NANOMEDICINE ROME 2018

Rome, 18-20 June 2018

Istituto Superiore di Sanità

DIAGNOSIS

THERAPY

THERANOSTICS

DRUG DELIVERY

TISSUE ENGINEERING

INVITED SPEAKERS

Patrick Couvreur

Fabrizio Gelain

Jan Grimm

Inge Katrin Herrmann

Carlotta Marianecci

Miguel Oliveira

Anna Salvati

Gert Storm

Thomas J. Webster

Frederik Wurm

Abstract Book

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Congress Coordinators

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

Agnese Molinari
National Centre for Drug Research and Evaluation - Istituto Superiore di Sanità
Tel. 06 4990 2228
E-mail: agnese.molinari@iss.it

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University of L'Aquila, Department of Physical and Chemical Sciences

PROGRAM

MONDAY JUNE 18

09.00 Registration
09.30 Opening Ceremony

Gualtiero Ricciardi	President of Istituto Superiore di Sanità, Rome
Massimo Inguscio	President of Consiglio Nazionale delle Ricerche, Rome
Patrizia Popoli	Director of Centre for Drug Research and Evaluation, ISS, Rome
Giovanna Mancini	Director of Institute of Chemical Methodologies, CNR, Rome
Agnese Molinari	Centre for Drug Research and Evaluation, ISS, Rome

Chairpersons: ***G. Storm, F. Wurm***

Invited lecture

10.00 ***P. Couvreur***
"Nanomedicines for the treatment of severe diseases"

Selected lectures

10.30 ***D. Alberti***
"A theranostic approach for Boron Neutron Capture Therapy (BNCT) treatment based on the use of Gd/B multimodal probes"

10.50 ***A. Fahr***
"Development of novel m-THPC-liposomes and layersomes for the oral treatment of cholangiocarcinoma and gastrointestinal tumors"

11.10 *Coffee break*

Invited lecture

11.30 **J. Grimm**
"When particles meet - utilizing the power of Cerenkov light with nanotechnology"

Selected lectures

12.00 ***S. Sennato***
"Temperature-dependent aggregation of PNIPAM microgels for controlled release of macromolecules"

12.20 ***S. Murgia***
"Bicontinuous cubic liquid crystalline dispersions as potential tools in nanomedicine"

12.40 ***N. Medard***
"SEEC Microscopy: a live and label-free analysis technique in the fields of materials and life sciences"

13.00 *Lunch and poster session*

MONDAY JUNE 18

Chairpersons: ***C. Marianecci, T.J. Webster***

Invited lecture

14.30 **I. Herrmann**

"Magnetic blood purification: from concept to clinics"

Selected lectures

15.00 ***C. Ferroni***

"Unprecedented behavior of (9R)-9-hydroxystearic acid loaded keratin nanoparticles on cancer cell cycle"

15.20 ***F. Sansone***

"Ammonium containing calixarenes as multivalent systems for the delivery of nucleic acids and mimics"

15.40 ***F. Caselli***

"High-throughput microfluidic impedance cytometer for label-free counting, localization and characterization of single cells"

16.00 *Coffee break and poster session*

Invited lecture

16.20 **F. Wurm**

"Poly(phosphoester)-functionalized nanocarriers: degradable alternatives to poly(ethylene glycol)"

Selected lectures

16.50 ***G. D'Avenio***

"Integrative cytotoxicity assessment of nanostructured medical devices"

17.10 ***C. Giordani***

"The role of the monosialoganglioside-GM1 in the interaction between model membranes and unstructured metastable amyloid oligomers of salmon calcitonin"

TUESDAY JUNE 19

Chairpersons: ***P. Couvreur, A. Salvati***

Invited lecture

9.00 **T.J. Webster**
"20 years of developing FDA approved nanomedicine"

Selected lectures

9.30 ***A. Kovačević***
"Development of ursodeoxycholic acid loaded nanostructured lipid carriers (NLC) for the therapy of liver diseases"

09.50 ***E. Markova***
"Protein binding capacity of nanostructured lipid carriers loaded with *Salvia off.* extract"

10.10 ***S. Di Gioia***
"Isolation of nanoparticles from *Brassica oleracea L.* (Broccoli) and study of their effect on the metabolic activity of lung tumor cell lines"

10.30 ***Y. Soleimanian***
"Propolis wax-nanostructured lipid carriers for improving oral delivery and cholesterol lowering activity of β-sitosterol

10.50 *Coffee break and poster session*

Invited lecture

11.10 **J.M. Oliveira**
"A multiscale approach in tissue engineering: from nano to tissues"

Selected lectures

11.40 ***M. Renault-Mahieux***
"Development and stability of liposomes co-encapsulating fisetin and cisplatin"

12.00 ***A. Arcovito***
"Drug delivery using protein nanocarriers: human or virus templates?"

12.20 ***C. Martini***
"Intercalation of bioactive molecules into nanosized ZnAl hydrotalcites for combined chemio and photo cancer treatment"

12.40 ***R. Santoliquido***
"Nanovectors: how to characterize size and concentration in liquid matrices"

13.00 *Lunch and poster session*

TUESDAY JUNE 19

Chairpersons: ***F. Ceccacci, I. Herrmann***

Invited lectures

14.30 **C. Marianecci**
"'Soft' nanocarriers: a versatile strategy for brain delivery"

15.00 **F. Gelain**
"Nanomaterials for nervous regeneration"

Selected lectures

15.30 ***M.G. Raucci***
"Eumelanin-based substrates as smart materials for neuronal regeneration"

15.50 ***L. Talamini***
"The role of nanocarrier physicochemical properties on the biodistribution and the blood brain barrier passage"

16.10 ***G. Erel***
"Radiation-induced targeted nanoparticle based gene delivery for brain tumors"

16.30 ***F. Garello***
"VCAM-1 targeted paramagnetic micelles for Magnetic Resonance Imaging of neuroinflammation"

20.00 ***Social Dinner and poster prizes***

WEDNESDAY JUNE 20

Chairpersons: ***J. Grimm, J.M. Oliveira***

Invited lecture

9.00 **G. Storm**
"The debate on (targeted) nanomedicine"

9.30 *Flash presentation for poster prizes*

Selected lectures

10.00 ***S. Bertoni***
"Reactive oxygen species-responsive nano-in-micro composite for targeted therapy of inflammatory bowel disease"

10.20 ***C. Conte***
"Redox responsive polymeric nanocarriers for the combined therapy of lung cancer"

10.40 ***A. Sarra***
"Study of membrane phase transition in bacterial vesicles"

11.00 ***H. Akbaba***
"Induction of apoptosis in the lung cancer cells using ultrasound-sensitive nanobubble formulations containing combination of survivin-siRNA and paclitaxel"

11.20 ***I. Russo Krauss***
"Functionalized superparamagnetic iron oxide nanoparticles as theranostic devices: from development to interaction with proteins"

11.40 *Coffee break and poster session*

Invited lecture

12.00 **A. Salvati**
"Dissecting how cells internalize and process nano-sized drug carriers for nanomedicine applications"

Selected lectures

12.30 ***D. Shalabalija***
"Influence of the surface properties of nanoliposomes on protein corona formation"

12.50 ***A. Fracassi***
"Preparation of synthetic Low Density Lipoprotein (sLDL) with an on-surface chemoselective ligation"

13.10 ***G. Celenza***
"Cerium oxide nanoparticles as potential antibiotic adjuvant. Effects of CeO_2 nanoparticles on bacterial outer membrane permeability"

13.30 ***L. Chronopoulou***
"Innovative nanofabrication methodologies for the preparation of drug delivery systems"

13.50 *Closing remarks and lunch*

Invited Lectures

Nanomedicines for the treatment of severe diseases

Patrick Couvreur

University of Paris-Sud, Université Paris-Saclay Institut Galien, UMR CNRS 8612, 5 rue J-B Clément F-92296 Chatenay-Malabry, France.
Email: patrick.couvreur@u-psud.fr

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

- The design of biodegradable doxorubicin-loaded polyalkylcyanoacrylate nanoparticles for the treatment of the multidrug resistant hepatocarcinoma (a nanomedicine with phase III clinical trials ended) [1].

- The construction of nanoparticles made of metal oxide frameworks (NanoMOFs) [2,10], a highly hyperporous material obtained by the complexation of iron oxide clusters with diacids. The nanopores of this material may be designed according to the molecular dimension of the drug molecule to be encapsulated.

- The "squalenoylation" [3,4], a technology that takes advantage of the squalene's dynamically folded molecular conformation, to link this natural and biocompatible lipid to anticancer drug molecules [5] to achieve the spontaneous formation of nanoassemblies (100–300 nm) in water, without the aid of surfactants. Surprisingly, these squalene-based nanoparticles are using the circulating LDL as "indirect" carriers for targeting cancer cells with high expression of LDL receptors [6]. The application of the "squalenoylation" concept for the treatment of brain ischemia and spinal cord injury will be discussed too (Figure 1). The possibility to use other terpenes (natural or synthetic) than squalene to design nanoparticles for the treatment of cancer will be discussed, too [9].

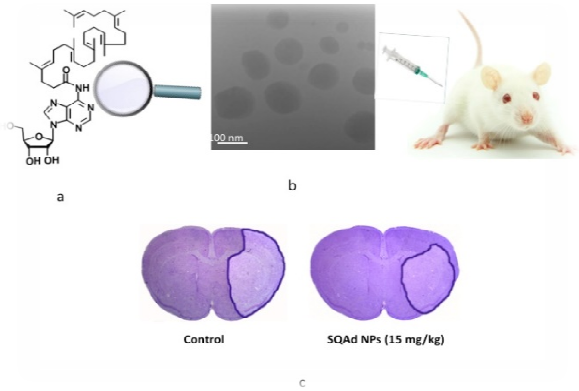

Figure 1: *Adenosine-Squalene bioconjugate (a) spontaneously self-assemble in water as nanoparticles (SQAd NPs) of ca. 100 nm (b). When injected into mice subject to brain ischemia, nanoparticles induce reduction of ischemic zone (c).*

The design of "multidrug" nanoparticles combining in the same nanodevice chemotherapy and imaging (ie., "nanotheranostics") or various drugs with complementary biological targets will be also discussed [7]. Finally, it will be shown that the construction of nanodevices sensitive to endogenous (ie. pH, ionic strength, enzymes etc.) or exogenous (ie., magnetic or electric field, light, ultrasounds etc.) stimuli may allow the spatio-temporal controlled delivery of drugs and overcome resistance to current treatments [8].

*1. L. Barraud et al., J. Hepatology, **2005**, 42, 736.*
*2. P. Horcajada et al., Nature Materials., **2010**, 9, 172.*
*3. P. Couvreur et al., Nano Letters, **2006**, 6, 2544.*
*4. A. Gaudin et al., Nature Nanotechnology, **2014**, 9, 1054.*
*5. A. Maksimenko et al., Proceedings of the National Academy of Science, **2014**, 111 (2), E217.*
*6. D. Sobot et al., Nature Communications, **2017**, 8, 15678. DOI: 10.1038/ncomms15678.*
*7. A. Maksimenko et al., ACS Nano, **2014**, 8, 2018.*
*8. S. Mura et al., Nature Materials, **2013**, 12, 991.*
*9. S. Harisson et al., Angewandte Chemie Int. Edition, **2013**, 52, 1678.*
*10. T. Simon-Yarza et al., Angewandte Chemie Int. Edition, **2017**. DOI: 10.1002/anie.201707346.*

When particles meet - utilizing the power of Cerenkov light with nanotechnology

Jan Grimm

Molecular Pharmacology Program & Department of Radiology, Memorial Sloan Kettering Cancer Center, New York, NY, USA.
Email: grimmj@mskcc.org

Nanotechnology has been used in cancer therapy and diagnosis for quite some time. Nanoparticles (NP) possess several desirable features for use in imaging and therapy. They serve as platforms for loading therapeutics and contrast agents while simultaneously anchoring targeting ligands or stealth polymer coatings. Their size and surface chemistry can be tuned such that they exhibit attractive biological properties, such as passive accumulation and retention in cancer, in contrast to the rapid washout often observed by small molecular imaging agents. Among the many imaging modalities that have adopted NP-based contrast agents are nuclear imaging techniques such as PET or SPECT. Consequently, radiolabeled NP are of great interest. Cerenkov luminescence (CL) is the low level of blue-light produced by particles traveling faster than the speed of a light through a dielectric medium such as tissue. CL imaging (CLI) utilizes clinical approved tracers, thus avoiding significant hurdles for approval of the imaging agent. By reverting to PET of the very same agent an internal standard is provided that allows for quantification as well as true multimodality imaging from the same imaging label. Clinical trials using Cerenkov imaging in patients are undergoing both in Europe and the US, and first commercial hardware is being developed and evaluated. The combination of radiotracers with NP also allows for some new imaging modalities due to the unique interaction of the NP with the radioactivity, which will be discussed in more depth.

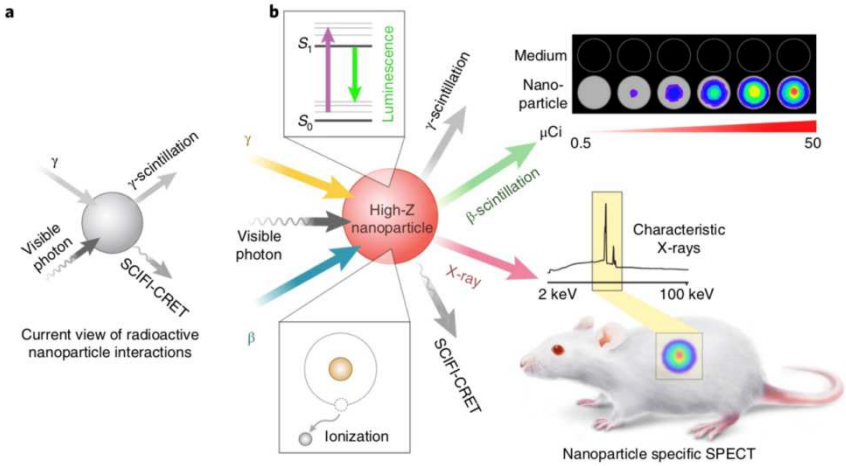

Figure 1. Interactions between ionizing radiation and nanoparticles. a, γ and visible photon sources are the excitation mechanisms of NPs by ionizing radiation. **b**, A more complete view of mechanisms resulting in excitation and ionization of NP from both β and γ radionuclides. [Pratt E et al., *Nature Nanotechnology*, **2018**, *13(5)*, 418]

Magnetic blood purification: from concept to clinics

Inge K. Herrmann

Swiss Federal Laboratories for Materials Science and Technology (Empa).
Email: Inge.Herrmann@empa.ch

Sepsis is a potentially life-threatening condition that requires immediate medical attention. Early administration of antibiotics has direct impact on patient outcome. However, sepsis is difficult to differentiate from non-infectious systemic inflammation (SIRS), a condition that is very common in intensive care unit patients. Treating all patients who show SIRS symptoms without proper diagnosis not only increases costs, but leads to other complications and increased microbial resistance, which is equally undesirable. There is a major unmet clinical need to better tailor antibiotic therapy to the patient's individual needs.
Here, we report on the design of functional magnetic capturing agents for theranostic magnetic separation-based blood purification. We designed iron oxide / polymer hybrid nanoclusters functionalized with a newly developed antibody to capture and remove pathogens from body fluids. Bacteria quantification in the supernatant and on the particle surface by plating and optical absorption revealed that bacteria were efficiently captured by antibody-functionalized beads with capturing efficacies > 98%. Then, we demonstrate rapid and sensitive detection of bacteria on magnetic beads recovered from the magnetic separator to allow speedy pathogen identification and diagnosis based on the enrichment of bacteria out of large volumes.
The present theranostic approach could significantly help to reduce the overuse of antibiotics by allowing speedy detection and identification of the causing pathogens and at the same time providing an effective treatment modality by decreasing the bacterial load to bridge the time before appropriate antibiotics can be administered. By rationally designing the magnetic particles (based on modelling of binding times and safety considerations), we address safety risks at an early stage and ensure that the approach leverages the unique benefits of nanoparticles while balancing associated risks.

