain conditions, depending on both chitosan concentration and on the different hydrophilic-hydrophobic balance of the surfactants, modification of the monolayer phase transition has been observed. Elucidating the molecular organization of chitosan-surfactant monolayers could be useful to better understand the release rate of model drugs from niosomes.

1. M. Rinaudo, *Prog. Polym. Sci.*, **2006**, *31*, 603.
2. C. Marianecci, L. Di Marzio, F. Rinaldi, C. Celia, D. Paolino, F. Alhaique, S. Esposito, M. Carafa, *Adv.Colloid Interface Sci.*, **2014**, *205*,187.

Acknowledgements The financial support of IIT and MIUR (FIRBRBFR12BUMH) is acknowledged.

New drug delivery systems: nanoemulsions and their potential applications

Patrizia Nadia Hanieh,[a] Monica Pianella,[a] Federica Rinaldi,[b] Simone Carradori,[c] Catia Longhi,[d] Elena Mattia,[d] Carlotta Marianecci,[a] Maria Carafa [a]

[a] *Department of Drug Chemistry and Technologies, "Sapienza" University of Rome, P.le A. Moro 5, 00185, Rome, Italy;* [b] *Center for Life NanoScience – IIT@Sapienza, Istituto Italiano di Tecnologia, Viale Regina Elena, 00185, Rome, Italy;* [c] *Department of Pharmacy, University "G. d'Annunzio", Chieti, Italy;* [d] *Dipartimento di Sanità Pubblica e Malattie Infettive, Università degli Studi di Roma La Sapienza, P.le A. Moro 5, I-00185 Roma, Italy.*

Email of presenting author: patrizianadia.hanieh@uniroma1.it

Advanced drug delivery systems have been developed to overcome the major drawbacks associated with the conventional ones [1].

The aim of our work is to develop nanoemulsions suitable for delivering bioactive substances.

Nanoemulsions (NEs) are nano-sized emulsions, manufactured for improving the delivery of active pharmaceutical ingredients. These carriers are composed by oil and surfactants; in particular, natural oils can exhibit anti-inflammatory, antioxidant, antibacterial, antiviral, anticancer and/or tissue regenerative activity, due to the presence of polyphenols and tocopherols [2]. The droplet size of NEs falls typically in the range 10–200 nm. They are kinetically more stable than microemulsions with no apparent flocculation or coalescence; they also improve intracellular penetration in biological tissues, bioavailability, tolerability and solubility of lipophilic drugs. As a result of the slow release of active compounds, the irritability described in tissues is low [1]. NEs are also non-toxic and can be formulated for different applications, such as topical, ocular, intravenous, intranasal and oral delivery [3].

In this study, nanoemulsions from Neem seed oil and Tween 20 or Tween 80 as non-ionic surfactants were prepared. In particular, Neem oil was selected since it shows many bioactive properties, such as antibacterial, antimalarial and antifungal activity [4]. A mean droplet size ranging from 10–100 nm was obtained by modulating the oil/surfactant ratio. Several nanoemulsion formulations were characterized in terms of size and ζ-potential [5,6] and physicochemical properties, such as microviscosity, polarity and turbidity, were evaluated. Furthermore stability studies were carried out for a period of 60 days at two different storage temperatures (4°C and 25°C). In order to evaluate the versatility of these systems in in vivo applications, further stability studies in different biological fluids, such as human and bovine serum, artificial cerebrospinal fluid (aCSF), artificial saliva, simulated gastric fluid (SGF) and simulated intestinal fluid (SIF), were conducted.

This work has shown that Neem oil and Tween surfactants are able to form stable nanoemulsions suitable as nanodelivery systems for different administration routes.

1. M. Jaiswal, R. Dudhe, P. K. Sharma, *3 Biotech*, **2015**, *5*, 123.
2. J. Rodriguez, M. J. Martin, M. A. Ruiz, B. Clares, *Food Res. Int.*, **2016**, *83*, 41.
3. A. Gupta, H. B. Eral, T. A. Hatton, P. S. Doyle, *Soft Matter*, **2016**.
4. R. Rajendran, R. Radhai, C. Balakumar, *J. Eng. Fiber. Fabr.*, **2012**, *7*, 136.
5. F. Rinaldi, P. N. Hanieh, C. Marianecci, M. Carafa, *Nanosci. Nanometrology*, **2015**, *1*, 8.
6. C. Marianecci, L. Di Marzio, F. Rinaldi, C. Celia, D. Paolino, F. Alhaique, S. Esposito, M. Carafa, *Advances in Colloid and Interface Science*, **2014**, *205*, 187.

Proper design of biofunctionalized ultra-small iron oxide superparamagnetic nanoparticles for biomedical applications

Mario Ledda,[a,≠] Daniela Fioretti,[a,≠] Sabrina Foglia,[b] Giovanna Iucci,[c] Giovanni Capellini,[c] Massimiliano Papi,[d] Sara Sepe,[e] Settimio Grimaldi,[a] Monica Rinaldi,[a,*] Antonella Lisi [a,*]

[a] *Institute of Translational Pharmacology, Department of Biomedical Sciences, National Research Council (CNR), Via del Fosso del Cavaliere 100, 00133 Rome, Italy;* [b] *Institute of Materials for Electronics and Magnetism (IMEM), Department of Engineering, ICT and technologies for energy and transportation, National Research Council (CNR), Italy;* [c] *Department of Science, University Roma Tre, Via della Vasca Navale 79, 00146 Rome, Italy;* [d] *Institute of Physic, Catholic University of the Sacred Heart Rome, L.go F. Vito 1, 00168 Rome, Italy;* [e] *Department of Science, LIME, University Roma Tre, Viale Guglielmo Marconi 446, 00146 Rome, Italy.*

Email of presenting author: mario.ledda@ift.cnr.it

[≠] *These authors shared first authorship.* [*] *These authors shared senior authorship*

Over the last decade, nanotechnology has become more relevant in medicine. Among magnetic nanomaterials the future of iron oxide nanoparticles (IONPs) for clinical applications relies on their biocompatibility in moderate doses, their ability to be produced in a wide range of sizes and shapes with biofunctionalization potential. Additionally, they show great promise to serve as a cell tracking system in cell-based therapies, and to generate local temperature increases in the magnetic thermotherapy of solid tumours. Thus, the study and development of novel magnetic nanoparticles for biomedical applications is one of the key topics in the field of nanotechnology.

Extremely small-sized Fe_3O_4 superparamagnetic nanoparticles were prepared by coprecipitation, thinly coated with silica and conjugated with FITC, as molecular model specimen. Nanoparticles were characterized by dinamic light scattering (DSL), transmission electron microscopy (TEM) and X-ray diffraction analysis (XRD) and surface functional groups and composition were analysed by infrared spectroscopy (FTIR).

The aim of this study was to determine the biocompatibility of the magnetic nanoparticles carriers with biofunctional coating (FITC-conjugated) on colon carcinoma CaCo-2 cell line as human cellular model. Phase contrast, immunofluorescence and confocal microscopy analyses were performed to study nanoparticles internalization and up-take. By transmission electron microscopy technique was investigated the effect of their internalization on ultrastructural features and intracellular compartments. Cellular growth and viability resulted unaffected following nanoparticles up-take and lack of toxicity was confirmed at transcriptional and translational level. Finally, even when used at high concentration, the cytotoxicity effect of the nanoparticles was not significant compared with control experiments, demonstrating their high potential in the applications of nanomedicines for a diagnostic and therapeutic tool.

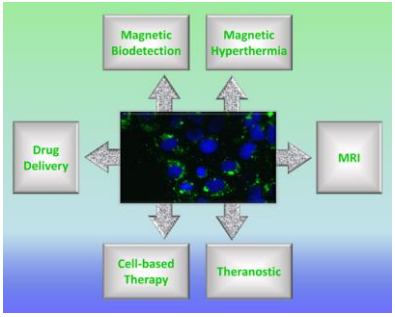


Figure 1. Biomedical applications of magnetic nanoparticles.

1. A.L. Cortajarena, *Nanobiomedicine*, **2014**, *1*, 2.
2. D. Ling, *Acc. Chem. Res*., **2015**, *48*, 1276.
3. L. Vayssieres, *Journal of colloid and interface science*, **1998**, *205*, 205.

Development and *in vitro* characterization of SLN encapsulating magnetic Heparin coated Iron Oxide for theranostic application

Eleonora Truzzi,[a] Federica Mazza,[a] Francesca Sacchetti,[a] Eleonora Maretti,[a] Valentina Iannuccelli,[a] Elena Vismara,[b] Eliana Leo [a]

[a] *Department of Life Sciences University of Modena and Reggio Emilia, Via Campi 103, 41125 Modena, Italy;* [b] *Department of Chemistry, Materials and Chemical Engineering "G. Natta" Politecnico di of Milano, via L. Mancinelli 7, 20131 Milan, Italy.*

Email presenting author: eliana.leo@unimore.it

Solid Lipid Nanoparticles (SLN) have been proposed for the oral delivery of drugs with poor oral bioavailability for their ability to be internalized directly by the lymphatic circulation, like chylomicrons, through intestinal absorption. Lymphatic system is considered an interesting target for anti-cancer drugs and contrast agents because exerts an active role in the cancer metastasis being the major route of the solid tumor spread. Recently, according to the potentiality of the iron oxide in diagnostic and of heparin in the cancer therapy, iron oxide nanoparticles non-covalently coated with heparin (Fe@hepa) have been proposed as delivery systems for theranostic application (1). The aim of this work was to encapsulate Fe@hepa in a biocompatible solid lipid shell in order to obtain a nanotheranostic tool for promoting the oral absorption through the lymphatic route.
SLN have been formulated by a modified self nanoemulsifying technique by using Gelucire50/13 and Geleol™. The resulted Fe@hepa-SLN were characterized regarding size, morphology, storage stability as well *in vitro* release of heparin and iron oxide. In addition, preliminary studies on Caco-2 cell line were carried out evaluating cytotoxicity by MTT tests and internalization by the direct quantification of Fe@hepa inside the cells. Fe@hepa-SLN displayed a mean diameter below 300 nm, suitable for the oral administration, and an incorporation efficiency of 75% ± 3.9. Morphology analyisis showed the lipid shell surrounding the Fe@hepa nanoparticles and the release studies demonstrated that this lipid envelop stabilized the heparin coating in physiological conditions. Finally, studies on Caco2 cells showed the low cytotoxicity of the Fe@hepa-SLN and their ability to be internalized in the cells used as intestinal permeability model. These results indicate that this novel nanotechnology strategy could be a promising tool for oral nanotheranostic approaches.

1. E.Vismara et al., *Int. J. Mol. Sci*, **2013**, *14*, 13469.

Sulfhur-containing chemotherapics delivery based on lamellar gold nanocrystals

Angela Longo, Gianfranco Carotenuto, Cosimo Carfagna

Insitute for Polymers, Composites and Biomedical Materials, National Research Council, Piazzale E. Fermi, 80055, Portici, Italy.

Email of presenting author: angela.longo@cnr.it

Chemotherapics are very aggressive pharmacological principles and their blood administrering by injection may cause tissue injuries as side-effect[1]. Therefore, the possibility to administer chemotherapics in non-active form, which slowly converts to the active principle inside the blood environment could be very suitable. Chemotherapics containing sulphydryl groups, -SH, (e.g., qumoxaline-2,3-dithiol, used for the Ehrlich ascites tumor, 6-mercaptopurine, etc.) can be chemisorbed on the surface of gold nanocrystals and slowly released in the body environment by a ligand-exchange mechanism, involving free ligand molecules present into the blood composition (e.g., urea, uric acid, creatinine, homocisteine, etc.)[2]. Lamellar gold nanocrystals have extremely large surface development, and consequently they are the best substrates for delivering aggressive chemoterapics. Owing to the surface plasmon absorption of lamellar gold nanocrystals located in the Near-IR region, the release rate can be even increased in the systemic treatment of skin cancers by exposing the sick tissue to IR radiation (optical hyperthermia). Here, the synthesis of gold nanoplatelets passivated by different thiol-containing pharmaceutical principle is presented. The nanoparticles have been prepared by mixing a very dilute tetrachloroauric acid ($HAuCl_4.3H_2O$, Aldrich) aqueous solutions with a L-ascorbic acid ($C_6H_8O_6$, Aldrich) solution at room temperature. After gold nanoparticles formation (blue colour solution) the –SH containing drug (e.g., N-acethyl-cysteine, L-glutatione, etc.) was added to the system. Extended thermal annealing treatments of these colloidal gold suspensions gave larger lamellar particles by a recrystallization mechanism. As visible in Figure 1a, the obtained nanoaprticles were characterized by a surface plasmon absorption band extending in the NIR spectral region. The precipitated nanostructured material was high purified and morphologically characterized by TEM (see Fig. 1b).