Poly(phosphoester)-functionalized nanocarriers: degradable alternatives to poly(ethylene glycol)

Frederik R. Wurm

Max-Planck-Institut für Polymerfoschung, Ackermannweg 10, 55128 Mainz, Germany.
Email: wurm@mpip-mainz.mpg.de

For decades now polyethylene glycol (PEG) is the standard for shielding biomolecules as well as nanoparticles from rapid clearance from the blood circulation [1]. Coating of surfaces and nanocarriers with PEG is supposed to strongly reduce protein adsorption and this reduced protein absorption is thought to be the main course of action, which is referred to as “stealth” effect. Nevertheless, it has been known that even on PEGylated surfaces a lower but still detectable amount of proteins attach [2].
We have developed a series of biodegradable poly(phosphoester)s that can substitute PEG but prevents any accumulation of nondegradable polymers [3]. The polyphosphoester synthesis platform allows further controlling the polymers’ hydrophilicity on the nanocarrier surface. We proved that the stealth effect is caused by a specific protein corona after contact with human blood, which depends on the hydrophilicity of the polymer. Therefore the protein pattern is crucial to understand the stealth effect in general. In addition, the same plasma protein is enriched on surfaces with hydrophilic polymers, which is clusterin that is a key player in the stealth behavior. Increasing hydrophobicity does not increase the protein amount, but the type of “recruited” protein from the blood plasma. In addition, targeting can be achieved by combining the stealth properties with carbohydrates, which can be recognized by immune cells.

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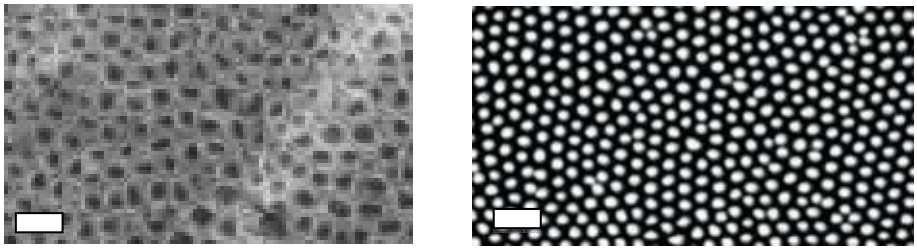
2. Schöttler S., Becker G., Winzen S., Steinbach T., Mohr K., Landfester K., Mailänder V., Wurm F.R., *Nat Nano,* **2016,** *11* (4), 372.
3. Bauer K.N., Tee H.T., Velencoso M.M., Wurm F.R., *Prog. Polym. Sci.,* **2017,** *73*, (Supplement C), 61-122.

20 years of developing FDA approved nanomedicine

Thomas J. Webster

Department of Chemical Engineering, Northeastern University, 313 Snell Engineering Center, 360 Huntington Avenue, Boston, MA 02115 USA.
Email: th.webster@northeastern.edu

Despite the fact that many researchers believe that nanomedicine is more hype than reality, there are numerous FDA approved medical products that incorporate nanomaterials improving human health today. This talk will highlight some of these FDA approved products with clinical data in the improvement of disease prevention, diagnosis, and treatment. In particular, it will highlight medical devices that use nanoscale features to improve tissue growth (such as bone, vascular, bladder, etc.) while limiting inflammation and inhibiting infection, all without the use of drugs. It will also highlight novel FDA approved nanoparticles that can simultaneously detect and treat diseases, such as infectious diseases and cancer. This talk will also review the future of medicine in implantable sensors and how nanomaterials are being used to assess immune system responses to an implant and then modify itself to ensure implant success. Lastly, this study will cover the fundamental reason why nanomaterials are able to control cell responses without using drugs. For example, how hip implants can be modified to possess nanoscale surface features that mimic those of insect wings to inhibit bacteria attachment and growth (Figure 1).



Medical device with nanofeatures Insect wing with natural nanofeatures

Figure 1: The development of medical devices to possess nanoscale features (left) which resemble those of an insect wing (right) to inhibit infection. Scale bar = 100 nm.

A multiscale approach in tissue engineering: from nano to tissues

Joaquim Miguel Oliveira [a,b,c]

[a] *3B's Research Group- Biomaterials, Biodegradable and Biomimetic, University of Minho, Headquarters of the European Institute of Excellence on Tissue Engineering and Regenerative Medicine, Avepark, 4805-017 Barco, Guimarães, Portugal;* [b] *ICVS 3Bs PT Government Associate Lab, Braga, Guimarães, Portugal;* [c] *The Discoveries Centre for Regenerative and Precision Medicine, Headquarters at University of Minho, Avepark, 4805-017 Barco, Guimarães, Portugal.*
Email: miguel.oliveira@i3bs.uminho.pt

Nanotechnology will have, in a near future, a crucial role in advanced and personalized treatment solutions for human diseases. The prospects for cell replacement and tissue regeneration in many musculoskeletal and neurological diseases are impeded by inefficient stem cell/drugs delivery. Through the development of nanoparticles, it is now possible to increase bioavailability and bioactivity of medical therapeutics and further selective targeting to damaged tissues. In the last few years, we have been developing several dendrimer nanoparticles [1] and gold nanoparticles that show great promise in tuning stem cells functions which open up different application possibilities in the intracellular drugs delivery aiming to treat bone, spinal cord and brain-related diseases/disorders. By its turn, tissue engineering (TE) aims combining engineering and biological properties to create functional substitutes for damaged and diseased tissues. Importantly, nanotechnology can open up a new era for TE, allowing the creation of nanostructures [2] that are comparable in size to those appearing in natural tissues. Despite our important advances in these fields, the convergence of nanotechnologies and TE strategies can allow us envisioning the development of safer and effective treatment solutions in tissue/organ regeneration. On the other hand, recent evidences indicate that 3D and flow models more closely resemble the *in vivo* function. In these contexts, our group has been exploiting several multi-scale strategies combining the use of nanoparticles, scaffolds and stem cells, and emerging technologies such as bioprinting and microfluidics towards developing both advanced treatment solutions and 3D *in vitro* models of disease (e.g. cancer). In particular, a semi-automated microfluidic platform for real-time investigation of nanoparticles' cellular uptake and cancer cells' tracking has been reported [3]. This microfluidic-based platform composed of microfluidic chip together with fluorescence-labeled dendrimer NPs can allow the validation of new chemotherapeutic agents and its potential use in the development of diagnostics platform and personalized therapies. Herein, the challenges associated with the multiscale approaches will be discussed, and examples of our most impactful achievements making use of nano- and micro-technologies combined with TE strategies will be also presented.

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2. Pina S., Oliveira J.M. et al., *Advanced Materials*, **2015**, *27 (7)*, 1143.
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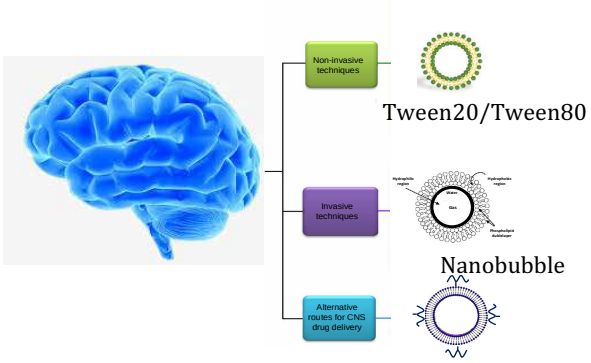
"Soft" nanocarriers: a versatile strategy for brain delivery

Carlotta Marianecci

Department of Chemistry and Technology of Drugs, University of Rome "Sapienza", P.le A. Moro 5, 00185, Rome, Italy.
Email: carlotta.marianecci@uniroma1.it

Diseases in the Central Nervous System (CNS) affect about the 20% of people worldwide and half of them are adults expected to develop degenerative CNS pathologies, such as Alzheimer's or Parkinson's. However, the greatest constraint in drug delivery to the brain is not the absence of drugs to treat CNS diseases, but rather the mechanism to transport such drugs through the nearly impenetrable blood brain barrier (BBB). Developing therapeutics for brain diseases is a major challenge; in particular, the most stimulating aspect of that challenge is to pass through the blood–brain barrier. Currently, strategies to increase drug delivery to the brain use invasive, non-invasive or alternative approaches to bypass the BBB. Nanomedicine has recently emerged as a promising field for innovative and effective approaches to cross the BBB and target brain diseases. In this presentation, the research activities performed in Nanomedicine_Lab of Rome Sapienza (M.Carafa, P.N. Hanieh, A. Imbriano, F. Rinaldi) will be presented. In particular, the application of different "soft" nanocarriers by different approaches to brain delivery will be illustrated (1-3).



Current approaches to CNS delivery

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2. F. Rinaldi, P.N. Hanieh, L.K.N. Chan, L. Angeloni, D. Passeri, M. Rossi, J.T.W. Wang, A. Imbriano, M. Carafa, C. Marianecci, *Pharmaceutics,* **2018**, *10*, 38.
3. M. Carafa, A. Bettucci, C. Marianecci, F. Rinaldi, A. Biagioni, "Nanobubbles and uses thereof" PCT/IB2017/052060.

Nanomaterials for nervous regeneration

Fabrizio Gelain [a,b]

[a]*ISBREMIT, IRCSS Casa Sollievo della Sofferenza, Opera di San Pio da Pietralcina, Viale Cappuccini 1, San Giovanni Rotondo (FG);* [b]*Center for Nanomedicine and Tissue Engineering (CNTE), ASST Niguarda Cà Granda, Piazza dell'Ospedale Maggiore 3, Milan.*
Email: f.gelain@css-mendel.it

Peptidic biomaterials have been receiving great interest because of their easiness of scale-up production, absence of pathogen-transfer risk, biomimetic properties, nanostructured morphology and customization potential for the specific tissue engineering application. However, their proper usage requires the understanding of the multiple-phenomena taking place at different scale levels during self-assembling. In this presentation, aiming at advancing the field of nervous regeneration, we will see some of our multi-disciplinary research and advances focused toward the regeneration of spinal cord injuries. This will bring us from molecular dynamics to cross-linking and electro-spinning of self-assembling peptides, from 3D neural stem cells cultures to in vivo testing.

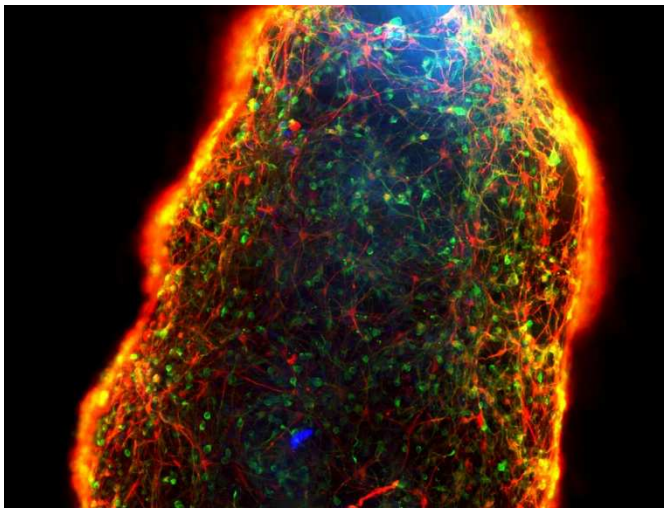

Figure 1. Human Neural Stem Cells cultured (7DIV) inside inner lumen of a microchannel entirely made of cross-linked self-assembling peptides. Astrocytes (red) and neurons (green) form an entangled network of differentiating cells.

1. R. Pugliese, A. Marchini, G.A. Saracino, R.N. Zuckermann, F. Gelain, *Nano Research,* **2017**, *11*, 586.
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3. F. Gelain, S. Panseri, S. Antonini, C. Cunha, M. Donega, J. Lowery, F. Taraballi, G. Cerri, M. Montagna, F. Baldissera, A. Vescovi, *ACS Nano*, **2011**, *5*, 227.

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The debate on (targeted) nanomedicine

Gert Storm

Dept. Pharmaceutics, Utrecht Institute for Pharmaceutical Sciences (UIPS), Utrecht University, PO Box 80082, 3508 TB Utrecht, The Netherlands; University Medical Centre Utrecht (UMCU), Division Imaging, Utrecht, The Netherlands; Dept. Biomaterials Science & Technology (BST), MIRA Institute for Biomedical Technology and Technical Medicine, University of Twente, Enschede, The Netherlands.
Email: G.Storm@uu.nl

One most active sector of research within the field of nanomedicine has been the design of nanopharmaceuticals for targeted drug delivery. In fact, novel nanomedicinal drug delivery systems continue to flourish in the research laboratory. However, the number of such nanomedicines that have been approved for the treatment of patients is still limited. Examples are Caelyx/Doxil (doxorubicin), Myocet (doxorubicin), DaunoXome (daunorubicin), Marqibo (vincristine), Onyvide (irinotecan), Onco-TCS (vincristine), Vyxeos (cytarabine and daunorubicin) and Abraxane (paclitaxel). While these examples illustrate that significant advances have been made over the years in making nanomedicines a clinical reality, there is nevertheless growing scepticism in the scientific literature regarding the future and clinical applicability of targeted nanopharmaceuticals. In this presentation, I will discuss the arguments raised to justify this negative attitude as well as my view on how targeted nanomedicine will face tomorrow.

Dissecting how cells internalize and process nano-sized drug carriers for nanomedicine applications

Anna Salvati

Groningen Research Institute of Pharmacy, University of Groningen, A. Deusinglaan 1, 9713Ave Groningen, The Netherlands.
Email: a.salvati@rug.nl

Nano-sized materials have the unique capacity to distribute in organisms and enter cells easily using cellular pathways. This has opened up tremendous opportunity in nanomedicine for using nano-sized carriers to deliver drugs more efficiently to their site of action. However, the molecular details of the mechanisms of uptake and intracellular trafficking of nano-sized drug carriers are in most cases still not clear. Such knowledge could allow us to further improve the design of truly targeted nanomedicines.
To this aim, we have combined different cell biology methods including the use of transport inhibitors and RNA interference to characterize the early steps of nanoparticle recognition by cells and the following uptake mechanisms. Additional efforts have been focused on developing *in vitro* cell barriers more closely resembling those nanomedicines encounter *in vivo* and in this way to elucidate whether the organization of cells into polarized cell barriers affects nano-carrier uptake and behavior.
Our results show that the same cells process nanoparticles in different ways when they are developed into a cell barrier rather than at different degrees of cell density, as commonly applied for *in vitro* studies. Furthermore we show that the corona molecules adsorbing from the environment on the nanoparticle surface not only can be recognized by cell receptors, as previously shown [1], but also can affect the details of the following uptake mechanism. Thus, in other words, the same nanoparticles enter cells via different pathways when different coronas are formed on their surface.

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Acknowledgments: This work was funded by the European Research Council (ERC) under the European Union's Horizon 2020 research and innovation programme under grant agreement Nº637614 (NanoPaths).

Selected Lectures

A theranostic approach for Boron Neutron Capture Therapy (BNCT) treatment based on the use of Gd/B multimodal probes

Diego Alberti,[a] Nicoletta Protti,[b] Silva Bortolussi,[b] Saverio Altieri,[b] Annamaria Deagostino,[c] Silvio Aime,[a] Simonetta Geninatti−Crich [a]

[a] *Department of Molecular Biotechology 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

This study aims at investigating a new theranostic approach for the treatment of primary tumours and metastasis based on the use of BNCT that combines low energy neutron irradiation with the presence of boron-containing compound at the targeted cells. This makes BNCT a promising option for the treatment of metastasis disseminated, for example, in the thoracic cavity that cannot be treated by methods requiring a precise localization, such as surgery or conventional radiotherapy. The innovation of this study lies on the development of novel theranostic agents, able to maximize the selective uptake of boron atoms in tumour cells and, at the same time, to quantify boron distribution in the tumour and in other tissues by Magnetic Resonance Imaging (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 tumour and healthy tissues. To this purpose a new dual BNCT/MRI agent has been synthesized and delivered to tumour cells using Low Density Lipoproteins as specific carriers. In particular, this study has been focused on the treatment of lung metastases generated by intravenous injection of a Her2 + breast cancer cell line (i.e. TUBO) in BALB/c mice, transgenic EML4-ALK mice used as primary lung tumor model [1] and of a subcutaneous tumour mouse model of Malignant Mesothelioma (MM). The latter is an aggressive tumour with a poor prognosis whose incidence and mortality is a function of past exposure to asbestos, after a latency period of 30-50 years. MM is a disseminated tumour against which conventional radiotherapy has limited effectiveness. Therefore, to improve both the clinical diagnostics and treatment, the discovery of new MM potential target molecules is of great interest. BNCT has been performed after MRI analysis at the TRIGA-Mark II reactor at the University of Pavia. With respect to controls, in boron treated group, tumour growth was significantly reduced.

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Development of novel m-THPC-liposomes and layersomes for the oral treatment of cholangiocarcinoma and gastrointestinal tumors

Gerhard D. Wieland, Dietrich Scheglmann, Arno Wiehe, Alfred Fahr, Volker Albrecht

Biolitec research GmbH, Otto-Schott-Strasse 15, D-07745 Jena, Germany.
Email of presenting author: alfred.fahr@uni-jena.de

Introduction: Photodynamic Therapy (PDT) as a combination of photoactivable pharmaceutical active ingredients (APIs, photosensitizers, PS) and PS-specific laser light is a well-proven method for local tumor therapy. Following illumination of the tumors with laser light of accurately defined wavelengths, reactive oxygen species built *in situ* can destroy the tumors. PDT therefore is a highly promising novel option addressing *cholangiocellular carcinoma (CCC)* and *tumors of the gastrointestinal tract (GIT)*. Physicochemical properties of PDT-APIs like solubility and stability often hinder sufficient oral bioavailability and targeted accumulation at tumor site. The goal of the projects summarized in this talk was the development of novel oral pharmaceutical formulations of BCS class IV APIs, administered parentally (for example: Foscan® with Temoporfin (m-tetrahydroxy-phenylchlorin (mTHPC)) as API) [1].
Liposomes have been often described as suitable drug carriers to increase the bioavailability of BCS Class IV APIs. So these projects aimed to elucidate the possibility to use liposomes or novel drug delivery systems like multilayered layersomes [2] amongst other nano-structured formulations, to deliver pharmaceutical APIs like photosensitizers (PS) into cells and tissues by oral administration.

Materials and methods: The ratio of the layersomes and the construction of the standard liposomes and the layersomes is documented in detail. The physicochemical and photochemical properties of the formulations are presented, as well as the stability of these formulations in biological fluids. MTT cytotoxicity assays determined the PDT efficacy of these novel liposomes on different cell lines. Life cell Fluorescence Microscopy shows the uptake of the liposomes in cells and tissues.

Results and Conclusion: Liposomal formulations are a promising novel option as oral administered pharmaceutical compositions for the treatment of cholangio carcinoma and GIT-tumours. Embedded in tablets the formulations cross the intestinal barriers to target CCC in the liver, or regionally targeted in the GIT may enter tumoral lesions in the intestine.

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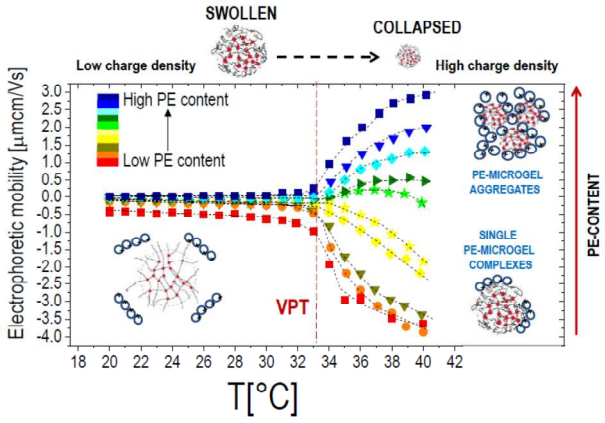
These projects were supported by the German Ministry for Education and Research (BMBF) (13N11386 *BioTrap for CCC* and 13N13422 *GITCare*).

Temperature-dependent aggregation of PNIPAM microgels for controlled release of macromolecules

Simona Sennato,[a] Domenico Truzzolillo,[b] S. Casciardi,[c] S. Sarti,[a] Federico Bordi [a]

[a] *Institute for Complex Systems ISC–CNR and Physics Department, Sapienza University of Rome, Italy;* [b] *Université de Montpellier, France;* [c] *INAIL Research, Roma, Italy.*
Email of presenting author: simona.sennato@roma1.infn.it

Microgels are crosslinked polymeric particles composed of water soluble/swellable polymer network and represent an important class of materials with interesting application potential since they be designed to exhibit volume changes in response to small changes in their environment such as pH, ionic strength, temperature, electric and magnetic field and solvent. Their high water content, biocompatibility and desirable mechanical properties, united to a tunable size, large surface area for multivalent bioconjugation and an interior network for biomolecules incorporation, offer unique advantages for drug delivery [1].
Due to the presence of a Volume Phase Transition around physiological condition, thermoresponsive microgels of Poly(N-isopropylacrylamide) (PNIPAM) are one of the most investigated systems [2]. By a combination of electrophoretic and light scattering techniques and TEM microscopy we have shown that PNIPAM microgels are able to physically adsorb and release a small biocompatible polycation (ε-polylysine ε-PLL) thanks to the reversibility of the electrostatic interaction between them. The adsorbent power increases dramatically above the VPT and large amount of ε-PLL can be load. We have demonstrated that this complexation is driven both by temperature through the VPT of microgels and by the PNIPAM/ε-PLL concentration ratio in a reentrant-condensation behavior [3]. Polyelectrolyte adsorption gives rise to a reentrant condensation of microgels for T > T_{VPT}, with formation of stable microgel aggregatesas opposed to a continuous enhancement of particle condensation observed for monovalent salt [3]. This peculiar electrostatically-driven controlled aggregation is here primarly tuned by the VPT-transition of PNIPAM and opens new intriguing scenarios for the controlled self-assembly of complex macromolecules employed for controlled release in nanomedicine.



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Acknowledgments: S.S. acknowledges E. Zaccarelli for discussions and funding (ERC MIMIC).

Bicontinuous cubic liquid crystalline dispersions as potential tools in nanomedicine

Sergio Murgia

Department of Chemical and Geological Sciences, University of Cagliari, s.s. 554 bivio Sestu, 09042, Monserrato (CA), Italy.
Email of presenting author: murgias@unica.it

New formulation strategies were recently developed with the purpose of combining imaging probes, drugs, and targeting agents in the same therapeutic/diagnostic (theranostic) nanocarrier to discover and treat diseases at the initial stage and with limited side effects. In this context, lipid-based nanoparticles can be considered as flexible platforms since they can be personalized depending on their application.
This presentation focuses on the possible use as theranostic tools of monoolein-based liquid crystalline nanoparticles, known as cubosomes, showing an inner structure characterized by a reverse bicontinuous cubic symmetry. Physicochemical and photophysical analysis demonstrated that cubosomes can effectively be loaded with anticancer drugs, UV-visible or NIR emitting fluorophores, and decorated with folic acid as cancer cells-targeting ligand. Their living cells imaging skills, cytotoxicity features, and biodistribution in vivo will be discussed.

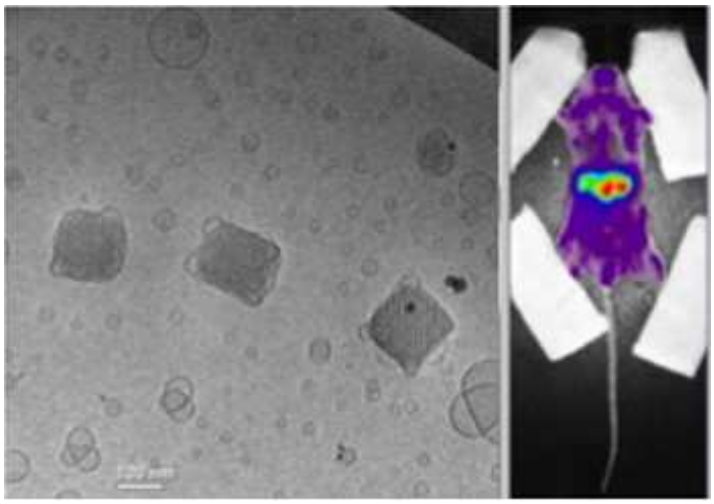

Figure 1. Cryo-TEM image of cubosomes (left), and whole body fluorescence intensity distribution of a healthy mouse that received an i.v. injection of fluorescent cubosomes (right).

1. V. Meli et al., *Langmuir*, **2015**, *31*, 9566.
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SEEC Microscopy: a live and label-free analysis technique in the fields of Materials and Life Sciences

Nicolas Medard, Imed Ayadi

NANOLANE, Pole Novaxud, 57 Boulevard Demorieux, 72100 Le Mans, France.
Email of presenting author: nicolas.medard@eolane.com

SEEC Microscopy is a label-free analysis technique offering new characterization capabilities such as the live nanoscale imaging, the multiplex molecular interaction analysis or the real-time quantitative study. The technique implements unique optical sensitive sensors (SEEC sensors) with specific contrast-enhanced properties enabling the live visualization of samples down to nanoscale (0.1nm). In addition, a proprietary algorithm (Q-SEEC) enables quantitative analyses (surface interaction and topography analyses) with an accuracy of 0.3nm.

SEEC Microscopy is dedicated to the study of samples in the fields of Materials and Life Sciences. Thanks to its high sensitivity, the technique can be applied for the analysis of nanofilms, nanopatterns, nanotubes, nanoparticles or DNA molecules…

Amongst successful analyses recently conducted, we will present studies concerning the role of the slime in bacteria motion, the tracking of dendritic cells behavior when used as drug carrier for cancer treatment. Others examples will be also presented concerning the multiplex molecular interactions analysis during enzymatic reactions [1] or also the label-free and real-time study of lipid vesicles behavior in changing biological environment.

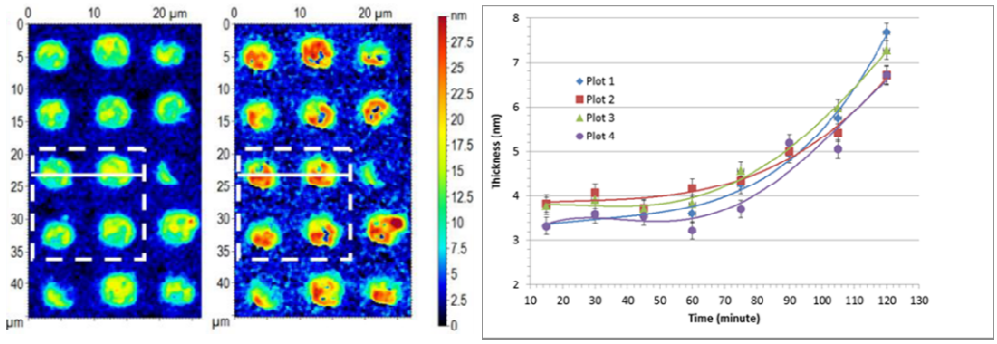

Figure 1. Dextransucrase-Based Enzymatic Reaction on patterns. 1. SEEC quantitative images at t=0 and t=120min. 2. Multiplex SEEC analysis of the kinetics of reactions on selected patterns.

1. A. Egea et al., *BioNanoScience*, **2014**, 4, 37.

Unprecedented behavior of (*9R*)-9-hydroxystearic acid loaded keratin nanoparticles on cancer cell cycle

Claudia Ferroni,[a] Alberto Busi,[b] Annalisa Aluigi,[a] Carla Boga,[b] Natalia Calonghi,[c] Andrea Guerrini,[a] Giovanna Sotgiu,[a] Tamara Posati,[a] Franco Corticelli,[d] Jessica Fiori,[e] Greta Varchi [a]

[a]*ISOF-CNR, Via Gobetti 101 - 40129 Bologna, Italy;* [b] *Department of Industrial Chemistry, Viale Risorgimento 4, 40136 Bologna, Italy;* [c] *Department of Pharmacy and Biotechnology, Via Irnerio 48, 40126 Bologna, Italy;* [d] *IMM-CNR, Via Gobetti 101 - 40129 Bologna, Italy;* [e] *Department of Pharmacy and Biotechnology, Via Belmeloro 6, 40126, Bologna, Italy.*
Email of presenting author: claudia.ferroni@isof.cnr.it

High level expression of histone deacetylase 1 (HDAC1) plays a pivotal role in the pathobiology of cancer [1]. The endogenous fatty acid *(9R)*-9-hydroxystearic acid, 9R, is a natural HDAC1 inhibitor, which exerts its anti-proliferative activity by arresting cancer cells growth in G0/G1 phase [2]. However, one of its major limitations is the unfavorable pharmacokinetic that hampers its therapeutic effectiveness. To overcome this constraint, 9R keratin nanoparticles (9R@Ker) were prepared and described here for the first time. Keratin was selected as carrier because possesses excellent biocompatibility and low toxicity to cells, thus resulting very promising material for drug delivery applications [3]. The formation of 200 nm nanoparticles (NPs) was induced by hydrophobic interactions between 9R and the hydrophobic protein domains, affording water-stable NPs with no need of toxic cross-linking agents. NPs were characterized in terms of particles size distribution, zeta potential, morphology, thermogravimetric behavior and drug release profile. Moreover, *in vitro* uptake and cytotoxicity was evaluated using human colorectal adenocarcinoma cells (HT29). Our data revealed that 9R@Ker are efficiently internalized by tumor cells, altering membrane lipidic composition. *In vitro* results demonstrate that the activity of 9R as free or loaded onto NPs is similar in terms of anti-proliferative effect. Remarkably, some significant differences were observed in the cell cycle analysis, showing that while free 9R caused a growth arrest in the G0/G1 phase, 9R@Ker induced a S phase arrest, thus promoting apoptosis (Fig.1). Additional studies are ongoing to better elucidate the underpinning biochemical mechanism of action of our newly developed delivery system.