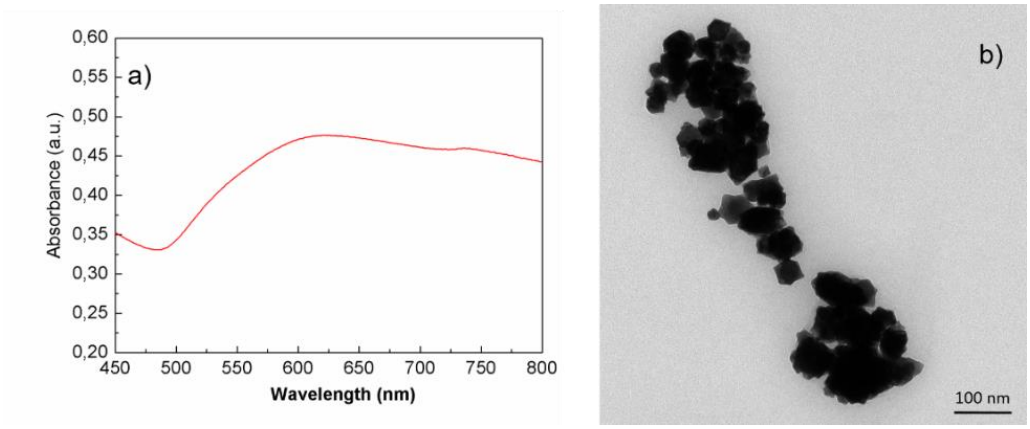


Figure 1. Au nanocrystals VIS spectrum (a), and TEM micrograph (b)

1. M. U. R. Naidu, G. V. Ramana, P. U. Rani, I. K. Mohan, A. Suman, P. Roy, *Neoplasia*, **2004**, *6*, 423.
2. J. Sittiwong, F. Unob, *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, **2015,** *138*, 381.

Self-organized multifunctional ORMOSIL nanoparticles

Marta Trevisan,[a] Venera Russo,[a] Elisa Lubian,[b] Luca Gabrielli,[b] Ignazio Castagliuolo,[a] Fabrizio Mancin[b]

[a] *Department of Molecular Medicine University of Padova, via Gabelli 63, 35121 Padova, Italy;*
[b] *Department of Chemical Sciences, University of Padova, via Marzolo 1, 35131 Padova, Italy.*

Email of presenting author: fabrizio.mancin@unipd.it

Silica nanoparticles have recently emerged as one of the most studied nanomaterials for biomedical applications. Such success is due to the possibility to obtain topologically and functionally complex structures, which has opened the way to the realization of sophisticated systems, such as gated porous particles, multimodal theranostic agents, or chemical sensors.
Recently, we developed a new synthetic protocol for the one-pot preparation of multifunctional PEGylated ORganically MOdified SILica (ORMOSIL) nanoparticles.[1] The protocol is based on the synthesis of ORMOSIL nanoparticles by base-catalysed condensation of vinyltriethoxysilane in aqueous solution containing a surfactant. We realized that surfactant aggregates could act not only as nanocontainers where the organosilane polymerization is confined, but also as a template that pre-organizes the nanoparticle components, placing them precisely in the sites where they are driven by their hydrophobic/hydrophilic balance. Water insoluble molecules (dyes, photosensitizers or drugs) locate themselves in the emulsion oil core, resulting entrapped in the polymerized ORMOSIL matrix. Amphiphilic species by the contrary locate at the oil/water interface and form, if capable to copolymerize with the ORMOSIL precursors, a surface functionalization layer. Taking full advantage of such approach, we show that densely PEGylated nanoparticles can be prepared. Functional groups for the subsequent conjugation with targeting agents can be introduced in the PEG coating during the nanoparticle synthesis. The dense PEG coating obtained improve the nanoparticles biocompatibility by reducing their toxic and pro-coagulant properties and avoiding capture by immune system cells, but also minimizes uptake by cells. Targeting with antibodies and other selective biomolecules allow specific delivery of dyes or drugs to the selected cells.

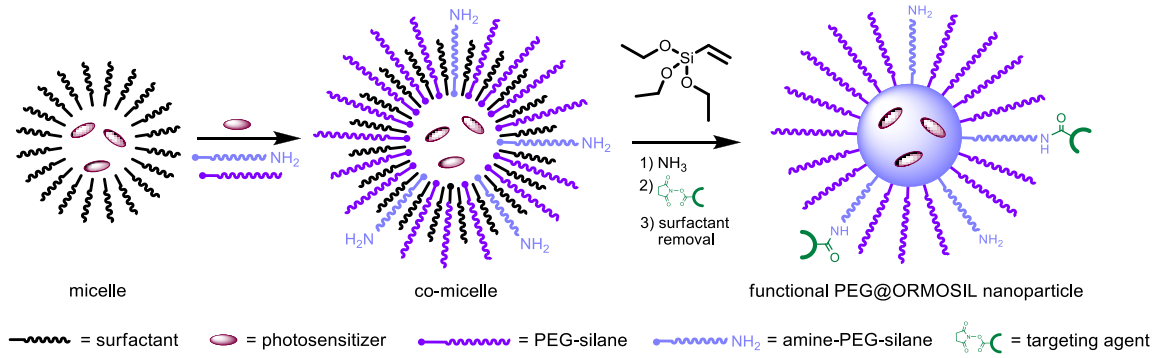


Figure 1. Synthetic protocol for the preparation of PEGylated ORMOSIL nanoparticles

1. a) I. M. Rio-Echevarria et al., *J. Mat. Chem.*, **2010**, *20*, 2780; b) D. Segat et al., *Nanomedicine*, **2011**, *6*, 1027; c) R. Tavano et al., *Nanomedicine*, **2010**, *5*, 881-896 d) F. Selvestrel et al., *Nanoscale*, **2013**, *5*, 6106.

Acknowledgments: Funding from MIUR (FIRB 2011 RBAP114AMK-RINAME) and University of Padova (Progetto Strategico NAMECA)

Anti-TB inhalation therapy: Design of mannose-based functionalised Solid Lipid Microparticles for an active targeting to alveolar macrophages

Eleonora Maretti, Cecilia Rustichelli, Luca Costantino, Eleonora Truzzi, Francesca Sacchetti, Eliana Leo, Valentina Iannuccelli

Department of Life Sciences, University of Modena and Reggio Emilia, Via G. Campi 103, 41125, Modena, Italy.

Email of presenting author: eleonora.maretti@unimore.it

Human tuberculosis (TB) is mainly a disease of the lung characterised by a long chronic stage of infection and progressive pathology that compromise the respiratory system. This is a curable infectious bacterial disease caused by the *Mycobacterium tuberculosis* (Mtb). TB therapies have exploited conventional routes of administration, such as oral and intramuscular.[1] The pulmonary route appears the most reasonable and effective way to target the alveolar macrophages (AM) and eradicate surviving Mtb at the primary infected site of TB, especially considering that 75-80% of cases remain localised in the lungs. The anti-TB therapy by inhalation offers benefits compared with the current treatment in terms of patient's compliance improvement, reduction in dose amount and frequency, treatment duration and TB diffusion in other organs, thus minimising the risk of drug-resistant mutants, toxicity and side effects.

For a direct intramacrophagic antitubercular therapy using Dry Powder Inhaler (DPI) devices, Solid Lipid Microparticles (SLM), produced using the melt emulsifying technique followed by freeze-drying, were developed to load rifampicin, a first-line antitubercular drug.

In the present project, SLM were modified to improve drug loading level and release as well as AM targeting. Several biocompatible lipid components such as fatty acids and their derivatives, diglycerides and triglycerides, were processed using mixtures of biocompatible stabilisers (sodium taurocholate and methyl mannopyranoside) in order to obtain SLM with maximum efficiency in terms of drug loading and release in simulated lung fluid. Lipids in the liquid physical state embedded into SLM provided Microstructured Lipid Carriers (MLC) that are known to exhibit superior advantages over SLM such as enhanced drug loading capacity and prevention of drug expulsion intended to maximise the drug concentration at the primary site of TB infection. The obtained microcarriers were examined for their intrinsic properties such as size and size distribution, morphology and shape, surface charge, bulk and tap density, aerodynamic diameter, physical state of the components, wettability, drug loading and release.

Macrophages, as is common knowledge, possess mannose-specific membrane receptors (MR) that can be recognised by carriers bearing mannose residues, facilitating their internalisation.[2, 3] Therefore, the functionalisation of SLM surface by mannose derivatives used as the co- stabiliser in the SLM formulation was used to achieve an active targeting. The actual presence of mannose on SLM surface was investigated by means of X-ray Photoelectron Spectroscopy for Chemical Analysis (XPS) and Energy Dispersive X-ray Analysis (EDX).

1. World Health Organization, Global Tuberculosis Report **2014**.
2. Y. Phanse, B.R. Carrillo-Conde, A.E. Ramer-Tait, R. Roychoudhury, N.L.B. Pohl, B. Narasimhan, M.J. Wannemuehler, B.H. Bellaire, *Acta Biomater*., **2013**, *9*, 8902.
3. S. Chono, T. Tanino, T. Seki, K. Morimoto, *J. Pharm. Pharmacol.*, **2007**, *59*, 75.

Mesenchymal Stromal Cells and innovative nanoparticles as multimodal therapy for osteosarcoma treatment

Elisa Martella,[a] Claudia Ferroni,[b] Chiara Bellotti,[a] Barbara Dozza,[a] Andrea Guerrini,[b] Enrico Lucarelli,[a] Greta Varchi,[b] Serena Duchi [a]

[a] *Osteoarticular Regeneration Laboratory, Rizzoli Orthopaedic Institute, 40136 Bologna, Italy;*
[b] *Institute of Organic Synthesis and Photoreactivity – Italian National Research Council, Via Gobetti 101, 40129 Bologna, Italy.*

Email of presenting author: elisa.martella@ior.it

Osteosarcoma (OS) is a highly malignant primary tumor frequently occurring in children and adolescents. The standard methods currently used to treat this tumor are unable to induce complete tumor necrosis, thus resulting in a 30% mortality. Our approach combines the chemotherapeutic drug Paclitaxel with a Photodynamic therapy (PDT) compound within a biodegradable albumin nanoparticle. Moreover, Mesenchymal stromal cells (MSC) are used as Trojan horse to deliver these NPs, by taking advantage of MSC ability to recognize and efficiently engraft the OS tumor stroma. HSA was conjugated with the photosensitizer chlorin e6 (Ce6) and the functionalized protein was used to produce PTX loaded nanoparticles through desolvation or drug-induced protein self-assembly techniques (PTX-Ce6@HSA NPs). To test the feasibility of the proposed approach, human MSC lines were loaded with different dosages of PTX-Ce6@HSA NPs and results showed that MSC efficiently internalize NPs (via clathrin mediated transport), release PTX by exocytosis (HPLC analysis) and the photosensitizer Ce6 remains active inside the cells for at least 3 days after loading (Figure 1). PTX-Ce6@HSA NPs loaded MSC co-cultured with the OS tumor cell line SaOS-2 showed a significant tumor cell growth reduction. From our preliminary *in vitro* data, the proposed multimodal therapy could minimize the side effects of the systemic chemotherapy administration and enhance its efficacy through the synergic effect of PTX and PDT, and could be intended as a future innovative co-adjuvant approach for OS treatment in patients.

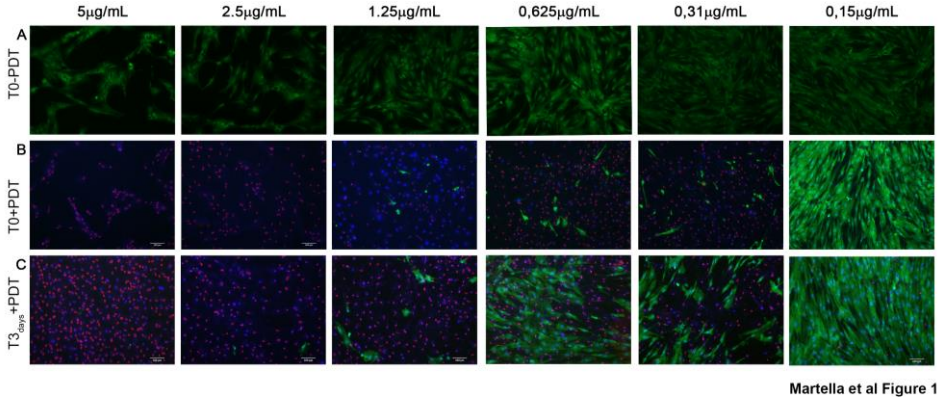


Figure 1. NPs internalization and Ce6 activation upon light irradiation. A) Calcein staining (in green) of MSC after 24 hours loading. B,C) Live&dead assay performed 24hr (B) and 3 days (C) after PDT treatment (live, green cells: dead, red cells: Hoechst, nuclei)

1. M. Serra, G. Reverter-Branchat, et al., *Ann Oncol*, **2004**, *15*, 151.
2. S. Duchi, G. Sotgiu, et al., *J Control Release*. **2013**, *168(2)*, 225.
3. LM. Wagner, H. Yin, et al., *Pediatr Blood Cancer*, **2014,** *61*(11), 2096.
4. YL. Hu, YH. Fu, et al. *J Control Release*. **2010**, *147*(2):154.
5. A. Aluigi, G. Sotgiu., et al *RSC Adv.*, **2016**, *6*, 33910.

Acknowledgments: This work is supported by Associazione Italina Ricerca sul Cancro (AIRC, grant 16491P.I. Serena Duchi).

Double targeting of biodegradable nanoparticles as an advanced approach to enhance antitumor effects

Francesca Moret,[a] Alessandro Venuta,[b] Diletta Esposito,[b] Giovanni del Poggetto,[c] Connie Avitabile,[b] Alessandra Romanelli,[b] Paola Laurienzo,[c] Fabiana Quaglia,[b] Elena Reddi [a]

[a] *Department of Biology, University of Padova, Via U. Bassi 58/B, 35100, Padua, Italy;* [b] *Department of Pharmacy, University of Naples Federico II, Via D. Montesano 49, 80131, Naples, Italy;* [c] *Institute for Polymers, Composites and Biomaterials, CNR, Via Campi Flegrei 34, 80078, Pozzuoli, Naples, Italy.*

Email of presenting author: francesca.moret@unipd.it

In this work, we propose core-shell nanoparticles (NPs) made of amphiphilic poly(ε-caprolactone)-polyethyleneglycol (PCL-PEG) block copolymers exposing on the surface both an anti-angiogenic peptide (anti-FLT1) and folic acid (FA) to encourage drug accumulation in tumors. While targeting by exploiting the high affinity folate receptors (FR), that is over-expressed by many cancer cells, is a widely used strategy to enhance the internalization of drug-loaded NPs, less is known about the role of anti-angiogenic factors functionalized on NPs in enhancing chemotherapeutic effects. Anti-FLT1 is an hexapeptide that specifically inhibits the binding of various vascular endothelial growth factor receptor 1 (VEGFR1) ligands, negatively affecting VEGF-induced endothelial cell migration and angiogenesis (1). Diblock PCL-PEG copolymers with PEG length guaranteeing exposition of anti-FLT1 and FA on NP surface (PCL_{4000}-PEG_{1000}, PCL_{4000}-PEG_{1500}-anti-FLT1 and PCL_{4300}-PEG_{1500}-FA) were synthesized by a ring-opening polymerization of ε-CL followed by coupling with the decorating unit. NPs were produced by nanoprecipitation technique in the size range of 110-150 nm with very low polydispersity. For FA-decorated NPs, the highly negative surface charge of NPs, more negative as the amount of FA-conjugated copolymer increased, was suggestive of the presence of FA moieties on the surface while anti-FLT1 decorated NPs showed surface charge and size similar to non-decorated NPs. Once formulation parameters were selected, entrapment of the antimitotic agent Docetaxel (DTX), a model poorly water-soluble drug, was attempted. NPs encapsulated DTX with high efficiency and were endowed of sustained release in complex biological media. In addition, in order to track the NPs inside the cells, Nile Red was entrapped in the PCL core or Rhodamine B was covalently linked to the hydroxyl end group of PCL-PEG. *In vitro* experiments in KB cancer cells over-expressing FRs showed significantly enhanced internalization of NPs exposing FA *vs.* undecorated NPs and *vs.* NPs having FA hidden inside the PEG brush, even in the presence of serum proteins in the culture medium. In addition, competition experiments carried out by saturating FRs with an excess of free FA (1 mM) prior to NP addition, demonstrated an exclusive inhibition of the uptake of NPs exposing FA, confirming the involvement of FRs. However, when DTX was included in FA-targeted NPs its cytotoxicity in KB cells, incubated up to 72 h, was comparable to that measured with un-targeted NPs but significantly higher with respect to that of the free drug, especially at very low drug concentrations. Forthcoming experiments on HUVEC endothelial cells will assess the anti-angiogenic potential of dual targeted anti-FLT1/FA NPs with the aim to potentiate DTX cytotoxicity in tumors by inhibiting the growth of the surrounding neo-vasculature.

1. D.G. Bae, T.D. Kim, *Cancer Therapy: Preclinical*, **2005**, *11*, 2651.

Acknowledgments: This work was supported by AIRC (IG2014 #15764)

From surface to core modification: development of gel-in-liposome hybrid systems

Martina Nardoni,[a,*] Settimio Pacelli,[b] Patrizia Paolicelli,[a] Stefania Petralito,[a] Maria Antonietta Casadei [a]

[a] *Department of Drug Chemistry and Technologies, Sapienza University of Rome, Italy;* [b] *BioIntel Research Laboratory, Department of Chemical and Petroleum Engineering, School of Engineering, University of Kansas, Lawrence, USA.*

Email of presenting author: martina.nardoni@uniroma1.it

Liposomes are likely the most studied colloidal carriers for the delivery of therapeutically active molecules due to their well-known unique properties. However, their use is still limited by a series of drawbacks including liposomes rapid degradation operated by the reticuloendothelial system (RES) and their inability to achieve a sustained drug delivery. To overcome these issues several strategies have been investigated over the past years. One of the most followed approach consists in the combination of different biomaterials within the same assembled system. So far, following this concept, either natural or synthetic hydrophilic polymers have been successfully combined in vesicle technology to change the surface properties of the bilayer, thus creating stealth phospholipid vesicles. More recently, a new step towards liposome improvement has been achieved by working on the internal structure of phospholipid vesicles with the aim to convert the aqueous inner core into a soft and elastic hydrogel (figure 1). Hybrid liposomes with a core composed of a cross-linked polymeric network offer several attractive features and properties.[1] In fact, the presence of a stable polymeric scaffold provides an internal mechanical support to the liposomal membrane, mimicking the elastic protein network of the cytoskeleton.[2] In this study, the effect of the polymer chain length on the stability of the final hybrid construct has been investigated. To this end, poly(ethylene glycol) dimethacrylate (PEG-DMA) with different molecular weights has been combined with hydrogenated soybean phosphatidylcholine/cholesterol (HSPC/Chol) liposomes with the intent to modify their liquid core into a soft and elastic hydrogel by UV photopolymerization. The obtained gel-in-liposome hybrid systems have been studied for their stability against surfactant chemical destabilization, as well as for their release properties and biocompatibility. The gelation step of the liposome core may affect the *in vivo* performance of the final system, as it may prevent any unwanted leakage of encapsulated molecules, due to defects provided by insertion into the bilayer of surfactants and proteins in the human body. In summary, this novel gel-in-liposome hybrid nanocostruct should meet the requirement for an effective drug delivery system and may offer a convenient new way of envisioning lipidic vesicles.

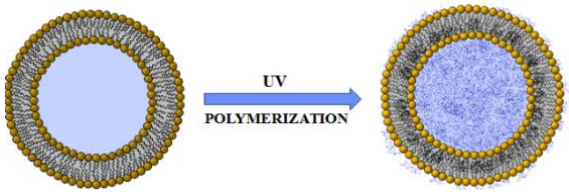


Figure 1. Development of gel-in-liposome hybrid systems by UV polymerization of PEG-DMA embedded in HSPC/Chol liposomes.

1. S. Tiwari, A.K. Gayal, K. Khatri, N. Mishra, S.P. Vyas, *J. Microencapsul.*, **2009**, *26*, 75.
2. S. Petralito, R. Spera, S. Pacelli, M. Relucenti, G. Familiari, A. Vitalone, P. Paolicelli, M.A. Casadei, *React. Funct. Polym.*, **2014**, *77*, 30.

Acknowledgments: This work was financially supported by a grant from the “Sapienza” University of Rome.

Microemulsion as a nanovector for the delivery of *Smyrnium olusatrum* L essential oil

Marco Cespi,[a] Luana Quassinti,[a] Diego Romano Perinelli,[a] Massimo Bramucci,[a] Romilde Iannarelli,[a] Fabrizio Papa,[a] Massimo Ricciutelli,[a] Luca Casettari,[b] Giulia Bonacucina,[a] Giovanni Filippo Palmieri,[a] Filippo Maggi [a]

[a] *School of Pharmacy, University of Camerino, Via G.III Da Varano, 62032, Camerino, Italy;*
[b] *Department of Biomolecular sciences, University of Urbino, Piazza Rinascimento, 61029, Urbino, Italy.*

Email of presenting author: diego.perinelli@unicam.it

The essential oil extracted from *Smyrnium olusatrum* L, also known as Alexanders (AEO) or wild celery, has been demonstrated to possess antimicrobial, anti-inflammatory, antiproliferative and antioxidant properties, thanks to the presence of bioactive constituents such as isofuranodiene and germacrone.[1] Due to the low solubility of water and stability concerns, the development of an appropriate formulation for the *in vivo* delivery of this essential oil is problematic. At the moment, nanoencapsulation technology appears to be the most suitable approach. In this work, the AEO was formulated as an "oil in water" microemulsion, using a central composite design to study the effect of the amount of AEO, surfactant (Polysorbate 80), co-surfactant (ethanol/glycerol mixture) and water on microemulsion preparation feasibility. The optimazed formulations were characterized by dynamic light scattering, polarised optical microscopy and tested in term of cytotoxicity (MTT assay) on human colon carcinoma cells (HCT116). AEO alone was not suitable as oil phase for the formulation of microemulsion due to the high amount of isofuranodiene that crystallizes during the preparation. The crystallization issue was overcome by the addition of ethyl oleate to AEO at the ratio 1:1. Two microemulsions were prepared according to the central composite design, made up of 0.75% (ME_AEO_0.75%) or 1.5% (ME_AEO_1.5%) oil phase. The prepared microemulsions remained stable over one year. MTT assay revealed that both AEO-loaded and unloaded microemulsions displayed cytotoxicity on HCT116 human colon carcinoma cell line. Although polysorbate 80 is responsible for the intrinsic cytotoxic activity of the vehicle, the calculated lower IC_{50} values for the AEO-loaded systems suggested that AEO maintains its activity when formulated as a microemulsion.

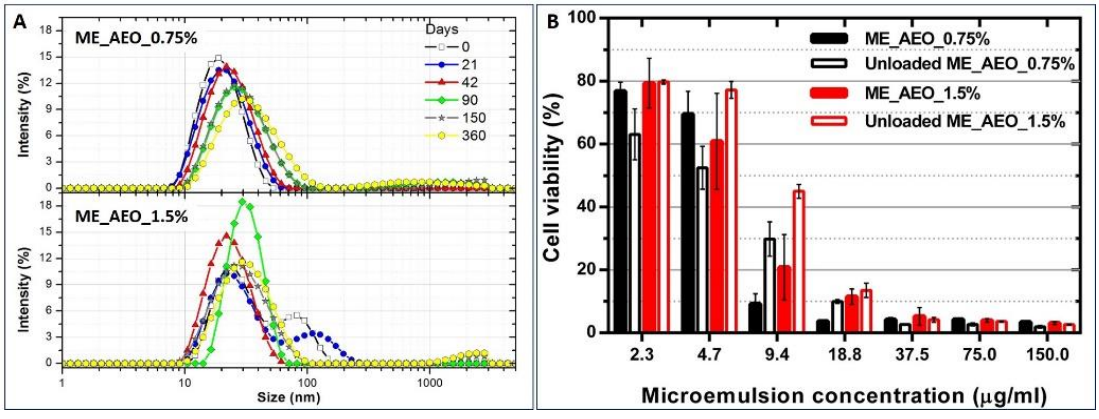


Figure 1. Microemulsion stability evaluated by DLS measurements (A); microemulsion cytotoxicity tested by MTT assay (B).

1. F. Maggi, L. Barboni, F. Papa, G. Caprioli, M. Ricciutelli, G. Sagratini, S.Vittori, *Food Chem.*, **2012**, *135*, 285.

New fluorescent liposome-based sensor for the detection of enzymatic biomarkers

Manuela Petaccia,[a,b] Luisa Giansanti,[a,b] Denise Gradella Villalva,[b,c] Giovanna Mancini [b]

[a] *Dipartimento di Scienze Fisiche e Chimiche, Università degli Studi dell'Aquila, Via Giovanni Falcone 25, 67100 L'Aquila, Italy;* [b]*CNR - Istituto di Metodologie Chimiche, Via Salaria km 29.300, 00016 Monterotondo Scalo Rome, Italy;* [c] *Dipartimento di Chimica, Università degli Studi di Roma "Sapienza", P.le A. Moro 5, 00185 Rome, Italy.*

Email of presenting author: manuela.petaccia@libero.it

5-fluorouracil (5-FU), a chemotherapic drug widely employed in the treatment of some of the most frequently occurring malignant tumours, has a narrow therapeutic window and a significant variability among patients in pharmacokinetics.[1] The detection of and the measurement of 5-FU target enzyme activity are necessary to achieve a personalized dose of the drug in order to reduce its toxics side effect. Liposomes, combined with fluorescent compounds, received considerable attention as sensors for chemical and biological detection because they generate a quick signal (thus reducing the diagnosis time), are very sensitive and are relatively inexpensive.[2] On these premises cationic and anionic fluorescent liposomes able to produce an optical signal upon the interaction with Thymidine Phosphorylase (TP), one of the target enzymes of 5-FU were investigated: cationic liposomes are composed by a natural phospholipid, 1,2-dioleoyl-sn-glycero-phosphocholine (DOPC), a cationic amphiphile tagged on the hydrophobic tail with a pyrene moiety (**1**) and a 5-FU derivative (**2**) whereas anionic liposomes were prepared replacing a fraction of **1** with lauric acid (12-A) or 11-bromoundecanoic acid (11-BrA). Upon the interaction of the target enzyme with 5-FU exposed on liposome surface, and hence with the liposome surface, lipid components reorganize and segregate in domains inducing a variation of the excimer/monomer ratio due to the consequent variation of the distance among the pyrene moiety located in the hydrophobic region of the bilayer. Among the investigated formulations, DOPC/11-BrA/**1**/**2** liposomes have a good potentiality for the development of a chemosensor for TP detection thanks to the ability of their lipid bilayer to act as transducer and to their high specificity.