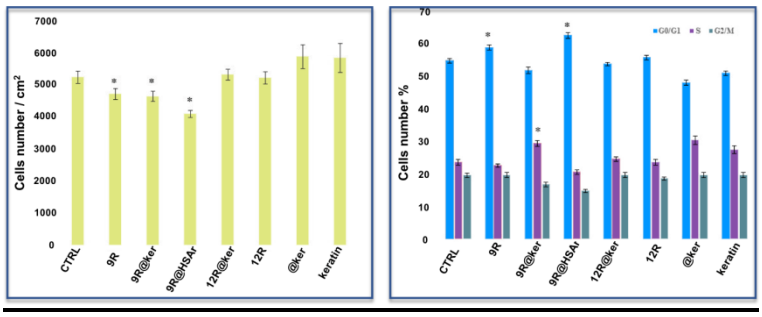

Figure 1. Figure illustrating the *In vitro* biological activity of (*R*)-9HSA@Ker nanoparticles.

1. B.M. Müller et al., *BMC Cancer*, **2013**, *13*, 215.
2. C. Parolin et al., *Biochimica et Biophysica Acta*, **2012**, *1821,* 1334.
3. A. Aluigi et al., *RSC Adv.*, **2016**, *6*, 33910.

Ammonium containing calixarenes as multivalent systems for the delivery of nucleic acids and mimics

Francesco Sansone,[a] Jessica Gasparello,[b] Michela Lomazzi,[a] Alessia Finotti,[b] Alex Manicardi,[a] Alessandro Casnati,[a] Roberto Corradini,[a] Roberto Gambari [b]

[a]*Dipartimento di Scienze Chimiche, della Vita e della Sostenibilità Ambientale, Università di Parma, Parco Area delle Scienze 17/a, 43124 Parma, Italy;* [b] *Dipartimento di Scienze della vita e biotecnologie, Università di Ferrara, Via Fossato di Mortara 74, 44121 Ferrara, Italy.*
E-mail of presenting author: francesco.sansone@unipr.it

Gene therapy is based on the possibility of delivering proper nucleic acids or mimics into the cells in order to block or restore processes and activities related with alterations in the genome of the patients. The first recent successes in this field exploit suitably modified viruses as carriers. However, their use is related with some possible drawbacks such as inflammation, toxicity, mutagenesis, limits in the cargo size, expensive procedures for large scale preparation. Therefore, it is relevant the development of non viral vectors based on organic molecules and polymers as safe and efficient delivery systems. In this context, some years ago we designed and synthesized molecular vectors characterized by a multivalent exposition of guanidinium or arginine units linked to a calixarene scaffold [1]. Some of them indeed showed a very high efficiency in the transfection of plasmid DNA associated with a very low or negligible citotoxicity, resulting better than commercially available formulations for transfection protocols. Very recently we interestingly demonstrated a remarkable activity of these macrocyclic vectors in the transfection of cells also with RNAs, such as miRNA and pre-miRNA [2], and nucleic acid mimics such as Peptide Nucleic Acids (PNAs) [3]. It is particularly relevant that for the latter ones no other significant vectors are currently available despite the importance of these molecules as potential therapeutics. These new findings make then our multivalent vectors promising non viral transfecting agents of interest for researchers and companies working on gene therapy and development of drugs based on nucleic acids and mimics.

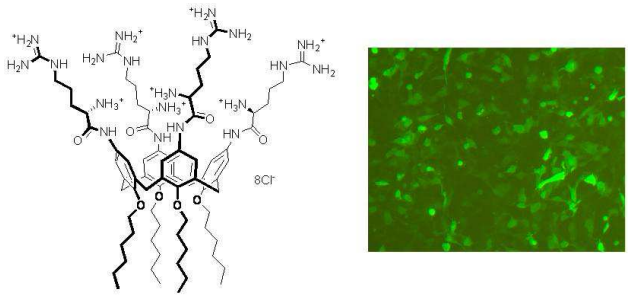

Figure 1. A fluorescence microscopy image of cells (right) transfected with the EGFP plasmid (encoding for the green fluorescence protein) by using a calixarene based vector (left).

1. V. Bagnacani, V. Franceschi, M. Bassi, M. Lomazzi, G. Donofrio, F. Sansone, A. Casnati, R. Ungaro, *Nat. Commun.*, **2013**, *4*, 1721.
2. Patent pending
3. Patent pending

High-throughput microfluidic impedance cytometer for label-free counting, localization and characterization of single cells

Riccardo Reale,[a] Adele De Ninno,[a,b] Luca Businaro,[b] Paolo Bisegna,[a] Federica Caselli [a]

[a]*Department of Civil Engineering and Computer Science, University of Rome Tor Vergata, Via del Politecnico 1, 00133, Rome, Italy;* [b] *Italian National Research Council, Institute for Photonics and Nanotechnologies, Via Cineto Romano 42, 00156, Rome, Italy.*

Email of presenting author: caselli@ing.uniroma2.it

Microfluidic impedance spectroscopy is an attractive label-free technique for high-throughput electrical characterization of single particles and cells. It is used in different biological assays, including particle sizing and counting, cell phenotyping, and disease diagnostics. An impedance-based flow microcytometer typically consists of a microchannel equipped with microelectrodes and filled with a conductive buffer. The current change upon passage of a cell between the electrodes under an AC voltage is measured and then analysed to determine cell properties.

In this work, we present innovative chip layouts and relevant operation modes that increase the accuracy and information content of the features embedded in the impedance signal traces [1,2,3]. In particular, novel electrical metrics encoding particle cross-sectional position are presented. They are exploited to achieve accurate sizing, overcoming the positional dependence issue, and to investigate particle inertial focusing mechanisms (Figure 1).

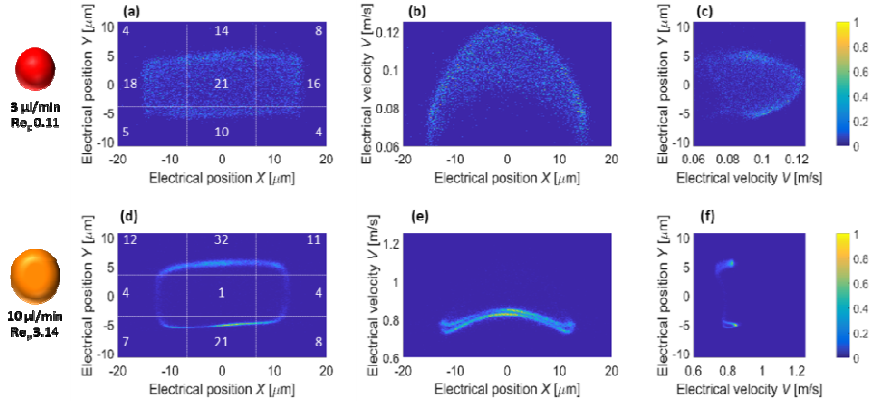

Figure 1. Electrical measurement of particle localization and focusing. Density plots of electrical position *Y* vs. electrical position *X* (a, d), electrical velocity *V* vs. electrical position *X* (b, e), electrical velocity *V* vs. electrical position *Y* (c, f). At higher particle Reynolds number Re_p (d-f), the hydrodynamic focusing is more pronounced.

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3. R. Reale et al, *Microfluid Nanofluid*, **2018**, *22*, 41.

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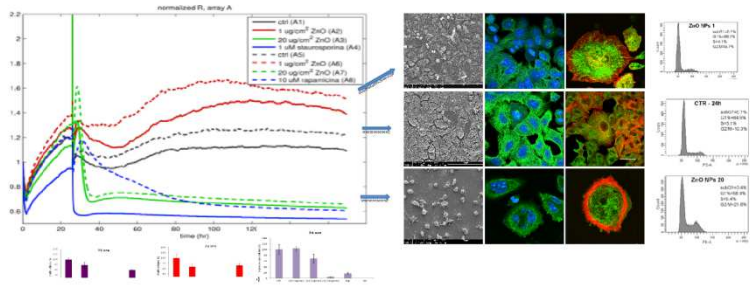
Integrative cytotoxicity assessment of nanostructured medical devices

Giuseppe D'Avenio,[a] Giuseppina Bozzuto,[b] Maria Condello,[b] Simona Sennato,[c] Giuseppe Familiari,[d] Ezio Battaglione,[d] Stefania Meschini,[b] Carla Daniele,[a] Agnese Molinari,[b] Mauro Grigioni [a]

[a]National Center for Technological Innovation in Public Health, Istituto Superiore di Sanità Rome, Italy; [b]National Center for Drug Research and Evaluation, Istituto Superiore di Sanità Rome, Italy; [c]Institute for Complex Systems ISC–CNR and Physics Department, Sapienza University of Rome; [d]Department of Anatomy, Histology, Forensic Medicine and Orthopaedics, Sapienza University of Rome.
Email of presenting author: davenio@iss.it

Nanostructured medical devices (MDs) are gaining a rapid diffusion. The recently introduced European Medical Device Regulation, for the first time, explicitly mentions such objects in the European regulatory framework.
Nanostructured MDs can be fabricated using many different processes. In this study, we addressed those MDs that present metallic nanoparticles (NPs) as a constituent, to be integrated in the final product upon proper handling (such as, e.g., in dental cements). Traditional cytotoxicity tests (e.g., Neutral Red, MTT), as per the EN ISO 10993-6 standard, together with studies of ultrastructural cellular pathology, were used in order to provide established references, based on the traditional approach to the evaluation of cytotoxicity presented by medical devices. Those tests were compared to Electrical Cell-substract impedance sensing (ECIS), a label-free, real-time test capable of recording continuously the electrical parameters of a cell layer subject to an external agent. In this case, different concentrations of ZnO NPs were delivered to cell layers of different type (A549, gingival fibroblasts). The utility of the proposed comparison is demonstrated by the evidence about criticalities in traditional cytotoxicity tests involving the use of dyes (e.g., MTT), as underlined by the recently issued ISO/TR 10993-22 Guidance on nanomaterials.
Electrical resistance of the cell-covered electrodes was found to decrease remarkably in the presence of ZnO NPs, especially at the highest concentration (20 μg/cm^2). The time course of the normalized resistance for the 20 μg/cm^2 ZnO was observed to be in striking resemblance to that of the positive control (a known apoptotic agent, STS), suggesting similar mechanisms of cell death, in the very first hours after treatment. ECIS test highlighted an enhanced proliferative activity at lower ZnO NPs concentration (1 μg/cm^2), not clearly evidenced by traditional tests. The comparison of MTT, NR cytotoxicity and cloning efficiency tests together with studies of ultrastructural cellular pathology allowed to clarify the interaction of NP and cell survival/death mechanisms. ECIS test has the potential to recapitulate requirements needed for the evaluation of nanomaterials cytotoxicity.



Acknowledgments: Funded by Regione Lazio - RinnovaReNano - FILAS - RU-2014-1041

The role of the monosialoganglioside-GM1 in the interaction between model membranes and unstructured metastable amyloid oligomers of salmon calcitonin

Cristiano Giordani,[a] Marco Diociaiuti,[b] Laura Zanetti-Polzi,[c] Raoul Fioravanti,[b] Cecilia Bombelli[d]

[a] *Instituto de Física, Universidad de Antioquia, Calle 70 No. 52-21, Medellín, Colombia;* [b] *Centro Nazionale Malattie Rare, Istituto Superiore di Sanità, I-00161 Rome, Italy;* [c] *Dipartimento di Fisica e Scienze Chimiche, Università dell'Aquila, via Vetoio (Coppito 1), 67010 L'Aquila, Italy;* [d] *CNR, Istituto di Metodologie Chimiche, Sezione Meccanismi di Reazione, c/o Dipartimento di Chimica, Università degli Studi di Roma "Sapienza", I-00185 Rome, Italy.*

Email of presenting author: cristiano.giordani@udea.edu.co

To investigate the molecular mechanisms of the interaction between amyloid aggregates and model membranes containing GM1, we applied Circular Dichroism (CD) spectroscopy and Transmission Electron Microscopy (TEM). In particular, we studied the interaction of sCT monomers, prefibrillar oligomers (PFOs), proto- and mature-fibers with liposomes made of DPPC, with and without GM1 and cholesterol. All data indicated that the presence of the negatively charged GM1 favored the interaction with all types of aggregates accelerating the formation of beta-structures. TEM data clearly showed that only PFOs were able to modify the bilayer structures by the formation of "amyloid channels" that were clearly visualized. Their structure was very similar to that proposed by Molecular Dynamics simulations for Abeta. CD data are compatible with this hypothesis. We speculate that the electrostatic interaction occurring between positively charged, native, flexible PFOs with the negatively charged GM1 localized in the outer part of the lipid bilayer, drives the initial binding while the hydrophobic interaction could be responsible for the subsequent incorporation in the membrane leading to the formation of the observed amyloid pores.

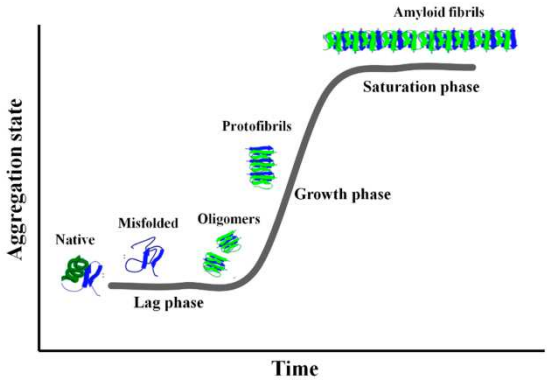


Figure 1. The typical aggregation curve of the amyloid proteins characterized by three zones: Lag-Growth- and Saturation-phases. The relative aggregation states of the protein are also depicted (for the courtesy of Iannuzzi et al. [1]).

1. C. Iannuzzi, G. Irace, I. Sirangelo, *Molecules,* **2015**, *20*, 2510.

Acknowledgments: This work was supported by the Italian "Ministero della Salute" with the "Progetto Ordinario di Ricerca Finalizzata (RF-2013-02355682)" and the research funds of Committee for Research Development from University of Antioquia (CODI, UdeA, Medellin, Colombia) through grant #IN641CE (Act 8700-3278, May 28, 2013).

Development of ursodeoxycholic acid loaded nanostructured lipid carriers (NLC) for the therapy of liver diseases

Anđelka Kovačević, Paola Luciani

Institute of Pharmacy, Faculty of Biological Sciences, Friedrich-Schiller University Jena, Lessingstraße 8, 07743, Jena, Germany.
Email of presenting author: andelka.kovacevic@uni-jena.de

Poor solubility in water and poor dissolution in the gastrointestinal fluids are the limiting factors for the *in vivo* bioavailability of numerous drug candidates administered orally. Ursodeoxycholic acid (UDCA) as a poorly soluble bile acid increasingly used in therapy of cholestatic liver diseases is available on the market in the form of solid dosage forms. However, commercial liquid formulations of UDCA for patients who cannot swallow capsules or tablets, are still missing. The aim of this study is development and characterisation of liquid formulation of UDCA in the form of NLC dispersions based on phospholipids. The potential benefits of phospholipids for treating liver diseases and maintaining liver function have been already well documented in the literature [1]. NLC, the second generation of the lipid nanoparticles have been marked as promising drug delivery systems for poorly water soluble drugs, because they can enhance their apparent solubility and dissolution in the GIT, and/or modulate the drug permeability and fate across the intestinal barrier [2]. In our study, UDCA-loaded NLC dispersions containing phospholipids were prepared by hot high pressure homogenization and physicochemical characterization of the developed carriers was performed. The lipid screening study performed using solid lipids and liquid lipids suitable for oral application indicated poor drug solubility. It was found that the highest amount of UDCA could be dissolved in the liquid lipid Transcutol® HP. In order to obtain solid lipid matrix Transcutol® HP was mixed with Cutina® CP and NLC dispersions with 10% and 20% of the lipid content were prepared. According to the results of particle size analysis average particle size from 182 nm at the day of production to 187 nm at day 7 was found for NLC dispersions with 10% solid lipid content. Increasing solid lipid concentration to 20% resulted in an increase in the particle size up to almost 300 nm. No difference was found in the size distribution of these formulations. PI below 0.25 for both formulations indicated a narrow size distribution favouring good physical stability. Zeta potential for all samples was above -40 mV which is sufficient to provide physical stability over time.
In conclusion, preparation of UDCA loaded NLC dispersions based on phospholipids was feasible using hot high pressure homogenization. Further study will be directed toward safety assessment of the developed carriers.