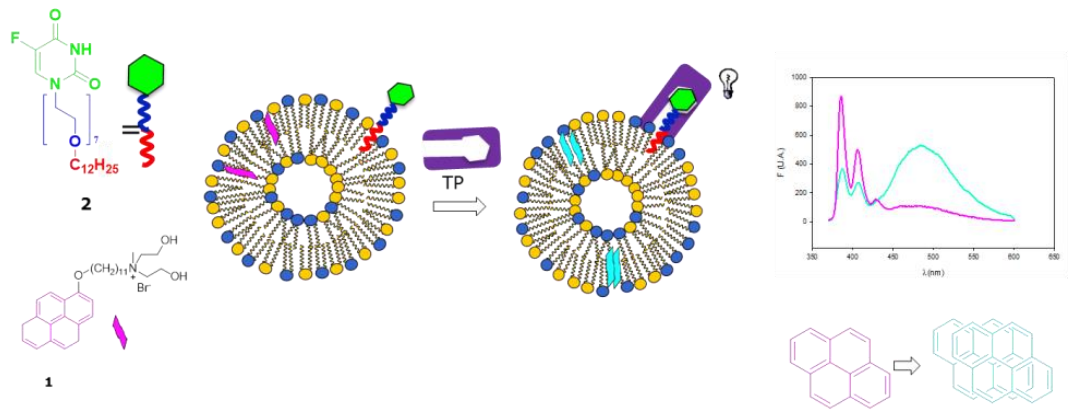


Figure 1. Liposome-based biosensor: the induction of an optical signal upon the interaction of liposomes functionalized with 5−FU derivatives with the target enzymes.

1. A. Pacia, et al. *Eur. J. Cancer*.**2014**, *12*, 2010.
2. C. Chen, Q. Wang, *Am. J. Nano Res. Appl.* **2015**, *3*, 13.

Acknowledgments: The authors thank the FIRB Project 2012 RBFR12BGHO and the Ciência sem Fronteiras, CAPES Foundation for the financial support.

Subcutaneous administration of niosomes encapsulating lidocaine long lasting reduce nociception in mice

Federica Rinaldi,[a] Carlotta Marianecci,[b] Maria Carafa,[b] Amalia Di Giannuario,[c] Francesca Marzoli,[c] Stefano Pieretti [c]

[a] *Center for Life Nano Science@Sapienza, Fondazione Istituto Italiano di Tecnologia, Viale Regina Elena 291, 00161 Rome, Italy;* [b] *Department of Drug Chemistry and Technology, University of Rome "Sapienza", Piazzale A. Moro 5, 00185 Rome, Italy;* [c] *Department of Pharmacology and Therapeutic Research, Istituto Superiore di Sanità, Viale R. Elena 299, 00161 Rome, Italy.*

Email of presenting author: stefano.pieretti@iss.it

Lidocaine is the most-frequently applied local anesthetic in addition to a wide range of applications in the management of neuropathic pain, postoperative pain, postherpetic neuralgia, centrally mediated pain, headache and infiltrative malignant neurological lesions (1, 2). As first step in our study on the possible use of lidocaine-loaded niosomes (LLNs) as analgesic, in this report we present results on the effects induced by subcutaneous injection of LLNs in an inflammatory-pain model, as the formalin test is in mice. LLNs, prepared as previously reported (3), were used as drug delivery systems and formulated according to their pharmacological activities on pain (330 μg/40 μl). Niosomes loaded with lidocaine were compared to: a) the unstructured surfactant formulation, b) the unstructured surfactant formulation with the same lidocaine concentration and c) lidocaine solution at the same concentration. Male CD-1 mice (Harlan, Italy) weighing 25-30 g were used for all experiments. The experiments were authorized by the Italian Ministry of Health, according to Legislative Decree 26/2014. Subcutaneous injection of a dilute solution of formalin (1%, 20 μl/paw) into the mice hind paw evokes nociceptive behavioural responses, such as licking, biting the injected paw or both, which are considered indices of nociception. The nociceptive response shows a biphasic trend, consisting of an early phase occurring from 0 to 10 min after the formalin injection, followed by a late prolonged phase occurring from 15 to 40 min, that reflects the response to inflammatory pain. The total time (s) that the animal spent licking or biting its paw during the formalin-induced early and late phase of nociception was recorded (4). Treatments a-c were performed 15 min before formalin in a volume of 40 μl, injecting the solutions into the mice hind paw. The significance among the groups was evaluated with the analysis of variance followed by Tukey's post-hoc comparisons using GraphPad Prism 6.03 software. The administration of the vesicles alone did not change the response to formalin both in the early and in the late phase of the test. When lidocaine was administered alone or together with the vesicles in the mice paw before formalin, we did not observe any differences in the paw licking induced by aldehyde. On the contrary, vesicles loaded with lidocaine were able to strongly reduce licking activity induced by formalin in both test phases. These encouraging results, prompt us to further studies in order to investigate the effects of lidocaine loaded niosomes also in other pain models.

1. H. Soleimanpour K. Hassanzadeh H. Vaezi, S.E. Golzari, R.M. Esfanjani, M. Soleimanpour, BMC Urol., **2012**, *12*, 13.
2. J. Sawynok, Eur J Pain., **2014**, *18*, 465.
3. C. Marianecci, L. Di Marzio, E. Del Favero, L. Cantù, P. Brocca, V. Rondelli et al., *Langmuir*, **2016**, *32*, 1241.
4. M. Colucci, F. Maione, M.C. Bonito, A. Piscopo, A. Di Giannuario, S. Pieretti, *Pharmacol Res*., **2008**, *57*, 419.

Identification of ssDNA aptamers targeting metastatic renal cell carcinoma using cell-SELEX technology

Karlis Pleiko, Liga Saulite, Una Riekstina

Faculty of Medicine, University of Latvia, Jelgavas street 1, LV-1004, Riga, Latvia

Email of presenting author: karlis.pleiko@lu.lv

Aptamers are oligonucleotides that bind to specific targets based on their tertiary structure interactions with target molecule. Identification of aptamers for specific target is achieved using SELEX (*systematic evolution of ligands by exponential enrichment*) method. Aptamers have been proven to be promising technique for use in theranostics and have been compared to antibodies with respect to their high specificity and mechanism of action. Cell-SELEX is a variation of SELEX method that uses live cells as a target and as a negative control.[1]

In the current study human clear cell renal cell carcinoma cells from tumor metastasis in lungs (RCC-MF) were used as target cells and human kidney epithelial cell line (RC-124) cells were used as negative control to identify aptamers through Cell-SELEX method that specifically bind to renal carcinoma cells. DNA oligonucleotide starting library with 40 nucleotide randomized region with two constant primer binding regions in each end of randomized region was used. Previously published cell SELEX was modified by adjusting PCR conditions, incubation time and DNA separation conditions to achieve the best results during the selection. Flow cytometry and confocal microscopy were used to monitor the progress of selection.

Renal cell carcinoma specific aptamers were selected using optimized Cell-SELEX method and characterized in this research to be further studied for therapeutic or diagnostic application.

In conclusion, by Cell-SELEX approach it was possible to select and enrich clear cell renal carcinoma aptamers which could be further functionalized by attachment to currently known chemotherapeutic agents to increase specificity or used for diagnostic purposes. Identifying target protein that aptamers bind to and adding post selection modifications could further increase the number of possible strategies to be used to apply selected aptamer in clinic.

1. K. Sefah, D. Shangguan, X. Xiong, M. O'Donoghue, W. Tan, *Nature Protocols*, **2010**, *5*, *6*, 1169.

Acknowledgements: This work was supported by Taiwan-Latvia-Lithuania research grant No. LV-LT-TW-/2016/6 and Students' council of the University of Latvia research project grant "Use of modified aptamer library for isolation of renal cell carcinoma specific aptamers".

Preparation and characterization of vesicular nanocarriers: different approaches to brain delivery

Federica Rinaldi,[a] Patrizia Nadia Hanieh,[b] Monica Pianella,[b] Andrea Bettucci,[c] Elena Del Favero,[d] Valeria Rondelli,[d] Maria Carafa,[b] Carlotta Marianecci [b]

[a] *Center for Life NanoScience – IIT@Sapienza, Istituto Italiano di Tecnologia, Viale Regina Elena 00185, Rome, Italy;* [b] *Department of Drug Chemistry and Technologies, "Sapienza", University of Rome, P.zzle A Moro 5 00185, Rome, Italy;* [c] *Dept. SBAI, "Sapienza", University of Rome, Via Scarpa, Rome, Italy;* [d] *Department of Medical Biotechnologies and Traslational Medicine, University of Milan, LITA - V. F.lli Cervi, 93 - 20090 Segrate, Milan, Italy.*

Email of presenting author: federica.rinaldi@iit.it

The blood–brain barrier (BBB) represents a quite insurmountable hurdle to the delivery of bio-active substances to the central nervous system. The tight junctions between the BBB cells prevent most compounds from entering brain tissue and only the 2% of small polar molecules is able to cross the BBB and, for this reason, the use of a lot of therapeutic agents for treating brain diseases has been limited.[1] The development of nanovesicular carriers able to go beyond this barrier is a promising approach to overcome problems related to the delivery of drugs to the brain. In particular, nanovesicles obtained by polysorbate 20 (Tween 20) could be useful because, in vivo, apolipoprotein E (apo E) or B (apo B) adsorb on their surface and this can promote the interaction with the LDL receptor followed by endocytotic uptake by BBB. To reach the brain, an alternative route of administration can be proposed, such as the intranasal delivery («nose to brain»). One of the major problems associated with nasal administration is the rapid removal of drugs or drug delivery systems by mucociliary clearance. This problem could be solved by coating nanocarriers with mucoadhesive agent (NioN 1, Nanobubbles A1, Nanobubbles B1).[2] Furthermore, an alternative strategy is the temporary disruption of the BBB to enhance the possibility of the carrier to reach the brain.[3] The circulating nanobubbles (vesicular systems loaded by a gas as contrast agent) could be able to produce a temporary BBB opening through the widening of tight junctions and the activation of transcellular transport mechanisms, with little effect on the surrounding parenchyma.
The aim of this project is the preparation and the characterization of different vesicular systems able, through different mechanisms, to reach the brain (Table 1) .

SAMPLE	NioN	NioN 1	Nanobubbles A	Nanobubbles A1	Nanobubbles B	Nanobubbles B1
POTENTIAL DELIVERY PATHWAY	Apo E interaction	Nose to brain	Temporary BBB disruption	Nose to brain	Temporary BBB disruption	Nose to brain

Table 1. Different vesicular systems and different mechanisms to reach the brain

1. C. Marianecci, F. Rinaldi, P.N. Hanieh, D. Paolino, L. Di Marzio, M. Carafa, *Curr. Pharm. Des*., **2015**, *21*, 5225.
2. F. Rinaldi, P.N. Hanieh, C. Marianecci, M. Carafa, *Nanoscience and Nanometrology*, **2015**, *1*, 8.
3. Arvanitis, *Adv. Drug Deliv. Rev*., **2014**, *72*, 94.

H-Ferritin nanoparticles allow doxorubicin targeted delivery in cancer cells, *in vitro* and *in vivo*

Maria Antonietta Rizzuto,[a] Michela Bellini,[a] Serena Mazzucchelli,[b] Luisa Fiandra,[b] Marta Truffi,[b] Fabio Corsi,[b] Davide Prosperi [a]

[a] *Department of Biotechnology and Biosciences, University of Milano-Bicocca, piazza della Scienza 2, 20126 Milan, Italy;* [b] *Department of Biomedical and Clinical Sciences "L. Sacco", University of Milan, via G.B. Grassi 74, 20157 Milan, Italy.*

Email of presenting author: m.rizzuto3@campus.unimib.it

Ferritin is an iron storage protein composed of 24 subunits to form a spherical cage, which plays a key role in iron metabolism, protecting cells from oxidative stress. Since its subunits can be disassembled and reassembled apoferritin can be exploited for the encapsulation of organic molecules thus representing an interesting scaffold for the development of a biocompatible drug delivery system.[1] Moreover, ferritin is specifically recognized by transferrin receptor 1, which is overexpressed in many tumors.[2] In this study a recombinant apoferritin variant consisting of heavy-chain subunits (HFn) was produced to achieve a cumulative delivery of doxorubicin (DOX), which exerts its cytotoxic action by targeting the DNA. After demonstrating the selectivity of HFn for cancer cells compared to healthy fibroblasts, HFn(DOX) was used to treat both human (HeLa) and murine (4T1) cancer cells. Confocal microscopy and DNA damage assays proved that the nanoformulation increased the nuclear delivery of DOX. HFn(DOX) acts as a "Trojan Horse": HFn was more efficiently internalized in cancer cells compared to free drug, then translocated into the nucleus following the DNA damage caused by the partial release of DOX in the cytoplasm. This self-triggered translocation allowed the drug to be directly released in the nucleus.[3] Furthermore *in vivo* experiments were carried out on highly invasive breast cancer model. Mice bearing 4T1 cells were treated with placebo, free DOX and HFn(DOX) for 21 days at 1.24 mg/Kg, significantly lower than the minimal clinical dosage (2.4 mg/kg). Our results demonstrate that treatment with HFn(DOX) is able to strongly affect the tumor progression and to show a significant improvement in DOX toxicity profile, even in a highly metastatic DOX-resistant breast cancer model *in vivo*. Histological analysis of cardiac tissues suggested that HFn-DOX allows overcoming cardiotoxicity, one of the most severe side effects of DOX. In conclusion, HFn(DOX) has a tremendous potential for the development of a novel chemotherapy strategy, based on more frequent and lower dose drug administrations.

1. Z. Yang et al., *Chem. Commun*., **2007**, *33*, 3453.
2. K. Fan et al., *Nature Nanotech*., **2012**, *7*, 459.
3. M. Bellini et al., *J. Controlled Rel*., **2014**, *196*, 184.

Spatiotemporal and chemical dynamics of silver nanoparticles in the burn wound

Marco Roman,[a,b] Chiara Rigo,[a] Hiram Castillo-Michel,[c] Dagmar S. Urgast,[d] Jörg Feldmann,[d] Ivan Munivrana,[e] Vincenzo Vindigni,[e] Federico Benetti,[f] Warren R.L. Cairns [b]

[a] *Department of Environmental Sciences, Statistics and Informatics (DAIS), Ca'Foscari University of Venice, Via Torino 155, 30172 Venice Mestre, Italy;* [b] *Institute for the Dynamics of Environmental Processes (IDPA-CNR), Via Torino 155, 30172 Venice Mestre, Italy;* [c] *European Synchrotron Radiation Facility (ESRF), 71 avenue des Martyrs, 38000 Grenoble, France;* [d] *Trace Element Speciation Laboratory (TESLA), University of Aberdeen, Aberdeen AB24 3UE, Scotland, UK;* [e] *Burns Centre, Division of Plastic Surgery, University Hospital of Padua, Via Giustiniani 2, 35128 Padua, Italy;* [f] *European Centre for the Sustainable Impact of Nanotechnology (ECSIN), Viale Porta Adige 45, 45100 Rovigo, Italy.*

Email of presenting author: marco.roman@unive.it

Silver nanoparticles are innovative antimicrobial agents used in key applications related to the care of burns and chronic wounds, but their chemical *in vivo* dynamics in human tissues has not been directly observed so far.[1-3] In this study, synchrotron radiation µXRF/µXANES and laser ablation ICP-MS were used to provide the first information on the spatiotemporal distribution and chemical speciation of silver in full-profile biopsies collected from the wound bed of burn patients treated with a dressing containing nanoparticles. A significant penetration of the metal was observed, inversely associated with the level of structural organization of the tissue, and accompanied by sequential processes of dissolution, chloride complexation, change into metal-thiol protein complexes, and final mobilization into deeper skin layers towards the vascular system. Hydrodynamic chromatography-single particle ICP-MS was used to confirm the high level of dissolved silver species and the absence of nanoparticles in the blood of analogous patients.[4] The results provide new realistic bases to design innovative silver nanomaterials with optimal antibacterial efficacy and minimum risks for the patient.

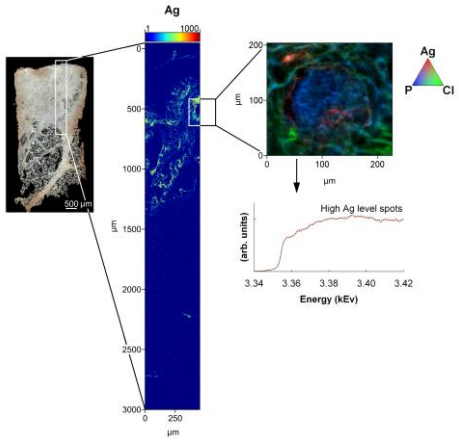


Figure 1. µXRF maps and µXANES spectrum of the full profile biopsy of a burn wound treated with silver nanoparticles.

1. C. Rigo, M. Roman, et al., *Burns*, **2012**, *38*, 1131.
2. C. Rigo, L. Ferroni, et al., *International Journal of Molecular Sciences*, **2013**, *14*, 4817.
3. M. Roman, C. Rigo, et al., *Talanta*, **2013**, *115*, 94.
4. M. Roman, C. Rigo, et al. *Analytical and Bioanalytical Chemistry*, **2016**, DOI: 10.1007/s00216-015-9014-6.

Topical application of choline-calix[4]arene nanocarrier of silibinin reduces retinal damage in a model of Age-Related Macular Disease

Clara La Marca,[a] Irene Deidda,[a] Patrizia Saladino,[a] Salvatore Papasergi,[a] Paola Pinto,[a] Giuseppe Granata,[b] Corrada Geraci,[b] Grazia Maria Letizia Consoli,[b] Anna Rita Blanco, [c] Patrizia Guarneri,[a].

[a]*CNR Institute of Biomedicine and Molecular Immunology - Neuroscience Unit, Via Ugo La Malfa 153, 90146, Palermo, Italy;* [b]*CNR Institute of Biomolecular Chemistry, Via Gaifami 18, 95126, Catania, Italy;.*[c] *SIFI SpA, Via Ercole Patti 36,95025, Catania, Italy.*

Email of presenting author: : irene.deidda@ibim.cnr.it; patrizia.saladino@ibim.cnr.it

Age-Related Macular Disease (AMD) is a multifactorial neurodegenerative disease that affects the macula, leading cause of blindness [1]. A pivotal role in AMD pathogenesis is played by dysregulation of iron homeostasis and currently no effective cure exists; controversial effects are indeed reported by treatments with anti-VEGF intraocular injections[2]. An important goal for the treatment of AMD is to obtain therapeutic drug concentrations in the eye posterior segment by a topical drug administration. Due to blood-ocular barriers and lacrimal drainage it is difficult to design non-invasive ocular formulations for the treatment of AMD. Nanoparticles have gained attention because of their ability to penetrate through the ocular tissues, enhancing drug bioavailability. As a possible treatment for AMD, we here investigated a new topical ophthalmic formulation based on choline-calix[4]arene (Ch-Cx) nanoparticles loaded with silibinin (Slb), a flavonoid extract of *silymarin (Silybum Marianum)*, which has multiple biological activities including anti-inflammatory, anti-angiogenic and antioxidant properties [3]. The synthesized Ch-Cx-Slb formulation showed to have good chemical, chemo-physical and pre-industrialization requirements for a drug delivery system: size smaller than 100 nm, low polydispersity index, stability, appropriate drug loading capacity, suitability of common sterilization and lyophilization processes and, finally, a positively charged surface that may increase eye adhesion and surface permanence [4]. In vitro screening was performed to analyze the cell viability and pharmacological effects of Ch-Cx-Slb using the human retinal pigment epithelial cells (ARPE-19) exposed or not to $FeSO_4$. In vivo assays were carried out using a rat model of AMD-like degeneration induced by a single intravitreal injection of $FeSO_4$ and following topical ocular application as collyrium of Ch-Cx-Slb, Ch-Cx uncomplexed with Slb or free Slb, once daily for a period of 10 days. Immunofluorescence and biochemical analyses demonstrated that Ch-Cx-Slb has a higher protective action against RPE/choroid alteration and retinal degeneration by reducing oxidative stress, inflammation and VEGF-induced proliferation, when compared to the free components. All data support the Ch-Cx as a potential drug delivery system of silibinin or other compounds for the treatment of AMD.

1. J. Ambati, BJ. Fowler, *Neuron*, **2012**, *75*, 26.
2. KG. Falavarjani, Q. D. Nguyen, *Eye*, **2013**, *27*, 787.
3. R .Gazák , D. Walterová D, V. Kren V, *Current Medicinal Chemistry*, **2007**, *14*, 315.
4. *International Patent, WO 2016/055976A1, 14.04.* **2016**.

Acknowledgements: This work was supported by HIPPOCRATES Project PON02_00355 to ARB, GMLC and PG, and by "PNR-CNR Aging Program 2012-201 PI.P02 to PG.

Oral Nanocarrier for Insulin Colon Delivery

Lucia Salvioni,[a] Luisa Fiandra,[b] Maria Dorly Del Curto,[c] Davide Prosperi,[a] Luca Palugan,[c] Fabio Corsi,[b] Miriam Colombo [a]

[a] Università degli Studi di Milano-Bicocca, NanoBioLab, Dipartimento di Biotecnologie e Bioscienze, piazza della Scienza 2, 20126 Milan, Italy; [b] Università degli Studi di Milano, Dipartimento di Scienze Biomediche e Cliniche "L. Sacco", via G.B. Grassi 74, 20157 Milan, Italy; [c] Università degli Studi di Milano, Dipartimento di Scienze Farmaceutiche, Sezione di Tecnologia e Legislazione Farmaceutiche "Maria Edvige Sangalli", via G. Colombo 71, 20133 Milan, Italy.

Email of presenting author: l.salvioni7@campus.unimib.it

The current treatment of diabetes disease (Type I and advanced Type II) relies on insulin subcutaneous injection.[1] Because of parenteral administration drawbacks, alternative administration routes have been investigated.[2] Among all, the oral administration may lead to a better glucose regulation exploiting the liver first-pass metabolism of insulin, thus preventing the risks of fluctuating glycaemia.[3] However, the oral bioavailability of peptides is very low and several efforts have been attempted to promote insulin bowel absorption. Despite all, the oral delivery of insulin remains an unmet need.[1] The aim of work was to prepare, characterize and evaluate both *in vitro* and *in vivo* a novel nanoformulated multiple-unit colon delivery system, i.e. coated pellets, as a possible oral nanocarrier for insulin. Insulin-loaded polymeric nanoparticles (NPs) were synthesized according to previously published protocols with some improvements.[4] The driving force of NPs formation was the opposite charges of polyethyleneimine and dextran sulphate resulting in the insulin entrapment into the polymeric matrix. NPs were incorporated into cores that were subsequently coated with three overlapping layers, aiming to release insulin into the large intestine: this gastrointestinal site is indeed characterized by a relatively low proteolytic activity. The system was evaluated *in vitro* for its physico-technological characteristics, NPs dispersion, disintegration and release performance, showing delayed release behaviour. Finally, the coated nanoformulation effect was tested in diabetic rats: a significant hypoglycaemic activity, due to the synergistic effect of NPs and colon delivery, was observed. Based on the *in vivo* efficacy, scalable process and safety profile, the proposed multitasking system appears a promising way to control diabetes.[5]

1. A. Maroni, L. Zema, M.D. Del Curto, A. Foppoli, A. Gazzaniga, , Adv. Drug. Delivery Rev. **2012**, *64*, 540.
2. F. Sousa, P. Castro, P. Fonte, B. Sarmento, , Ther. Deliv. **2015**, *6*, 83.
3. M.M. Patel, , Expert Opin. Drug Deliv. **2013**, *10*, 731.
4. W.Tiyaboonchai, J. Woiszwillo, R.C. Sims, C.R. Middaugha, , Int. J. Pharm. **2003**, *255*, 139.
5. L. Salvioni, L. Fiandra, M.D. Del Curto, S. Mazzucchelli, R. Allevi, M. Truffi, L. Sorrentino, B. Santini, M. Cerea, L. Palugan, F. Corsi, M. Colombo, , Pharm. Res., in press

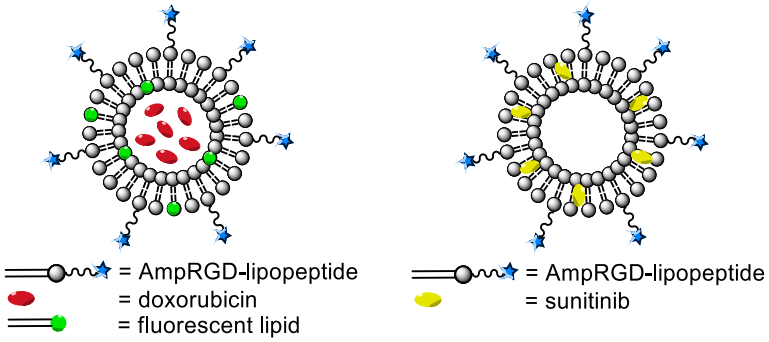
AmpRGD-Functionalized liposomes: from targeted delivery vehicles to integrated targeted antiangiogenic tools