1. A. Beloqui, A. del Pozo-Rodríguez, A. Isla, A. Rodríguez-Gascon, M.A. Solinís, *J. Drug Deliv. Sci. Tech.,* **2017**, *42*, 144.
2. J.S. Cohn, E. Wat, A. Kamili, S. Tandy, *Curr. Opin. Lipidol.,* **2008**, *19*, 257.

Acknowledgments: Lipoid GmbH is gratefully acknowledged for the endowment to FSU Jena.

Protein binding capacity of nanostructured lipid carriers loaded with *Salvia off.* extract

E. Markova, M. Kostovska, L. Taneska, Lj. Cambuleva, D. Shalabalija, M.G. Dodov, M.S. Crcarevska

Institute of pharmaceutical technology, Center of pharmaceutical nanotechnology, Faculty of pharmacy, Ss. Cyril & Methodius University, Majka Tereza 47, 1000 Skopje, R. Macedonia.
Email of presenting author: elena.markova1@yahoo.com

Having in mind the current options for Alzheimer's disease (AD) treatment and its limitations on the one hand, and advantages and possibilities offered by drug delivery carriers (DDS) on the other hand, nanostructured lipid carriers (NLC) would be potentially efficient in AD treatment. One of the most important factors that influence DDS in vivo faith is their plasma protein binding capacity, critical for formation of protein corona that largely defines their biological identity [1]. Understanding the influence of formulation composition and DDS physico-chemical properties upon affinity for protein corona formation might significantly contribute to development of new innovative functional therapies in AD. NLC loaded with freeze-dried *Salvia off.* methanolic extract (FSE) were formulated using solvent evaporation method previously described [2]. Phospolipon 90H (Phospholipid, Germany) and oleic acid (Sigma-Aldrich, Germany) were used as lipid phase (1:0.43). Ratio of total lipid to ethanol as organic solvent was 1:20, while total lipid to Tween 80 (Merck, Germany) ratio was 1:0.47, 1:0.84 and 1:1.16 for NLC-FSE5, NLC-FSE6 and NLC-FSE 7, respectively. Organic solvent with 0.58% FSE to water phase (0.5% Poloxamer 407, BASF, Germany) ratio was 1:2. Prepared formulations were characterized in terms of particle size and zeta potential (Zetasizer nano ZS, Malvern, UK). Protein binding capacity was determined by protein adsorption studies [3]. Obtained results indicated that by increasing the amount of surfactant (Tween 80) the particle size decreased (from 236±4 to 132±3 nm) probably due to the lower surface tension thus resulting with smaller NLC-FSE. Zeta potential values were in a range of -10±2.1 to -18±2.8 mV. At the same time protein binding capacity increased (from 33.74±3.2 to 50.76±4.1%), most likely due to the larger surface area exposed to the proteins as well as zeta potential values.

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Isolation of nanoparticles from *Brassica oleracea L.* (Broccoli) and study of their effect on the metabolic activity of lung tumor cell lines

Lorena di Toma,[a] Stefano Castellani,[a] Rosanna Tamborra,[a] Vincenzo De Leo,[b] Simona de Nittis,[a] Lucia Catucci,[b] Massimo Conese,[a] Sante Di Gioia [a]

[a] *Department of Medical and Surgical Sciences, University of Foggia, Viale Luigi Pinto 1, 71122, Foggia, Italy;*
[b] *Department of Chemistry, University of Bari "Aldo Moro", Via Orabona, 4, 70126 Bari, Italy.*
Email of presenting author: sante.digioia@unifg.it

Objectives

The use of natural products in medicine is still elusive, partly due to the compounds' low bioavailability. Nanoparticles from plant cells can significantly increase the bioavailability of natural molecules with anti-inflammatory or anti-proliferative properties [1], both *in vitro* and *in vivo*. In this study, we test our hypothesis that Broccoli Derived Nanoparticles (BDNPs) can exert an effect on the metabolic activity, *in vitro,* on lung cancer cell lines.

Methods

BDNPs were isolated from homogenized fresh broccoli (*Brassica oleracea* L.) using a sucrose gradient ultracentrifugation method [2]. Briefly, broccoli extract was sequentially centrifuged and the pellet transferred to a sucrose gradient (8%/30%/45%/60%). After centrifugation, three bands of BDNPs were obtained and the particle size was measured by using Dynamic Light Scattering (DLS). The *in vitro* effect of BDNPs on the metabolic activity of H441 (bronchiolar) and A549 (type II pneumocytes) cell lines, was evaluated by incubating cells with five doses of nanoparticles (2.5, 5, 11, 22, 45 µg/ml). After 24h, MTT assay was performed to test the metabolic activity. To study the cell uptake of BDNPs, H441 cells were incubated with Nile red-labelled BDNPs (11 µg/ml) and evaluated by epifluorescence microscopy after 15, 60 and 180 min.

Results

After ultracentrifugation, BDNPs mainly accumulated at the 8/30% (band 1) and 30/45% (band 2) interfaces of the sucrose gradient while a smaller band was also detected at the 45/60% interface (band 3). The average size measured by DLS was about 191 nm (band 1, BDNP1), 440 nm (band 2, BDNP2), and 984 nm (band 3, BDNP3). BDNP3 were excluded from subsequent study because of their dimensions. The treatment of A549 and H441 cells for 24h suggested that the effects of both BDNP1 and BDNP2 could differ in dependence on cell types. Indeed, BDNP1 and BDNP2, in the range 2.5-22 µg/ml, resulted in an increase of A549 cell proliferation while the higher concentration tested (45 µg/ml) inhibited the cell growth. On the other end, H441 cells were almost insensitive to the treatment with BDNPs. Microscopy revealed that fluorescent punctate spots were observed mainly in the cell cytosol, indicating that BDNPs were taken up by the cells. Moreover, the quantitative analysis of the images, by ImageJ software [3], showed that the cell uptake at 180 min was ~ 3- fold higher than after 15 min of incubation.

Conclusions

In this preliminary *in vitro* study, we have found, for the first time that nanoparticles from broccoli can modify the metabolic activity and hence the growth of two lung tumor cell lines upon their uptake. Further studies should be performed with a broader range of BDNP concentrations and other tumor cell lines. Our findings open to the possibility to find an alternative approach for cancer treatment, focused on using nanoparticles from natural substances.

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Propolis wax-nanostructured lipid carriers for improving oral delivery and cholesterol lowering activity of β-sitosterol

Yasamin Soleimanian,[a] Sayed Amir Hossein Goli,[a] Francesca Maestrelli,[b] Lorenzo Di Cesare Mannelli [c]

[a] *Department of Food Science and Technology, Isfahan University of Technology, 84156-83111, Isfahan, Iran;* [b] *Department of Chemistry, University of Florence, via Schiff 6, 50019 Sesto Fiorentino, Florence, Italy;* [c] *Department of Neuroscience, Psychology, Drug Research and Child Health, Viale Pieraccini 6, 50139, Florence, Italy.*

E mail of presenting author: Y.Soleimanian@ag.iut.ac.ir

Nanostructured lipid carriers (NLCs) were produced for the delivery of β-sitosterol to increase its extremely low water solubility and oral bioavailability. NLCs were prepared via melt emulsification-ultrasonication technique, using functional pomegranate seed oil (as liquid lipid) and mixture of propolis wax and glyceryl behenate (1:1). NLCs exhibited nanosized (105 nm) spherical morphologies with narrow size distribution and high drug entrapment efficiency (97%), sustained drug release pattern, and negative surface charge (zeta potential of -26 mV) that imparts sufficient electrostatic physical stability. Using three step simulated gastrointestinal model, the bioavailability of β-sitosterol was evaluated around 73%. When tested in vivo, β-sitosterol NLCs demonstrated improved reduction in the total and low density lipoprotein (LDL) cholesterol level (Figure 1). This could be attributed to the improved solubility and dissolution of the drug associated with the NLC administration. Overall, NLCs might provide efficient nanodevices for the management of hypercholesterolemia and promising drug delivery systems to enhance β-sitosterol oral bioavailability.

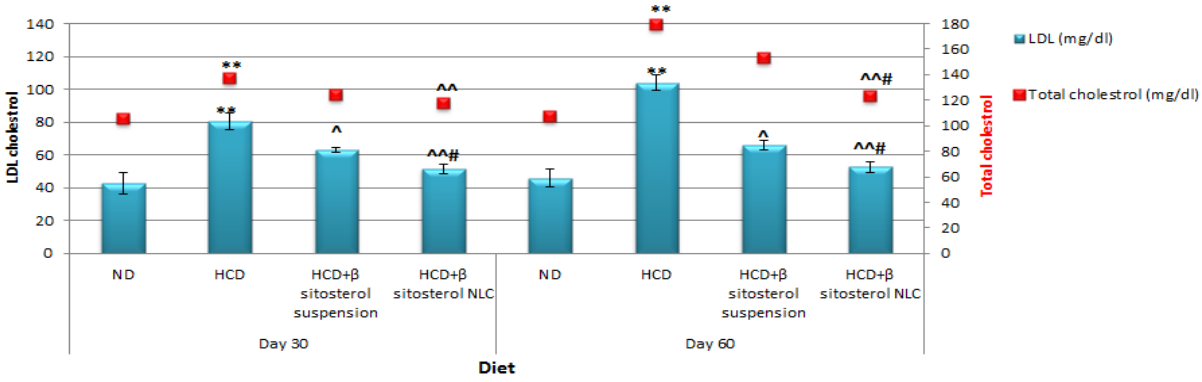

Figure 1. Changes in cholesterol level in mice after administration of Normal diet (ND) and High cholesterol diet (HCD) (Normal diet supplemented with 2 % cholesterol) treated with either vehicle (1% carboxymethylcellulose) or β-sitosterol formulations over 60 days. **P<0.01 vs normal diet + vehicle; ^P<0.05 and ^^P<0.01 vs HCD + vehicle; #P<0.05 vs HCD + β sitosterol.

A.I. Katsarou , A.C. Kaliora, A. Chiou, N. Kalogeropoulos, A. Papalois, G. Agrogiannis, N.K. Andrikopoulos, *European Journal of Nutrition*.,**2016**, *55*, 1283.

Development and stability of liposomes co-encapsulating fisetin and cisplatin

Morgane Renault-Mahieux,[a, b] Muriel Paul,[a] Karine Andrieux [b]

[a]*AP-HP, Henri Mondor Hospital Group, Pharmacy Department, 51 avenue du Maréchal de Lattre de Tassigny, 94010 Créteil, France;* [b]*UTCBS, INSERM U1022 UMR CNRS 8258, Paris Descartes University, 4 avenue de l'observatoire, 75006 Paris, France.*
Email of presenting author: morgane.renaultm@hotmail.com

In order to fight against glioblastoma, the antiangiogenic fisetin and the anticarcinogenic cisplatin were proposed to be co-encapsulated in liposomes, aiming at a synergistic effect.
Liposomes suspensions were prepared using the film-hydration method with various ratios of DOPC, cholesterol and DODA-GLY-PEG2000 and characterized using DLS and TEM. The encapsulation efficiency (EE) and the storage stability were assessed using HPLC for fisetin and GF-AAS for cisplatin.
Two formulations (F3 and F4) encapsulating either cisplatin or fisetin were selected amongst seven, F4 having more cholesterol and less DOPC than F3. They had a size below 200 nm, a polydispersity index below 0.2 and a satisfactory EE. However, fisetin quickly leaked from the liposomes during storage (more than 60% of fisetin release after only 13 days). The release of cisplatin was around 25% for the two formulations.
Double encapsulation of fisetin and cisplatin was then investigated. The particle sizes of F3 and F4 were below 200 nm with a polydispersity index below 0.2. However, TEM highlighted a disruption of the liposomal bilayers explaining that the liposomal suspension was not stable, and that the leaking of the two drugs was higher when co-encapsulated than when alone in the liposomes (above 70% for fisetin and 35% for cisplatin after 10 days) (Figure 1) .

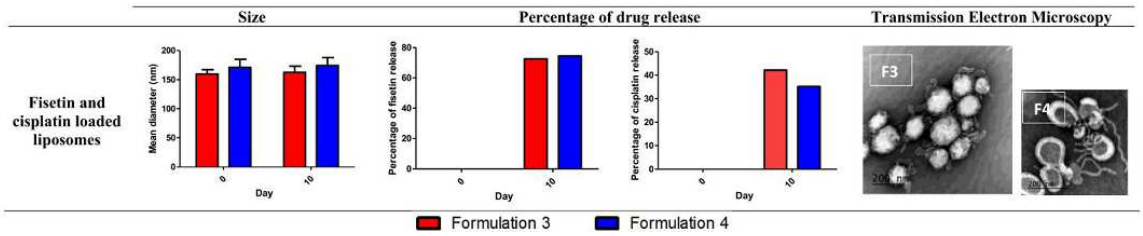

Figure 1. Aspect and stability of the different liposomal suspensions.

In order to increase the stability of the preparations, freeze-drying of empty liposomes was investigated. The protective capacity sucrose and trehalose, at 3 concentrations, 5, 10 and 20%, was investigated on empty liposomes. Only a 20% concentration of lyoprotectant could prevent the macroscopically aggregation of liposomes. Sucrose 20% had the best protective effect concerning size (154.7 ± 1.3nm before freeze-drying and 155.5 ± 2.2nm after rehydration, NS) and PDI (0.094 ± 0.023 before freeze-drying and 0.121 ± 0.019 after rehydration, $p < 0.05$) of empty liposomes but the aspect of the lyophilized cake was crystalline. Addition of adjuvant and water content were optimized to promote best lyophilized cakes and maintain the protective effect.
Further investigation will concern freezing protocol and effect of encapsulation of cisplatin and fisetin on freeze-drying and rehydration processes.

Drug delivery using protein nanocarriers: human or virus templates?

Alessandro Arcovito,[a] Adriana Amalfitano,[a] Francesca Bugli,[b] Pierpaolo Ceci,[c] Gianni Colotti,[c] Elisabetta Falvo,[c] Cecilia Martini,[b] Giuseppina Nocca,[a] Massimiliano Papi,[d] Alberto Vitali [e]

[a] Institute of Biochemistry and Clinical Biochemistry, Catholic University of Sacred Heart, Largo F. Vito 1, 00168, Rome, Italy; [b] Institute of Microbiology, Catholic University of Sacred Heart, Largo F. Vito 1, 00168, Rome, Italy; [c] Institute of Molecular Biology and Pathology, CNR - National Research Council of Italy, Piazzale A. Moro 5, 00185 Rome, Italy; [d] Institute of Physics, Catholic University of Sacred Heart, Largo F. Vito 1, 00168, Rome, Italy; [e] Institute of Chemistry of Molecular Recognition, CNR - National Research Council of Italy, Largo F. Vito 1, 00168, Rome, Italy.
Email of presenting author: alessandro.arcovito@unicatt.it

In the modern view of selective drug delivery of bioactive molecules, the attention is moving onto the setup of the perfect carrier more than in the optimization of the active compound. In this respect, nanoparticles (NPs) are intensively studied for novel therapeutic use and are acquiring a special interest in drug-delivery and regenerative medicine, due to the ability of release an active molecule in a more specific time and spatial window, with respect to the systemic administration of a free drug. A subclass of NPs is made of self-assembling protein subunits. A great advantage of using this last type of polymerizing agents, is the possibility to use at its maximum extent the whole biotechnological support of protein engineering, in order to produce both wild-type and designed mutants of the desired protein, as recombinant constructs of a specific expression vector. Two different proteins have been deeply studied from our group to accomplish this task: VP6, a viral protein from the viral capsid of human rotavirus A and human ferritin [1-5]. Results will be presented and discussed in light of defining the characteristics that a modern drug nanocarrier should have to be effective and safe.

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Intercalation of bioactive molecules into nanosized ZnAl hydrotalcites for combined chemio and photo cancer treatment

Cecilia Martini,[a,b] Claudia Ferroni,[a] Tamara Posati,[a] Marzia Bruna Gariboldi,[c] Morena Nocchetti,[d] Annalisa Aluigi,[a] Giovanna Sotgiu,[a] Paolo Dambruoso,[a] Greta Varchi [a]

[a] CNR-ISOF, Via Gobetti 101, 40129, Bologna; [b] Department of Chemistry, Life Sciences and Environmental Sustainability, University of Parma, Parco Area delle Scienze 17/A, 43124, Parma; [c] Department of Biotechnology and Life Science (DBSV), University of Insubria, Via Ravasi 2, 21100, Varese; [d] Department of Chemistry, University of Perugia, Via Elce di Sotto 8, 06123, Perugia.
Email of presenting author: cecilia.martini@isof.cnr.it; cecilia.martini@studenti.unipr.it

Hydrotalcites (HTlc), also known as anionic clays or layered double hydroxides, represent the only example of lamellar solid with positively charged layers and exchangeable interlayer anions [1]. The present study focuses on the synthesis and characterization of ZnAl HTlc with formula $[Zn_{0,72}Al_{0,28}(OH)_2]Br_{0,28}\bullet 0{,}69H_2O$, as excellent materials for designing drug delivery systems due to their biocompatibility, pH-dependent stability and low toxicity [2]. In this regard, two different molecules were selected for being separately intercalated: the anticancer drug norcantharidin (NCTD), known for inducing cell cycle arrest at G2/M phase, and the tetrasulfonated aluminum phtalocyanine (ftl-Al), a photosensitizer largely used in conventional photodynamic therapy (PDT), that upon near-infrared irradiation, generates reactive oxygen species, ROS, and singlet oxygen, $^1O_2$, inducing cell death. The nanostructured ZnAl-HTlc, were synthesized by the double micro-emulsion technique and characterized in terms of X-ray powder diffraction pattern, thermogravimetric analysis, SEM microscopy, drug release profile and ability to produce ROS and $^1O_2$ upon irradiation. Our results clearly indicate that the two selected compounds are efficiently intercalated within HTlc layers. Moreover, *in vitro* preliminary studies on a panel of cancer cells lines account for a greater cytotoxicity of the two drugs once loaded on HTlc either when administrated singularly or in combination.

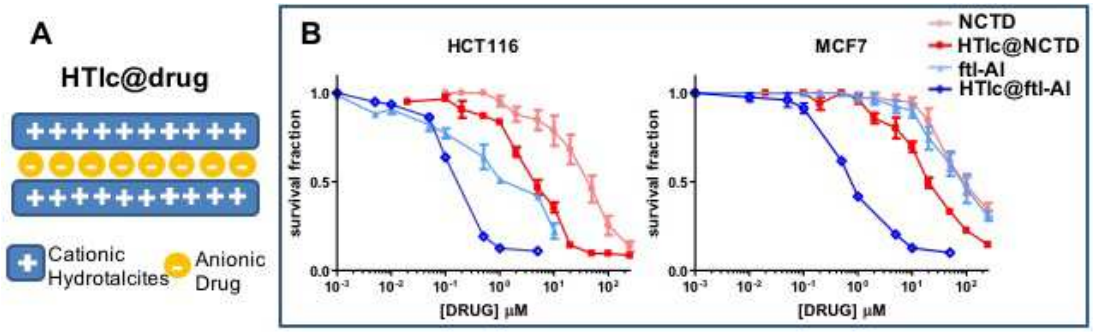

Figure 1. A) Schematic representation of the structure of HTlcs; **B)** *In vitro* cytotoxicity of the tested drugs in different cancer cells lines.

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Nanovectors: how to characterize size and concentration in liquid matrices

Roberto Santoliquido

ALFATEST s.r.l., Via Giulio Pittarelli, 00166, Rome, Italy.
Email: roberto.santoliquido@alfatest.it

Nanomedicine is daily evolving discovering new methods to deliver the drugs and control the bioavailability, the size of the nanovector and its interaction with the biological fluids being key factors for successful drug delivery. This presentation will show some of the latest technologies available to quantify by number the concentration of different kind of nanovectors (viruses, SLN solid lipid nanoparticles, exosomes, polymer nanoparticles or liposomes), monitor the protein corona formation and measure the size distribution to control the purification steps. The technologies based on light scattering phenomena allow the physical characterization of size and concentration while Field Flow Fractionation can increase dramatically the resolution to provide accurate information about the physical state of the samples.

Eumelanin-based substrates as smart materials for neuronal regeneration

Maria Grazia Raucci,[a] Ines Fasolino,[a] Irene Bonadies,[a] Alessandra Soriente,[a] Cosimo Carfagna,[a] Alessandro Pezzella,[b] Luigi Ambrosio [a]

[a] *Institute of Polymers, Composites and Biomaterials (IPCB) - CNR, Viale J.F. Kennendy 54 – Mostra d'Oltremare Pad.20, 80125 Naples (Italy);* [b] *Department of Chemical Sciences, University of Naples "Federico II", Via Cintia 4, I-80126 Naples (Italy).*
Email of presenting author: mariagrazia.raucci@cnr.it

The regeneration of neurite network constitutes a strategy for the treatment of neurodegenerative disorders characterized by a loss and dysfunction of trophic factors such as BDNF and NGF. The fabrication of eumelanin-coated microfibrous structures represents a novel strategy in order to realize tissue-engineering scaffolds for neuronal cells growth and control by providing both mechanical support for growing cells and biological signals to direct the axonal growth cone to the distal stump. Our results show that eumelanin coatings (random and aligned fibres distribution), eliminating the need of differentiating factors in the media, expand the scope of substrate driven cell culture growth and maturation. Indeed, biological results showed that both random and aligned eumelanin microfibers support cell survival and adhesion. Furthermore, eumelanin random microfibers induced the formation network of neuritic processes and stimulated GAP-43 expression over culture time, thus confirming differentiation processes.

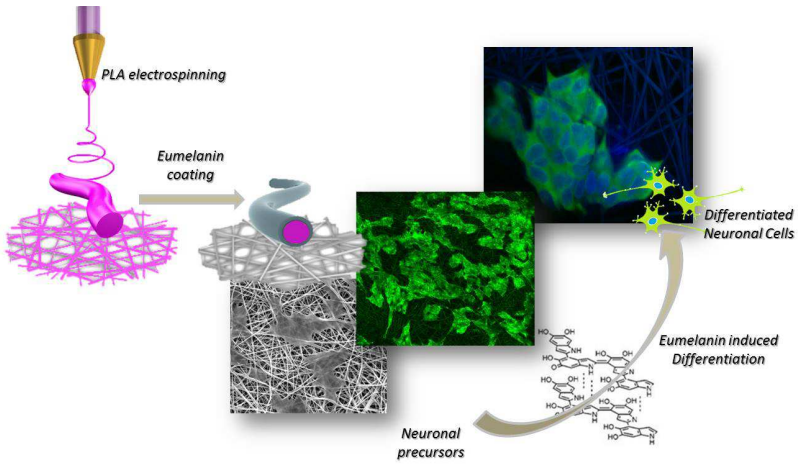

Figure 1. Scheme of Eumelanin-based substrates preparation for neuronal regeneration

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The role of nanocarrier physicochemical properties on the biodistribution and the blood brain barrier passage

Laura Talamini, Elisa Zanier, Mario Salmona, Paolo Bigini

IRCCS - Istituto di Ricerche Farmacologiche "Mario Negri", Milano, Italy.
Email of presenting author: laura.talamini@marionegri.it

Nanocarriers (NC) are a big hope and, at the same time, a hard challenge for the medicine of the XXI century. Ever since the discovery of liposomal formulation to reduce the anti-cancer compounds toxicity, many efforts have been carried out, however the clinical relapses have been poor and controversial. One of the major hurdles was the extreme heterogeneity of preclinical studies, both in terms of materials and biological tools. In this context, the passage of NCs through the blood brain barrier (BBB) remains far from being elucidated. Although different studies seem to correlate the NC physicochemical features and the rate of brain accumulation, nowadays, reliable rules are strongly needed for successful clinical translation. Our study was therefore aimed at understanding IF and HOW NC parameters (such as size, shape and surface functionalization) may actually influence the BBB passage.

Through a step-by-step approach, we measured the amount of nano-material in the mouse brains after systemic administration using a measurable and repeatable technique. To minimize biological variables, healthy, immunocompetent mice were used and housed in specific pathogen free conditions.

Firstly, the role of the size was assessed. LPS-free spherical nanogolds (NGs) having two sizes (100 and 5 nm) were intravenously injected. Mice were sacrificed at 1 hour, 1 day and 5 days after the treatment and the percentage of injected dose (ID%) was calculated in the brain and other peripheral organs. In parallel, the role of the shape was investigated following the same procedure described above.

Our work suggests that the tissue distribution of NGs is size- and shape- dependent but the brain tropism is not affected by this.

Subsequently, the effect of Apoε coating and its specific architecture on 100 nm silica NCs was investigated. Quite surprisingly, neither the presence of the peptide nor its orientation improved the BBB passage.

Next, we moved to consider the animal model of traumatic brain injury (TBI), and we treated them with Apoε silica NCs. Expectedly, the data achieved pinpointed that the levels of silica in the traumatized hemisphere was manifold higher than those measured in controlateral side. Quite interestingly, similarly to the results obtained from healthy mice, no influence of the surface functionalization was observed.

The last experiment was carried out treating healthy mice with sugar-coated silica NCs (20 and 5 nm). Whilst, similarly to NGs, we did not find a robust size-dependent effect, we demonstrated that the presence of sugar significantly improved the BBB passage of 5 nm NCs exclusively.

Although preliminary, this study can be considered a reliable attempt to provide a correlation between the geometry, the nature of the material, the type of ligand linked to NCs and the BBB passage in both undamaged and injured brain. Ex vivo analyses on brain sections are in progress to evaluate the overall distribution of NCs in brain parenchyma and their cellular tropism.

Radiation-induced targeted nanoparticle based gene delivery for brain tumors

Gülşah Erel,[a,b,c] A. Gülten Kantarci,[c] Bakhos A. Tannous [a]

[a] *Experimental Therapeutics and Molecular Imaging Lab, Department of Neurology, Neuro-Oncology Division, Massachusetts General Hospital, 02129, Boston, MA, USA;* [b] *Department of Pharmaceutical Biotechnology, Faculty of Pharmacy, Izmir Katip Celebi University, 35620, Izmir, Turkey;* [c] *Department of Pharmaceutical Biotechnology, Faculty of Pharmacy, Ege University, 35100, Izmir, Turkey.*
Email of presenting author: gulsah.erel.akbaba@ikc.edu.tr

Gliomas are the most common group of primary brain tumors. Despite the therapeutic approaches, median survival for glioblastoma is only 14 months. The development of an effective and innovative treatment in the presence of blood-brain barrier is challenging and emerging issue. Hence, tumor penetrating peptides conjugating delivery systems give promising results for cancer therapy. Furthermore, radiation was shown to enhance nanoparticle uptake by tumor, recently [1]. Here, we propose a novel and efficient method to develop tumor-specifically targeted nanoparticles to deliver the immune checkpoint inhibiting and tumor suppressing siRNAs in combination with radiation therapy against glioblastoma, for the first time.
In this study, the DSPE-PEG-DBCO containing functional solid lipid nanoparticles (f(SLN)) were obtained by microemulsion dilution method. The iRGD peptide which exhibits high affinity to integrin $\alpha_v\beta_3$ was bound to the outer surface of nanoparticles by click chemistry and f(SLN)-iRGD was formed. The-iRGD dependent targeted ability of nanoparticles was demonstrated *in vitro* and *in vivo*. Finally, *in vivo* studies which were carried on glioblastoma xenograft model with GL261-FLuc cells on C57BL/6 mice have shown that the developed siRNA-EGFR and siRNA-PD-L1-carrying targetable nanoparticles extends survival significantly. Moreover, combining this therapy with low dose radiation contributes the survival ($p < 0.05$).

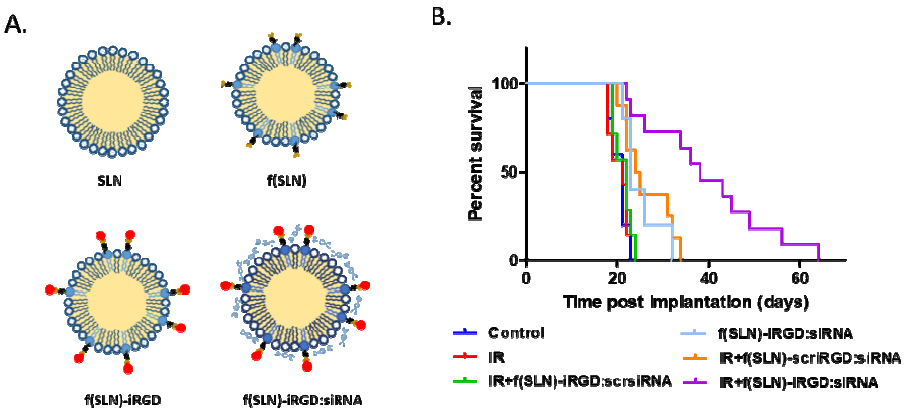

Figure 1. Targeted Therapy of Glioblastoma Cells following the Systemic Delivery of f(SLN)-iRGD:siRNA complexes combining with radiation. A. Schematic overview of SLN, f(SLNP), f(SLN)-iRGD and f(SLN)-iRGD:siRNA. B. Kaplan–Meier survival curves for specified groups.