Andrea Sartori,[a] Elisabetta Portioli,[a] Augusta De Santis,[b] Francesca Bianchini,[c] Gerardino D'Errico,[b] Lucia Battistini,[a] Franca Zanardi [a]

[a] *Department of Pharmacy, University of Parma, P.co Area delle Scienze 27A, 43124, Parma, Italy;*
[b] *Department of Chemical Sciences, University of Naples Federico II, Via Cintia, 80126, Napoli, Italy;*
[c] *Department of Biomedical, Experimental and Clinical Sciences 'Mario Serio', University of Florence, Viale Morgagni 50, 50134, Firenze, Italy.*

Email of presenting author: andrea.sartori@unipr.it

Tumor endothelial cells show increased levels of expression of several cell surface molecules that potentiate cell proliferation, invasion, and survival during tumor vascular remodeling and angiogenesis. One such molecule is the $\alpha_V\beta_3$ integrin, whose overexpression in both tumor-associated vascular endothelial cells and various tumor types renders it an eligible biomarker of the cancerous disease.[1] Enlightened by successful results in our laboratories dealing with the discovery of a new class of $\alpha_V\beta_3$-integrin ligands featuring a cyclic tetrapeptide AmpRGD sequence (Amp = aminoproline), we recently envisioned the possibility to use such peptides as decorating moieties of suitable lipopeptides to be used in the assemblage of targeted liposomial nanoparticles (LNPs).[2] The increased cytotoxicity of doxorubicin when delivered by AmpRGD-labeled liposomes as compared to the free drug and untargeted LNPs was assessed, pointing to the conclusion that these LNPs could constitute good vehicles for targeted delivery of cytotoxic drugs.[2] As a further advancement in the field, and aware of the close physical and functional connection between the $\alpha_V\beta_3$ integrin receptor and the vascular endothelial growth factor receptor (VEGFR2), we designed new liposomial nanoparticles wherein the exquisite integrin-targeting capability and possible antiangiogenic activity of the AmpRGD decorating moieties is complemented by the antiangiogenic activity of popular on-market drugs such as sunitinib, a cargo to be loaded within the NPs. Physico-chemical characterization of such liposomes and sunitinib-loading procedures will be discussed, along with preliminary in vitro biological results aimed at the evaluation of the anti-angiogenic activity of these integrated targeted nanoplatforms vis-à-vis the single components and covalent counterparts.



1. J. S. Desgrosselier, D. A. Cheresh, *Nature Rev. Cancer,* **2010**, *10*, 9.
2. L. Battistini, P. Burreddu, A. Sartori, D. Arosio, L. Manzoni, L. Paduano, G. D'Errico, R. Sala, L. Reia, S. Bonomini, G. Rassu, F. Zanardi, *Mol. Pharmaceutics,* **2014**, *11*, 2280.

Laser light triggered release of Silibinin from metal/polymer nanocomposites

Angela Scala,[a] Simona Grimato,[a] Giovanni Grassi,[a] Fortunato Neri,[b] Enza Fazio [b]

[a] *Department of Chemical, Biological, Pharmaceutical and Environmental Sciences, University of Messina, V.le F. Stagno d'Alcontres 31, 98166, Messina, Italy;* [b]*Department of Mathematics, Computer Sciences, Physics, and Earth Sciences, University of Messina, V.le F. Stagno d'Alcontres 31, 98166, Messina, Italy.*

Email of presenting author: ascala@unime.it

Nowadays, due to the optical properties of metallic NPs,[1] the design of stimuli-responsive metal/polymer nanocomposite plays an important role in the development of novel smart drug delivery systems (DDS), that allow on-demand control of the dose, the timing and the duration of the drug release, upon laser light irradiation.
With this in mind, new remotely-triggered DDS based on both PEG–PLGA_Au and PEG-PLA_Ag nanocomposites have been prepared,[2] and loaded with Silibinin (SLB), a well-known hepatoprotective drug whose employment in other pathological diseases is limited by a really low water solubility. The metallic NPs have been synthesized by laser ablation and subsequently embedded into the copolymer via a modified emulsion–diffusion method.
A combination of analytical techniques including nuclear magnetic resonance (NMR), static and dynamic light scattering (SLS, DLS), gel permeation chromatography (GPC), thermogravimetric analysis (TGA), X-ray photoelectron spectroscopy (XPS), infrared (FTIR) spectroscopy and scanning/transmission electron microscopies (SEM/STEM/TEM), have been used to study the structural and morphological properties of the nanocomposites.
The ability of the nanoplatforms to release SLB, working as a controlled light triggered DDS, has been evaluated. The effects of different laser wavelengths have been investigated pointing out that that the drug diffusion shows strong dependences on laser wavelength and metallic NPs Surface Plasmon Resonance (SPR).

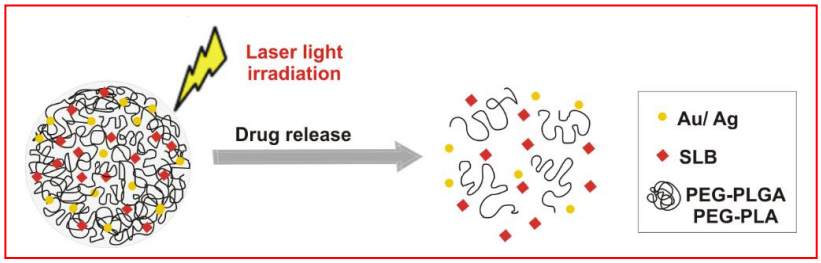


Figure 1. Schematic representation of the stimulated laser light drug release from the metal/polymer nanocomposites.

1. A. Stalmashonak, G. Seifert, A. Abdolvand, *SpringerBriefs in Physics.*, **2013**, *Chap. 3*, 17.
2. E. Fazio, A. Scala, S. Grimato, A. Ridolfo, G. Grassi, F. Neri, *J. Mater. Chem. B*, **2015**, *3*, 9023.

Acknowledgments: This work was partially funded by the European Community through the Programma Operativo Nazionale Ricerca e Competitività 2007–2013 (PON02_00355_2964193 Hippocrates Project).

Development of targeting drug delivery systems based on hyaluronic acid bioconjugates

Angela Scala,[a] Nicola Micale,[a] Nawal Mahfoudh,[b] Placido G. Mineo,[c] Anna Piperno,[a] Giovanni Grassi [a]

[a]*Department of Chemical, Biological, Pharmaceutical and Environmental Sciences, University of Messina, V.le F. Stagno d'Alcontres 31, 98166, Messina, Italy;* [b]*Department of Medicinal and Organic Chemistry, University of Granada, Faculty of Pharmacy, 18071 Granada, Spain;* [c]*Department of Chemical Sciences, University of Catania, V.le A. Doria 6, 95125, Catania, Italy.*

Email of presenting author: ascala@unime.it

Hyaluronic acid (HA), the main component of the extracellular matrix, has shown huge success in a variety of biomedical applications, including drug delivery, tissue engineering, and molecular imaging.[1] One unique feature of HA relies in the active tumor targeting through selective interactions with specific receptors, such as CD44 and RHAMM, typically over-expressed on the surface membranes of various tumor cells.[2] Additionally, HA is an attractive targeting ligand specifically recognized and internalized by macrophages that are known to express HA receptors for endocytosis (HARE/Stab2).[3] Exploiting these ligand-receptor interaction, the use of HA is now rapidly-growing to improve anticancer therapies and macrophages mediated pathologies. Herein, we exploit different synthetic strategies to obtain drug/HA bioconiugates. HA-drug systems were characterized to elucidate structure, size, charge surface, morphology, drug contents and biological profiles.

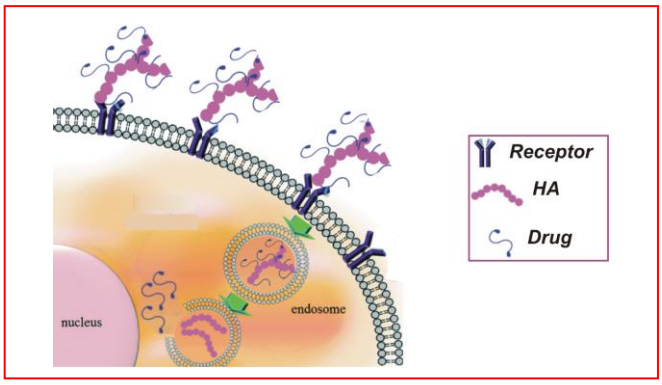


Figure 1. Illustration of HA cellular recognition.

1. F. Dosio, A. Arpicco, B. Stella, E. Fattal. *Adv. Drug Delivery Rev.*, **2016**, *97*, 204.
2. G. Mattheolabakis, L. Milane, A. Singh, M.M. Amiji. *J. Drug Target,* **2015**; *23*, 605.
3. N. Micale, A. Piperno, N. Mahfoudh, U. Schurigt, M. Schultheis, P.G. Mineo, T. Schirmeister, A. Scala, G. Grassi, *RSC Adv.*, **2015**, *5*, 95545.

Detection of breast cancer biomarkers in cell lysates using a biosensing platform based on Bloch surface waves

Alberto Sinibaldi,[a] Camilla Sampaoli,[b] Peter Munzert,[c] Leonardo Sibilio,[d] Elisa Tremante,[b] Norbert Danz,[c] Agostino Occhicone,[a] Patrizio Giacomini,[b] Francesco Michelotti [a]

[a] *Department of Basic and Applied Science for Engineering, Sapienza University of Rome, Via A. Scarpa 16, 00161 Rome, Italy;* [b] *Regina Elena National Cancer Institute, Via Elio Chianesi 53, 00144 Rome, Italy;* [c] *Fraunhofer Institute for Applied Optics and Precision Engineering IOF, Albert-Einstein-Str. 7, 07745 Jena, Germany;* [d] *IBI- Istituto Biochimico Italiano Giovanni Lorenzini Spa, Via Fossignano 2, 04011 Aprilia, Rome, Italy*

Email of presenting author: alberto.sinibaldi@uniroma1.it

The ongoing and worldwide challenge in diagnostics is to detect cancer biomarkers at very low concentrations to prevent cancer cell proliferation and metastatic diseases. In particular, the estimated numbers of new breast cancer cases and deaths in Europe in 2012 is approximately 464,000, collocating the breast cancer as the commonest potentially fatal cancer of women.[1] In this work, we report on the use of one-dimensional photonic crystals (1DPC) to detect clinically relevant concentrations of breast related cancer biomarkers in cell lysates. To this aim, we developed an optical platform that combines both label-free and fluorescence detection. Such platform makes use of 1DPC coated glass microscope slides tailored with monoclonal antibodies (BSW biochip) for high specific biological recognition. Similar to Surface Plasmon Polaritons (SPP), the excitation of a Bloch Surface Wave (BSW) can be obtained by a prism coupler leading to a dip in the angular reflectance spectrum.[2] The angular position of such dip is very sensitive to perturbations of the refractive index at the interface and is exploited for bio-sensing applications. Moreover, in presence of fluorescent molecules at the 1DPC surface, the platform can interrogate the BSW biochip also in the enhanced fluorescence mode obtaining further information on the cancer biomarker assay (see Fig.1).[3] Results on a clinically relevant breast cancer biomarker will be shown and analysed in the extended contribution.

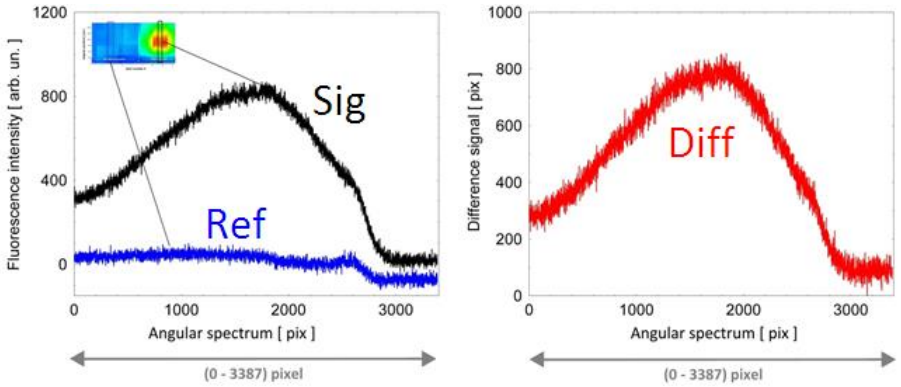


Figure 1. Fluorescence spectra obtained at the end of the bio-recognition assay in the reference (absence of the cancer biomarker, blue curve) and signal (presence of cancer biomarker, black curve) regions, respectively. The red spectrum is the differential signal.

1. J. Ferlay et al., *European Journal of Cancer*, **2013**, *49*, 1374.
2. A. Sinibaldi et al., *Sensors and Actuators B*, **2012**, *174*, 292.
3. N. Danz et al., *Proc. SPIE*, **2015**, *9506*, 95060V.