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VCAM-1 targeted paramagnetic micelles for Magnetic Resonance Imaging of neuroinflammation

Francesca Garello,[a] Amerigo Pagoto,[a] Francesca Arena,[a] Annalisa Buffo,[b,c] Francesco Blasi, [a] Diego Alberti, [a] Enzo Terreno [a]

[a] *Department of Molecular Biotechnology and Health Sciences, University of Torino, Via Nizza 52, 10126, Torino, Italy;* [b] *Department of Neuroscience Rita Levi-Montalcini, University of Torino, 10126, Torino, Italy;* [c]*Neuroscience Institute Cavalieri Ottolenghi, Regione Gonzole 10, 10043, Orbassano, Italy.*
E-mail of presenting author: francesca.garello@unito.it

The need for specialized non invasive imaging techniques is increasing in order to better clarify the role and the spatio-temporal correlation between neuroinflammation and the onset of neurodegenerative diseases [1]. Magnetic Resonance Imaging (MRI) with its remarkable spatial resolution and poor toxicity could be the technique of choice. In the herein reported work paramagnetic micelles targeting Vascular Cell Adhesion Molecule-1 (VCAM-1), over-expressed in case of inflammation, were designed and *in vivo* tested in a model of neuroinflammation. The cyclic nonapeptide CNNSKSHTC [2], able to bind VCAM-1 with high specificity, was synthesized and conjugated to DSPE-PEG2000 to formulate a lipid-based nanosystem including Gd-DOTAMA$(C18)_2$ and Rhodamine-DOPE. The scrambled version HSCNKNSCT was tested as control. VCAM-1 targeted micelles (size ca. 20 nm) showed a longitudinal relaxivity of 35 $s^{-1}mM_{Gd}^{-1}$ at around 1T. Targeted micelles were first tested *in vitro,* on brain endothelial cells, displaying higher affinity towards VCAM-1 expressing cells in comparison to the untargeted nanosystem. Subsequently targeted micelles were *iv* injected in mice (n=20) bearing LPS induced neuroinflammation. The inflamed region was clearly identified and detected only after targeted micelle administration, with a MRI T1 signal enhancement calculated over pre images of 39.3 ± 4.4 %, 24 h *p.i.* Whereas scrambled micelles and the clinical agent MultiHance® showed a comparable T1 SE in the inflamed region, probably due to passive extravasation (18.9 % and 13.4 % respectively). Histological studies proved micelle extravasation at the lesion site. In conclusion, the developed probe allowed for the early detection of the disease, with higher contrast and more precise localization in comparison to untargeted micelles or to the clinical agent MultiHance®. Moreover, the relatively long blood half-life of the nanosystem (ca. 6.3 h) guaranteed a good accumulation in the inflamed regions, paving the way to future diagnostic/theranostic applications, implying the loading of neuroprotective or even anti-cancer drugs inside the core of the micelles.

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Reactive oxygen species-responsive nano-in-micro composite for targeted therapy of inflammatory bowel disease

Serena Bertoni,[a,b] Wei Li,[a] Zehua Liu,[a] Alexandra Correia,[a] João Pedro Martins,[a] Antti Rahikkala,[a] Flavia Fontana,[a] Marianna Kemell,[c] Beatrice Albertini,[b] Nadia Passerini,[b] Hélder A. Santos [a]

[a] *Drug Research Program, Division of Pharmaceutical Chemistry and Technology, Faculty of Pharmacy, University of Helsinki, 00014, Helsinki, Finland;* [b] *PharmTechLab, Department of Pharmacy and Biotechnology, University of Bologna, via San Donato 19/2, 40127, Bologna, Italy;* [c] *Laboratory of Inorganic Chemistry, Department of Chemistry, University of Helsinki, 00014, Helsinki, Finland.*
Email of presenting author: serena.bertoni4@unibo.it

Oxidative stress and abnormally high levels of reactive oxygen species play an essential role in the pathogenesis and progression of inflammatory bowel disease (IBD) [1]. In this study we designed a nanoplatform for the encapsulation and "on demand" delivery of rifaximin (RIF) at sites of oxidative stress. Nanoparticles (NPs) were formulated from a phenylboronic esters-modified dextran (OxiDEX), that degrades selectively in response to hydrogen peroxide (H_2O_2) [2]. OxiDEX NPs with and without a chitosan (CS) coating were then encapsulated by microfluidics in a pH-sensitive polymer to produce nano-in-micro composites called NPs@MF and CS-NPs@MF, respectively (Figure 1b). The final composites were spherical with homogeneous particle size (53±3 µm) and maintained integrity at acidic pH, preventing the premature release of the NPs in simulated gastric conditions. The degradation of NPs was highly responsive to the level of H_2O_2, and the release of the loaded drug was sustained in the presence of physiologically relevant H_2O_2 concentrations (Figure 1a). The presence of CS on the particles surface significantly enhanced NPs stability in intestinal pH and the interactions between NPs and cells. Compared to a traditional enteric formulation, the composites showed ten-fold decreased drug permeability across intestinal cell monolayer, representing an advantage in terms of unspecific absorption and systemic side effects. Based on the results, the developed nano-in-micro composite has great potential for selective drug delivery in IBD treatment.

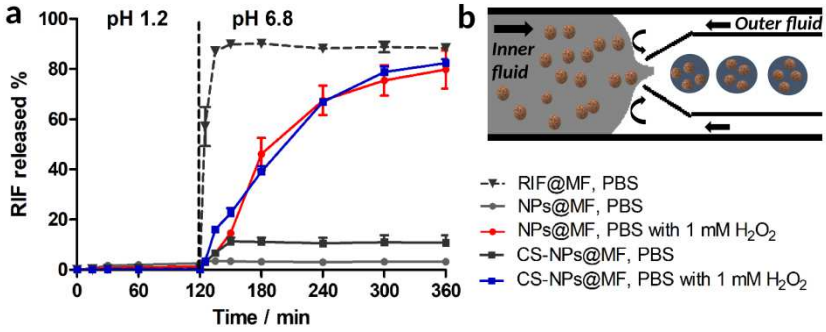

Figure 1. (a) Drug release profiles of NPs@MF and CS-NPs@MF and reference formulation consisted in pure drug encapsulated in the same enteric polymer (RIF@MF). (b) Schematic illustration of the fabrication process of the nano-in-micro composites by microfluidics.

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Redox responsive polymeric nanocarriers for the combined therapy of lung cancer

Claudia Conte,[a,b] Francesca Ungaro,[a] Fabiana Quaglia,[a] Snjezana Stolnik,[b] Maria Kavallaris,[c] Cameron Alexander [b]

[a] *Department of Pharmacy, University of Napoli Federico II, Via D. Montesano 49, 80131, Napoli, Italy;* [b] *MTF Division, School of Pharmacy, University of Nottingham, NG7 2RD, Nottingham, UK;* [c] *Children's Cancer Institute, C25/9 High St, Kensington, NSW 2750, Australia.*
Email of presenting author: claudia.conte@unina.it

Redox-responsive nanoparticles (RR-NPs) are of interest for anticancer nanomedicines, owing to the possibility to 'design in' selective modulation of drug release at target sites [1,2]. With this in mind, herein we have developed new RR-NPs based on polyethylene glycol (PEG)-poly(lactic-co-glycolic acid) (PLGA) block copolymers and novel poly (β-aminoesters) (PβAE) containing bioreducible disulfide units for the *in situ* delivery of combination drugs. In particular, we have focused our attention on docetaxel (DTX) and TUBB3-siRNA, in order to have a synergistic effect in the treatment of lung cancer. Spherical NPs of around 150 nm with negative zeta potential and high loading efficiencies of both drugs were obtained. NPs showed high stability in the most relevant simulated biological fluids whereas disassembly of NP structure was shown at a simulated intracellular level of reducing agents, thus triggering drug release. Unloaded NPs were well-tolerated by lung cancer cells, while in terms of intracellular siRNA delivery, demonstrated a fast and high gene-silencing efficiency. Uptake and intracellular trafficking of NPs showed a high internalization of NPs and no co-localization of the siRNA with the lysosomes, thus suggesting that NPs were able to escape to the reducing cytosolic regions. Finally, viability of cells treated with combined DTX/TUBB3-siRNA-NPs remarkably decreased as compared to NPs loaded only with DTX, thus showing an efficient synergistic anticancer effect. In fact, through immunofluorescence microscopy, significant reduction of class III β-tubulin expression was found, thus potentiating DTX activity. Taken together, these results demonstrate that PLGA/PβAE RR-NPs represent a promising new therapeutic approach with great potential for the dual siRNA/drug therapy of lung cancer.

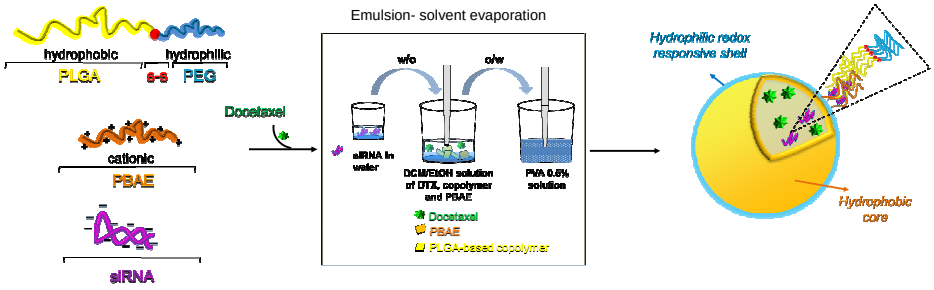

Figure 1. Schematic illustration of NP preparation.

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Study of membrane phase transition in bacterial vesicles

Angelo Sarra,[a] S. Sennato,[b] A. Celluzzi,[c] A. Masotti,[c] C. Ricci,[d] M.G. Ortore,[d] F. Bruni,[a] F. Bordi,[b] P. Postorino [b]

[a] Department of Science, University of Roma Tre, Via della Vasca Navale, 00146, Rome, Italy; [b] Department of Physics, Sapienza University of Rome, Pz.le A.Moro, 00185, Rome, Italy;[c] Gene Expression Microarrays Laboratory, Bambino Gesù Children's Hospital of Rome, Via di S.Paolo, 00146, Rome, Italy; [d] SAIFET department, Polytechnic University of Marche, Via B.Bianche, 60131, Ancona, Italy.
Email of presenting author: angelo.sarra@uniroma3.it

In the last few years, it has been discovered that the majority of eukaryotic and prokaryotic cells release small lipid vesicles. These vesicles, generally called micro-vesicles or exosomes, are becoming the object of a great scientific interest, because of their role as carrier of information about progenitor cells, through their cargo of proteins and nucleic acids and the biochemical composition of their membrane [1]. Here we present a study of the temperature-induced phase transitions in the membrane of bacterial Outer-Membrane-Vesicles (OMVs). In particular, we study the OMVs produced by Escherichia Coli because they are considered a model system for biological studies. Indeed, as known from the literature [2], Escherichia Coli membrane presents different temperature phase transitions due to structural changes in the organization of the lipid bilayer. In our study, we have studied the phase transition behaviour of the OMVs membrane by Light Scattering measurements at varying temperature [3]. OMVs were characterized by the presence of phase transitions similar to those revealed for E. Coli membrane but occurring at slightly different temperatures. In order to better understand the membrane behaviour at the transition temperature, SAXS measurements have been made and will be analysed in the next future.

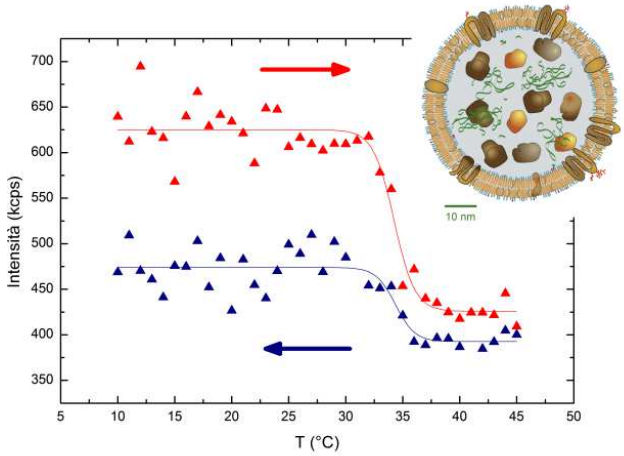

Figure 1: Light scattered intensity as function of temperature. For both increasing (red) and decreasing (blue) temperature it is clear that a membrane phase transition occurs.

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Induction of apoptosis in the lung cancer cells using ultrasound-sensitive nanobubble formulations containing combination of survivin-siRNA and paclitaxel

Hasan Akbaba,[a] Gülşah Erel,[b] Yücel Başpınar [a]

[a]*Department of Pharmaceutical Biotechnology, University of Ege, 35100, İzmir, Turkey;* [b]*Department of Pharmaceutical Biotechnology, University of İzmir Katip Çelebi, 35620, İzmir, Turkey.*
Email of presenting author: hasan.akbaba@ege.edu.tr

Lung cancer remains 23 % of cancer-related death worldwide, ranking on the first place for men and the second place for women. Almost each cancer type has a great deal in common, an overexpression of the apoptosis inhibitor survivin [1].
The aim of this research is to develop ultrasound sensitive nanobubble (NB) formulations containing paclitaxel and survivin-siRNA, and investigate their effects on lung cancer. Nanobubbles as gene delivery vectors were prepared by using phosphatidylcholine, water, perfluorocarbon, chitosan, ethanol and palmitic acid. Gel retardation assay was performed to evaluate the complex formation ability and protection capability of NBs against serum nucleases. Particle size, polydispersity index (PDI), and zeta potential of NB formulations were measured by DLS method. The viability of cells was determined by colorimetric XTT cell proliferation assay prior to the transfection studies. Gene silencing efficiency and Apoptosis was evaluated by qPCR and Apoptosis kits.
Particle size was measured as 206,8 nm for NB formulations. Furthermore, NB formulations have the positive electrical charge (43,9 mV) in order to be used for siRNA complexation and able to protect survivin-siRNA against serum nucleases. It was found that cell viability is reduced in relative order against increasing dose of NB formulation and no sufficient toxicity was observed for the required transfection doses. Gene silencing and apoptosis studies w/wo ultrasound showed that NB formulations were able to silence survivin expression and induce apoptosis in A549 cell line.

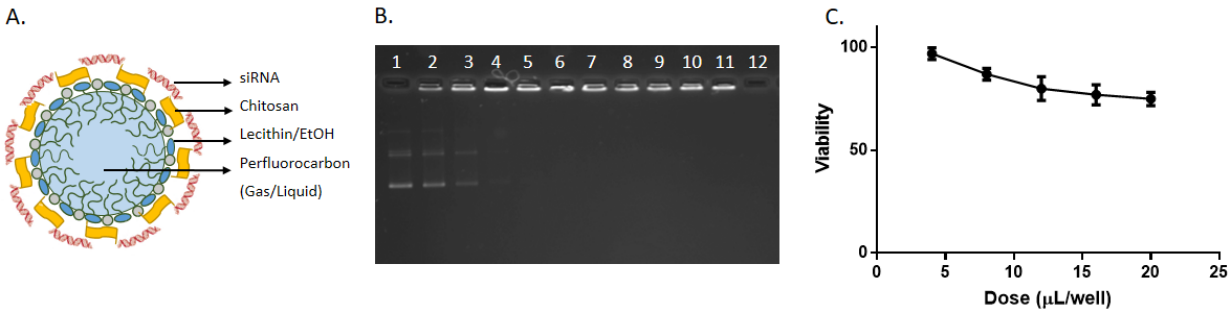

Figure 1. A. Schematic illustration of synthesized NB formulation, B. Gel retardation assay and agarose gel electrophoresis image, C. Viability percentages of A549 cells treated with NB formulations.