Coenzyme Q_{10} nanosuspensions for pulmonary delivery

Irene Rossi, Isabella Orsola Tomasoni, Fabio Sonvico, Francesca Buttini

Department of Pharmacy, University of Parma, Parco Area delle Scienze 27/a, 43125, Parma, Italy.

Email of presenting author: fabio.sonvico@unipr.it

Many studies have investigated the use of Coenzyme Q_{10} (CoQ_{10}) as a therapeutic adjuvant in the treatment of cancer patients with radiation and chemotherapy[1]. The aim of this study was the production and characterization of three different CoQ_{10} nanosuspensions for nebulisation.
Three CoQ_{10} suspensions were prepared through high pressure homogenization with highly biocompatible excipients (drug excipient ratio 1:1): Lipoid S 45, Gelucìre 48/16, Vitamin-E TPGS. Suspensions were dispersed at the concentration of 0.5% w/v in water or hydro alcoholic solution (70:30, only for lecithin) and then homogenized at 1500 bar with 40 subsequent passages. Ethanol, when present, was removed using a rotary evaporator (Rotavapor, Büchi, Switzerland). The formulations were characterized in terms of particle size, zeta-potential and median volume diameter. *In vitro* nebulisation efficiency and aerodynamic performance (total drug delivered, DD; drug delivery rate, DDR; median mass aerodynamic diameter, MMAD; respirable fraction, RF) was assessed nebulising CoQ_{10} with Pari LC Sprint® ampoule (PARI Pharma GmbH, Germany).
All preparations were identified as nanosuspensions: particle size of 85.8 ± 1.6 nm for CoQ_{10}:Gelucìre, 77.9 ± 1.1 nm for CoQ_{10}:lecithin, but the smallest particles were obtained with Vit-E TPGS (38.7 ± 0.1 nm). Furthermore, all nanoparticles obtained were negatively charged (about -20mV).

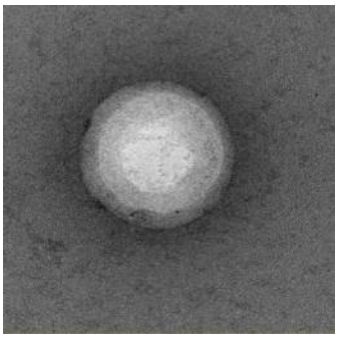


Figure 1. TEM image of CoQ_{10}: Lipoid S 45 nanosuspension

After the nebulisation, differences were less evident as median volume diameter of the aerosol was similar (3.93 µm-3.52 µm). The DDR using LC Sprint® was about 2%/min for all the formulations. The DD from the nebulisation system was higher for the preparation containing lecithin (42.17% ± 0.25), while for the remaining it was similar (around 30%). The nanosuspension containing Gelucìre presented the highest RF (70.6% ± 0.05) and smallest MMAD (3.02 µm ± 0.49). The other two formulations presented RF values higher than 55% and MMAD around 4 µm, with the CoQ_{10}:lecithin preparation which reported the less bright aerodynamic performance. However, preliminary stability tests showed that the only stable formulation after 90 days, was the one containing Vitamin-E TPGS.

1. T. C. Carvalho, J. P. McCook, N. R. Narain, J. T. McConville, *Journal of liposome research*, **2013**, *23*, 276

Ultrastructural characterization of Membrane Vesicles (MVs) produced by *Lactobacillus reuteri*.

Annarita Stringaro,[a] Marisa Colone,[a] Rosita Primavera,[b] Gabriella Mincione,[c] Martina Di Francesco,[d] Maria Carmela Di Marcantonio,[c] Luca Savino,[c] Valentina Puca,[e] Cristina Geminiani,[b] Rossella Grande [b]

[a] *Department of Technology and Health, Istituto Superiore di Sanità (ISS), Roma, Italy;* [b] *Departments of Pharmacy, "G. d'Annunzio" University of Chieti-Pescara Chieti, Italy;* [c] *Departments of Medical, Oral and Biotechnological Sciences "G. d'Annunzio" University of Chieti-Pescara Chieti, Italy;*
[d] *Department of Health Sciences, University "Magna Graecia", Catanzaro, Italy;* [e] *Department of Biotechnological and Applied Clinical Sciences, University of L'Aquila, L'Aquila, Italy.*

Email of presenting author: annarita.stringaro@iss.it

Nanotechnology represents a great promise for drug delivery opening new therapeutic opportunities for agents that cannot be effectively used for therapeutic use. In fact, nanomedicine has recently received increasing attention for its ability to improve the efficacy of cancer therapeutics, imaging tools, antibacterial agents and gene delivery vesicles (1, 2, 3).
As a natural phenomenon, bacteria release Membrane Vesicles (MVs) at a various stages of growth, under environmental conditions, or in response to chemical signals. MVs are a demonstrated form of communication used by bacteria.
Since the probiotic Gram positive *Lactobacillus reuteri* DSM 17938 develops biofilm *in vitro* producing factors which give health benefit to the host, the aim of this study was the ultrastuctural characterization of MVs produced from *L. reuteri* planktonic (pMVs) and biofilm (bMVs) phenotypes.
The microorganism is capable of generating MVs in both planktonic and biofilm phenotypes. *L. reuteri* DSM 17938 biofilm formation was evaluated by syto 9 staining and Confocal Laser Scanning Microscopy (CLSM) analysis. The data obtained in this study demonstrated that *L .reuteri* developed a well-structured biofilm after 24 h of incubation. Moreover, MVs production, in the two phenotypes, was confirmed by Scanning Electron Microscopy (SEM) observations. In order to evaluate MVs biological composition (as lipids and proteins), pMVs and bMVs were isolated by filtration and ultracentrifugation and subsequently submitted at an enzymatic digestion with DNase I, Proteinase K and Phospholipase C and finally analyzed by Transmission Electron Microscopy (TEM) and SEM.
The structure and composition of MVs may provide a relevant information about the use of such structures in the development of vesicles-based therapeutic systems. The analysis performed by TEM and SEM demonstrated that there are interesting differences both in the ultrastructural organization and in dimensions between pMVs and bMVs .

1. H. K. Sajja, M. P. East et al., *Curr. Drug. Discov. Technol*., **2009**, *6*, 43.
2. J. M. Miller-Kleinhenz, E. N. Bozeman et al., *Advanced Review*, **2015**, *7*, 797.
3. N. J. Alves, K. B. Turner et al., *Ther. Deliv*., **2015**, *7*, 873.

Albumin and hyaluronic acid coated superparamagnetic iron oxide nanoparticles for biomedical applications

Elena Vismara, Chiara Bongio, Andrea Serafini

Department of Chemistry, Materials and Chemical Engineer, Polytechnic of Milan, via Mancinelli 7, 20131, Milan, Italy.

Email of presenting author: elena.vismara@polimi.it

Magnetic iron oxide nanoparticles targeted to specific markers on cancer cells have recently emerged as a promising technology for delivering and as potential platforms to provide both imaging and therapy. Animal studies have demonstrated the potential for successful therapy following systemic or intravenous delivery of nanoparticles[1]. The objectives of the nanoparticles are two-fold: to reduce the amount of systemic distribution of the cytotoxic drug, thus reducing the associated side-effects; and to reduce the dosage required by more efficient, localized targeting of the drug. The novelty of our approach to *BSA-HA@Fe_3O_4* summarized in Figure 1 is to combine two types of coating: both BSA and HA could have an important synergic role. The protein could stabilize the nanoparticle preventing the aggregation of the nanoparticles, while the polymer could prolong the circulation in the bloodstream and bind the nanoparticles to receptors of the cancer cells. Since both the biological elements are suitable carriers for various anti-cancer drugs, it allows to manage and to test different drugs.

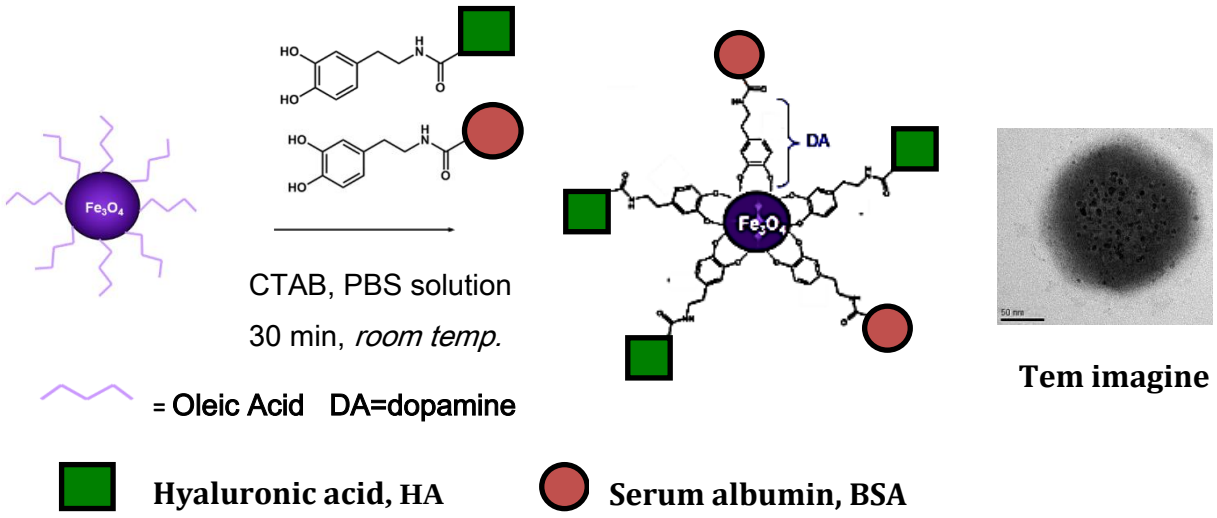


Figure 1. BSA-HA@Fe_3O_4 preparation

BSA and HA were thought to be covalently linked to the surface of Fe_3O_4 magnetic core, in order to obtain a strong interaction between the bioorganic layer and the inorganic core. The covalent bond between HA/BSA and Fe_3O_4 was realized by using a linking moiety, dopamine (DA), bearing suitable functional groups to react with both HA or BSA and Fe_3O_4. Indeed, dopamine was shown to easily bind to Fe_3O_4 as a bidentate enediol ligand and to be a robust and stable anchor group to functionalise Fe_3O_4 nanoparticles with functional molecules. Oleic acid coating was used to stabilize Fe_3O_4 and to enhance the exchange with dopamine (DA).

1. R. Ivkov et al, *Nanomedicine*, **2012**, *7*, 1697.

Acknowledgments: Ministero degli Affari Esteri (MAE) for partial funding.

List of Participants

1	Abu Saleh	Doaa	Technion-Israel Istitute of Tecnology, Haifa, Israel *Mail: sdoaaas@gmail.com*	**P1**
2	Alberti	Diego	Università di Torino, Italia *Mail: diego.alberti@unito.it*	**SL2**
3	Alongi	Jenny	Università degli Studi di Milano, Italia *Mail: jenni.alongi@unimi.it*	**P2**
4	Altieri	Barbara	Università dell'Aquila, Italia *Mail: barbara.altieri@gmail.com*	**P3**
5	Amalfitano	Adriana	Università Cattolica del Sacro Cuore, Roma, Italia *Mail: adry_amalfitano@libero.it*	**P4**
6	Ambrosio	Luigi	CNR – IPCB – Roma, Italia *Mail: luigi.ambrosio@cnr.it*	**IL8**
7	Antimisiaris	Sophia G.	University of Patras, Greece *Mail: santimis@upatras.gr*	**IL9**
8	Arcovito	Alessandro	Università Cattolica del Sacro Cuore, Roma, Italia *Mail: alessandro.arcovito@unicatt.it*	**P5**
9	Barenholz	Yechezkel	The Hebrew University, Jerusalem, Israel *Mail: chezyb1@gmail.com*	**IL1**
10	Battista	Sara	Università dell'Aquila, Italia *Mail: sara.battista@student.univaq.it*	**P6**
11	Bertalot	Thomas	Università di Padova, Italia *Mail: thomas.bertalot@unipd.it*	**P7**
12	Boccalon	Mariangela	Centro Ricerche Bracco, Bracco Imaging s.p.a., Basovizza, Trieste, Italia *Mail: mariangela.boccalon@bracco.com*	**P8**
13	Bombelli	Cecilia	CNR - IMC - Roma, Italia *Mail: cecilia.bombelli@uniroma1.it*	
14	Bondì	Maria Luisa	CNR - ISMN - Palermo, Italia *Mail: marialuisa.bondì@ismn.cnr.it*	**P9** **P10**
15	Borocci	Stefano	Università della Tuscia, Viterbo, Italia *Mail: borocci@unitus.it*	
16	Bozzuto	Giuseppina	Istituto Superiore di Sanità, Roma, Italia *Mail: giuseppina.bozzuto@iss.it*	**P11**
17	Bukchin	Alexandra	Technion-Israel Istitute of Tecnology, Haifa, Israel *Mail: alexbukchin@gmail.com*	**P12**
18	Cacciapaglia	Roberta	CNR - IMC - Roma, Italia *Mail: roberta.cacciapaglia@uniroma1.it*	
19	Calcabrini	Annarica	Istituto Superiore di Sanità, Roma, Italia *Mail: annarica.calcabrini@iss.it*	
20	Cantarutti	Cristina	Università di Udine, Italia *Mail: cantarutti.cristina@spes.uniud.it*	**P13**
21	Capitani	Donatella	CNR – IMC – Montelibretti, Roma, Italia *Mail: Donatella.capitani@cnr.it*	
22	Capozza	Martina	Centro Ricerche Bracco, Bracco Imaging s.p.a., Collereto Giacosa, Torino, Italia *Mail: Martina.capozza@bracco.com*	**SL12**

23	Castellani	Stefano	Università di Foggia, Italia *Mail: stefano.castellani@unifg.it*	**SL6**
24	Ceccacci	Francesca	CNR - IMC - Roma, Italia *Mail: francesca.ceccacci@uniroma1.it*	
25	Centi	Sonia	CNR- IFAC- Sesto Fiorentino, Italia *Mail: s.centi@ifac.cnr.it*	**SL23**
26	Chronopoulou	Laura	Università di Roma "Sapienza", Italia *Mail: laura.chronopoulou@uniroma1.it*	**SL17**
27	Ciranna	Fabrizio	Università di Roma "Sapienza", Italia *Mail: fabrizio.ciranna@uniroma1.it*	**P14**
28	Clementino	Adryana	Università di Parma, Italia *Mail: adryanarc@gmail.com*	**SL4**
29	Colone	Marisa	Istituto Superiore di Sanità, Roma, Italia *Mail: marisa.colone@iss.it*	**P15**
30	Comes Franchini	Mauro	Università di Bologna, Italia *Mail: mauro.comesfranchini@unibo.it*	**SL10**
31	Condello	Maria	Istituto Superiore di Sanità, Roma, Italia *Mail: maria.condello@iss.it*	**P16**
32	Conese	Massimo	Università di Foggia, Italia *Mail: massimo.conese@unifg.it*	
33	Consoli	Grazia Maria Letizia	CNR - ICB - Catania, Italia *Mail: grazia.consoli@icb.cnr.it*	
34	Crucho	Carina	University of Lisboa, Portugal *Mail: carina.crucho@tecnico.ulisboa.pt*	**P17**
35	D'Acunzo	Francesca	CNR – IMC – Montelibretti, Roma, Italia *Mail: francesca.dacunzo@cnr.it*	**P18**
36	Dawre	Shilpa	Deemed University, Mumbai, India *Mail: shilpadawre@gmail.com*	**P19**
37	Deidda	Irene	CNR – IBIM – Palermo, Italia *Mail: irene.deidda@ibim.cnr.it*	**P44**
38	Di Bari	Ivana	Università degli Studi di Catania, Italia *Mail: ivanadibari@hotmail.it*	**P20**
39	Di Giannuario	Amalia	Istituto Superiore di Sanità, Roma, Italia *Mail: amalia.digiannuario@iss.it*	**P21**
40	Dozio	Samuele Maria	CNR - ISTEC – Faenza, Ravenna, Italia *Mail: samuele.dozio@istec.cnr.it*	**SL21** **P22**
41	Ferroni	Claudia	CNR - ISOF – Bologna, Italia *Mail: claudia.ferroni@isof.cnr.it*	**SL9**
42	Fracassi	Alessandro	ETH, Zürich, Switzerland *Mail: afracassi@org.chem.ethz.ch*	**P23**
43	Galantini	Luciano	Università di Roma "Sapienza", Italia *Mail: luciano.galantini@uniroma1.it*	
44	Galeazzi	Roberta	Università Politecnica delle Marche, Ancona, Italia *Mail: r.galeazzi@univpm.it*	**P24**
45	Garduño-Ramirez	Maria Luisa	Universidad Autónoma del Estado de Morelos, México *Mail: lgarduno@uaem.mx*	**P25** **P26**

46	Giansanti	Luisa	Università degli Studi dell'Aquila, Italia *Mail: luisa.giansanti@univaq.it*	
47	Giordani	Maria	Università della Tuscia, Viterbo, Italia *Mail: m.giordani@unitus.it*	
48	Guarneri	Patrizia	CNR – IBIM – Palermo, Italia *Mail: patrizia.guarneri@ibim.cnr.it*	
49	Hanieh	Patrizia Nadia	Università di Roma "Sapienza", Italia *Mail: patrizianadia.hanieh@uniroma1.it*	**P27** **P28**
50	Laghezza Masci	Valentina	Università della Tuscia, Viterbo, Italia *Mail: laghezzamasci@unitus.it*	**SL22**
51	Lai	Sarah	CNR - IFAC - Sesto Fiorentino, Firenze, Italia *Mail: s.lai@ifac.cnr.it*	**SL15**
52	Laudadio	Emiliano	Università Politecnica delle Marche, Ancona, Italia *Mail: e.laudadio@univpm.it*	
53	Ledda	Mario	CNR - IFT - Roma, Italia *Mail: mario.ledda@ift.cnr.it*	**P29**
54	Leo	Eliana	Università di Modena e Reggio Emilia, Italia *Mail: eliana.leo@unimore.it*	**P30**
55	Lolli	Maria Grazia	CNR - IFT - Roma, Italia *Mail: Mariagrazia.lolli@ift.cnr.it*	
56	Longo	Angela	CNR - IPCB - Portici, Napoli, Italia *Mail: angela.longo@cnr.it*	**P31**
57	Luciani	Paola	Friedrich Shiller University, Jena, Germany *Mail: paola.luciani@uni-jena.de*	**IL4**
58	Maggi	Vito	Istituto Italiano di Tecnologia, Arnesano, Lecce, Italia *Mail: vito.maggi@iit.it*	**SL14**
59	Mancin	Fabrizio	Università di Padova, Italia *Mail: fabrizio.mancin@unipd.it*	**P32**
60	Mancini	Giovanna	CNR – IMC – Montelibretti, Roma, Italia *Mail: giovanna.mancini@cnr.it*	
61	Maretti	Eleonora	Università di Modena e Reggio Emilia, Italia *Mail: eleonora.maretti@unimore.it*	**P33**
62	Martella	Elisa	Istituto Ortopedico Rizzoli, Bologna, Italia *Mail: elisa.martella@ior.it*	**P34**
63	Maysinger	Dusica	McGill University, Montreal, Canada *Mail: dusica.maysinger@mcgill.ca*	**IL3**
64	Mazzaglia	Antonino	CNR - ISMN – Messina, Italia *Mail: antonino.mazzaglia@ismn.cnr.it*	**SL8**
65	Meschini	Stefania	Istituto Superiore di Sanità, Roma, Italia *Mail: stefania.meschini@iss.it*	
66	Minnelli	Cristina	Università Politecnica delle Marche, Ancona, Italia *Mail: cristina.minnelli@outlook.it*	
67	Mobbili	Giovanna	Università Politecnica delle Marche, Ancona, Italia *Mail: g.mobbili@univpm.it*	
68	Molinari	Agnese	Istituto Superiore di Sanità, Roma, Italia *Mail: agnese.molinari@iss.it*	

69	Moret	Francesca	Università di Padova, Italia *Mail: francesca.moret@unipd.it*	**P35**
70	Motiei	Leila	Weizmann Istitute of Science, Rehovot, Israel *Mail: leila.motiei@weizmann.ac.il*	**SL1**
71	Nardoni	Martina	Università di Roma "Sapienza", Italia *Mail: martina.nardoni@uniroma1.it*	**P36**
72	Nocca	Giuseppina	Università Cattolica del Sacro Cuore, Roma, Italia *Mail: giuseppina.nocca@unicatt.it*	
73	Novelli	Federica	Università di Roma "Sapienza", Italia *Mail: federica.novelli@uniroma1.it*	**SL19**
74	Oliva	Paolo	Centro Ricerche Bracco, Bracco Imaging s.p.a., Colleretto Giacosa, Torino, Italia *Mail: paolo.oliva@bracco.com*	**SL25**
75	Papini	Emanuele	Università di Padova, Italia *Mail: Emanuele.papini@unipd.it*	**SL3**
76	Pellegrini	Evelin	Istituto Superiore di Sanità, Roma, Italia *Mail: evelin.pellegrini.guest@iss.it*	
77	Perinelli	Diego Romano	Università degli Studi di Camerino, Macerata, Italy *Mail: diego.perinelli@unicam.it*	**P37**
78	Petaccia	Manuela	Università dell'Aquila. Italia *Mail: manuela.petaccia@libero.it*	**P38**
79	Pieretti	Stefano	Istituto Superiore di Sanità, Roma, Italia *Mail: stefano.pieretti@iss.it*	**P39**
80	Pigozzo	Andrea	Alfatest srl, Roma, Italia *Mail: andrea.pigozzo@alfatest.it*	**IR1**
81	Pleiko	Karlis	University of Latvia, Riga, Latvia *Mail: karlis.pleiko@lu.lv*	**P40**
82	Ribeiro	Tânia	University of Lisboa, Portugal *Mail: tania.ribeiro@tecnico.ulisboa.pt*	**SL20**
83	Ricci	Francesco	Università di Roma "Tor Vergata", Italia *Mail: francesco.ricci@uniroma2.it*	**IL2**
84	Rinaldi	Federica	Istituto Italiano di Tecnologia, Roma, Italia *Mail: federica.rinaldi@iit.it*	**P41**
85	Rizzuto	Maria Antonietta	Università degli Studi di Milano-Bicocca, Italia *Mail: m.rizzuto3@campus.unimib.it*	**P42**
86	Rodrigues	Ana Sofia	University of Lisboa, Portugal *Mail: anagrodrigues@tecnico.ulisboa.pt*	**SL16**
87	Roman	Marco	Ca'Foscari Università di Venezia, Italia *Mail: marco.roman@unive.it*	**P43**
88	Saladino	Patrizia	CNR – IBIM – Palermo, Italia *Mail: patrizia.saladino@ibim.cnr.it*	**P44**
89	Salvio	Riccardo	Università di Roma "Sapienza", Italia *Mail: riccardo.salvio@uniroma1.it*	
90	Salvioni	Lucia	Università degli Studi di Milano-Bicocca, Italia *Mail: l.salvioni7@campus.unimib.it*	**P45**
91	Sartori	Andrea	Università di Parma, Italia *Mail: andrea.sartori@unipr.it*	**P46**

92	Scala	Angela	Università di Messina, Italia *Mail: ascala@unime.it*	**P47** **P48**
93	Schlachet	Inbar	Technion - Israel Institute of Technology, Haifa, Israel *Mail: nbarschlachet@gmail.com*	
94	Scipioni	Anita	Università di Roma "Sapienza", Italia *Mail: anita.scipioni@uniroma1.it*	
95	Segal	Ester	Technion-Israel Institute of Technology, Haifa, Israel *Mail*: *esegal@technion.ac.il*	**IL5**
96	Sinibaldi	Alberto	Università di Roma "Sapienza", Italia *Mail: alberto.sinibaldi@uniroma1.it*	**P49**
97	Sonvico	Fabio	Università degli Studi di Parma, Italia *Mail: fabio.sonvico@unipr.it*	**P50**
98	Storm	Gert	Utrecht University, The Netherlands *Mail: g.storm@uu.nl*	**IL6**
99	Stringaro	Annarita	Istituto Superiore di Sanità, Roma, Italia *Mail: annarita.stringaro@iss.it*	**P51**
100	Tei	Lorenzo	Università del Piemonte Orientale, Alessandria, Italia *Mail: lorenzo.tei@uniupo.it*	**SL13**
101	Toccacieli	Laura	Istituto Superiore di Sanità, Roma, Italia *Mail: laura.toccacieli@iss.it*	
102	Torchilin	Vladimir P.	Northeastern University, Boston, USA *Mail: v.torchilin@neu.edu*	**IL7**
103	Trzcinski	Jakub Waldemar	Università di Padova, Italia *Mail: jakubwaldemar.trzcinski@unipd.it*	**SL5**
104	Turjeman	Keren	The Hebrew University, Jerusalem, Israel *Mail: Kerenbarhum@gmail.com*	**SL7**
105	Varchi	Greta	CNR - ISOF- Bologna, Italia *Mail: greta.varchi@isof.cnr.it*	**SL11**
106	Villa	Silvia	Università degli Studi di Genova, Italia *Mail: silvia.villa@chimica.unige.it*	**SL18**
107	Vismara	Elena	Politecnico di Milano, Italia *Mail: elena.vismara@polimi.it*	**P52**
108	Webster	Thomas J.	Northeastern University, Boston, USA *Mail: th.webster@neu.edu*	**IL10**
109	Yamakoshi	Yoko	ETH, Zürich, Switzerland *Mail: yamakoshi@org.chem.ethz.ch*	**SL24**

The NANOMEDICINE VITERBO 2016
Conference is organized under the patronage of

ISBN 978-88-97987-12-3

Organized by
Institute of Chemical Methodologies, CNR, Roma
Department of Technology and Health, Istituto Superiore di Sanità, Roma