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Functionalized superparamagnetic iron oxide nanoparticles as theranostic devices: from development to interaction with proteins

Irene Russo Krauss,[a,b] Alessandra Picariello,[a] Alessandra Luchini,[b] Luigi Paduano [a,b]

[a] *Department of Chemical Science, University of Naples Federico II, Via Cinthia, I-80126, Naples, Italy;* [b] *CSGI, Via Lastruccia, I-50019, Florence, Italy.*
Email of presenting author: irene.russokrauss@unina.it

Metal-based nanoparticles (NPs) have attracted great interest in the last years for different uses in biomedical field, including diagnostic, drug delivery and therapeutic applications [1]. Among metal-based nanoparticles, iron oxide nanoparticles with a radius less than 20 nm are very promising for the design of diagnostic and theranostic devices, because they exhibit a strong magnetic response to an applied external magnetic field, which allow their use as contrast agents, and are inherently poorly toxic [2]. However, in order to be safely employed *in vivo*, NPs need to be water soluble, biocompatible and non toxic. The first two targets can be achieved through a proper coating of NP surface. In this respect we have developed small Fe_3O_4 NPs coated with a double layer of oleic acid/oleylamine and 1-octadecyl-2-hydroxy-sn-glycero-3-phosphocholine (18LPC) [3], which can be further functionalized employing drugs [4] or different targeting or diagnostic agents bearing a proper hydrophobic tail. The possible toxicity of the NPs is a far serious issue. Apart from organ/tissue accumulation and removal of NPs, the first event to take in account is the interaction of the NP with proteins, because interaction between NPs and proteins can cause toxic effects by altering, unfolding or inducing aggregation of proteins [5], but also change NP properties and functionalities, through extensive covering of nanoparticle surface by proteins. In this respect, NP behaviour strongly depends on their chemical nature and geometrical properties (dimensions, curvature of the surface and so on) [6] as well as on their coating. With the aim at fully developing our nanosystems for biomedical applications, we have investigated their interaction with two abundant serum proteins, human apo transferrin (HTF) and human serum albumin (HSA), by means of spectroscopic and scattering techniques. We found that interaction with NPs does not cause the unfolding of the investigated proteins and has only limited effects on their tertiary structure. In the case of HSA a protein layer on the NP surface forms. None of these effects is observed with control 18LPC solutions, suggesting that they depend on the geometrical properties of the NP and on protein structural features. Our results confirm the high biocompatibility of 18LPC as coating molecule for development of biomedical NPs.

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Influence of the surface properties of nanoliposomes on protein corona formation

Dushko Shalabalija,[a] Ljubica Cambuleva,[a] Ivana C. Karanfilova,[b] Maja S. Crcarevska,[a] Krtistina Mladenovska, [a] Vladimir Ivanovski, [c] Marija Glavas Dodov[a]

[a] *Institute of Pharmaceutical Technology, Faculty of Pharmacy, University Ss. Cyril and Methodius, Majka Tereza 47, 1000, Skopje, R. Macedonia;* [b] *Institute of Pharmacognosy, Faculty of Pharmacy, University Ss. Cyril and Methodius, Majka Tereza 47, 1000, Skopje, R. Macedonia;* [c] *Department of Chemistry, Faculty of Natural Science and Mathematics, University Ss. Cyril and Methodius, Arhimedova, 1000, Skopje, R. Macedonia.*
Email of presenting author: d_salabalija@hotmail.com

One of the biggest problems upon nanoparticles' administration *in vivo* is their interaction with blood proteins and the formation of the protein corona (PC), followed by the rapid recognition and uptake of the particles by the mononuclear phagocyte cells, thus leading to quick removal from the circulation. Therefore, understanding of the particle–PC complex formation is a prerequisite for successful development of drug carrier system and proper characterization is essential.
This problem could be partially overcome by the decoration of the particles' surface with polyethylene glycol (PEG) [1]. In order to investigate the effect of PEG on the PC formation, as well as to characterize the interaction, two formulations of nanoliposomes (NLs) (lechitin:cholesterol:PEG = 8.7:1:1.7 and 9:1:0.17 for *sample 1* and *2*, respectively) loaded with rosmarinic extract (RE) were prepared by the modified lipid film hydration technique [2]. Blank samples were prepared for comparison. The mean size of the prepared NLs was ~120 nm, with unimodal narrow size distribution and high encapsulation efficiency (~90%). *In vitro* release studies showed that the prepared vesicles had prolonged drug release properties (26 and 46% for 24h, for *sample 1* and *2*, respectively). The slower release of RE from *sample 1* could be probably due to the fast hydration process of the higher amount of PEG present on the surface of the vesicles. NLs–PC complex formation was confirmed using SDS-PAGE. Our previous investigations using Bratford assay confirmed that the PEG increases the hydrophilicity of the NLs surface thus resulting in reduced PC formation. These results were confirmed using FTIR and quantitative information of protein–NLs interactions was gained. From the obtained spectra it could be concluded that NLS-protein interaction was mainly due to the hydrogen bonds formed between C = O...N-H or C = O...H-OH. These interactions were stronger between the NLs prepared with lower PEG amount and the protein. Incorporation of RE into the NLs did not affect the intensity of the interactions.

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Preparation of Synthetic Low Density Lipoprotein (sLDL) with an *on-Surface* Chemoseletive Ligation

Alessandro Fracassi,[a] Sean Orian,[a] Naoko Yoshizawa-Sugata,[b] Hisao Masai,[b] Yoko Yamakoshi [a]

[a] *Laboratorium für Organische Chemie, ETH Zürich, Vladimir-Prelog-Weg 3, 8093 Zürich, Switzerland;* [b] *Tokyo Metropolitan Institute of Medical Sciences, Kamikitazawa 2-1-6, Setagaya, Tokyo 156-8506, Japan.*
Email of presenting author: afracassi@org.chem.ethz.ch

Low density lipoprotein (LDL) has been used as a powerful carrier for the targeted delivery of drugs and imaging probes [1,2,3]. Since natural LDL (nLDL) suffers from disadvantages such as limited supply and deleterious side effects, the development of synthetic LDL (sLDL) is an attractive alternative. In this study, we report a versatile method for the chemical functionalization of sLDL using a chemoselective ligation reaction of potassium acryltifluoroborate (KAT) with *N*-hydroxylamine (HA), which allows rapid amide bond formation under dilute aqueous conditions with equimolar amounts of ligation partner, in the presence of unprotected functional groups [4]. The sLDL was prepared by mixing a KAT derivative of oleic acid (OA-KAT, 5% mol) with the lipidic contents used in the preparation of previously reported sLDL [5] (phosphatidyl choline (PC), triolein (TO), and cholesterol oleate (CO) in 3:2:1 mol ratio). All starting materials were solubilized in a mixture of $CHCl_3$ - MeOH - acetone (2:1:1) and concentrated to provide a uniform lipid film, which was subsequently hydrated with Tris-HCl buffer (pH 8.0), sonicated and extruded through membranes (with pore size of 50 and 100 nm) to give lipidic nanoparticles (NP) with KAT moiety (NP-KAT). The obtained NP-KAT was well-dispersed, with particle diameters of 60-75 nm by DLS. Separately, HA derivatives of apoB100-mimetic peptide **1** (HA-GTT*RLTRKRGLK*LA) and fluorescein **2** were synthesized and purified by HPLC. The "on-particle" KAT ligation of NP-KAT with both **1** and **2** was carried out in NH_4Cl saturated solution (pH 5.5) to provide fluorescent sLDL. Using a macrophage cell line RAW 264.7, sLDL was shown to accumulate in cells, as observed by fluorescent microscopy, demonstrating the potential of sLDL as a carrier.

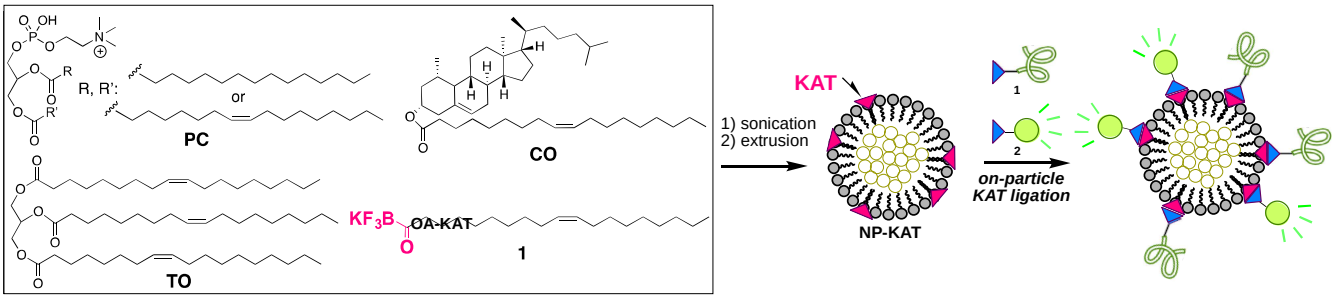

Figure. NP-KAT preparation and subsequent surface modification by KAT ligation with HA derivatives of apoB100-mimetic peptide **1** and fluorescein **2**.

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Cerium oxide nanoparticles as potential antibiotic adjuvant. Effects of CeO_2 nanoparticles on bacterial outer membrane permeability

Pierangelo Bellio,[a] Carla Luzi,[a] Alisia Mancini,[a] Salvatore Cracchiolo,[a] Maurizio Passacantando,[b] Letizia Di Pietro,[a] Mariagrazia Perilli,[a] Gianfranco Amicosante,[a] Sandro Santucci,[b] Giuseppe Celenza [a]

[a]Department of Biotechnological and Applied Clinical Sciences, University of l'Aquila, via Vetoio, 67100, l'Aquila, Italy; [b]Department of Physical and Chemical Sciences, University of L'Aquila, via Vetoio, 67100, l'Aquila, Italy. Email of presenting author: giuseppe.celenza@univaq.it

Background: Therapeutic options against Multi Drug Resistant (MDR) pathogens are limited and the overall strategy would be the development of adjuvants able to enhance the activity of available antibiotics. Unspecific outer membrane permeabilizer, like metal-oxide nanoparticles, can be used to increase the activity of antibiotics in drug-resistant pathogens. The study aims to investigate the effect of cerium oxide nanoparticles (CeO_2 NPs) on bacterial outer membrane permeability and their application in increasing the antibacterial activity of antibiotics in MDR pathogens (Figure 1).

Methods: The ability of CeO_2 NPs to permeabilize Gram-negative bacterial outer membrane was investigated by calcein-loaded liposomes. The extent of the damage was evaluated using lipid vesicles loaded with FITC-dextran probes. The effect on bacterial outer membrane was evaluated by measuring the coefficient of permeability at increasing concentrations of CeO_2 NPs. The interaction between CeO_2 NPs and beta-lactams was evaluated by chequerboard assay against a *Klebsiella pneumoniae* clinical isolate expressing high levels of resistance against those antibiotics.

Results: Calcein leakage increases as NPs concentrations increase while no leakage was observed in FITC-dextran loaded liposomes. In *Escherichia coli* the outer membrane permeability coefficient increases in presence of CeO_2 NPs. The antibacterial activity of beta-lactam antibiotics against *K. pneumoniae* was enhanced when combined with NPs.

Conclusions: CeO_2 NPs increases the effectiveness of antimicrobials which activity is compromised by drug resistance mechanisms. The synergistic effect is the result of the interaction of NPs with the bacterial outer membrane. The low toxicity of CeO_2 NPs makes them attractive as antibiotic adjuvants against MDR pathogens.

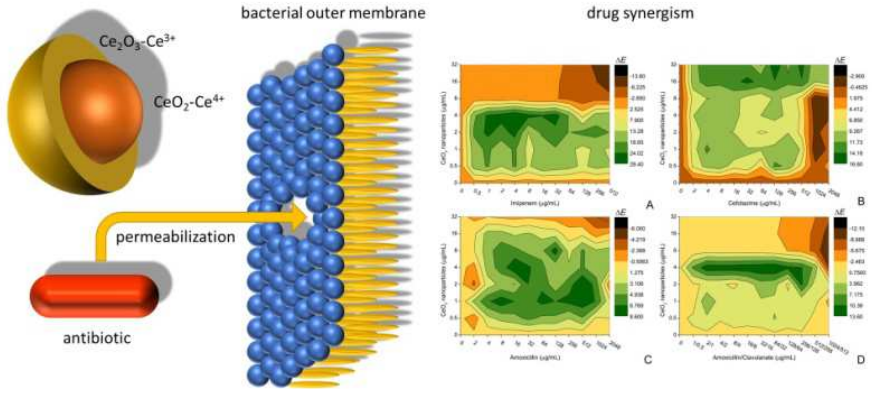

Figure 1. CeO_2 NPs increase the permeable area allowing the passive diffusion of antibiotics, but not disruption of the membrane. CeO_2 NPs exert synergistic action enhancing the activity of β-lactam antibiotics in *Klebsiella pneumoniae*.

Innovative nanofabrication methodologies for the preparation of drug delivery systems

Laura Chronopoulou,[a] Antonio DI Nitto,[a] Adriana Amalfitano,[b] Giuseppina Nocca,[b] Alessandro Arcovito,[b] Ida Silvestri,[c] Fabio Dominici,[d] Sabrina Giantulli,[c] Francesco Brasili,[d] Cleofe Palocci [a]

[a] *Department of Chemistry, Sapienza University of Rome, P.le A. Moro 5, 00185, Rome, Italy;* [b] *Biochemistry and Clinical Biochemistry Institute, Catholic University Sacro Cuore, L.go F. Vito 1, 00168, Rome, Italy;* [c] *Department of Molecular Medicine, Sapienza University of Rome, V.le Regina Elena 324, 00161, Rome, Italy;* [d] *Department of Chemical Science and Technology, University of Rome Tor Vergata, V.le della ricerca scientifica 1, 00133, Rome, Italy.*

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

In recent years, novel nanofabrication approaches have attracted the growing interest of researchers in the biomedical field. Nanomaterials are highly versatile tools that can interact with cells in general, including bacteria, animal and plant cells. When used as drug carriers, NPs can afford improved circulation and biodistribution, in addition to high drug loading and controlled release rates, as well as protection from degradation that may occur both *in vitro* and *in vivo*. Biopolymers have been extensively explored in recent years for biomedical applications thanks to their properties such as biocompatibility and biodegradability.

Our research group has been designing biopolymeric nanovectors for the controlled delivery of bioactive molecules by using different nanoprecipitation approaches. Conventional methods such as ionotropic gelation have been used for the development of positively charged chitosan-based NPs for the delivery of nucleic acids (plasmid DNA, siRNA) or antibiotics for bacterial biofilm management [1]. Moreover, we have patented a methodology based on the use of semipermeable membranes to fabricate polymeric NPs by exploiting their solution properties at the interface in different media. Size modulation can be obtained by changing experimental conditions such as temperature, polymer concentration and solvent polarity. The NPs obtained are free of contaminants such as surfactants, initiator residues, and their decomposition products. Moreover, this methodology allows to prepare in one step drug-loaded NPs. We used this methodology for the production of dexamethasone (DXM)-loaded biopolymeric NPs that were able to induce human gingival fibroblasts to acquire an osteogenic phenotype [2]. Also, we developed stable poly-(lactic-co-glycolic) acid (PLGA) NPs loaded with doxorubicin (DOX). We tested these preparations on human breast cancer cells and we found that the uptake efficiency of DOX was dramatically increased when loaded in PLGA NPs that improved the antitumor efficacy with a reduced toxicity in cell cultures. More recently, we have assembled a novel and versatile capillary-based microfluidic system for the reproducible production of stabilized polymeric NPs with low polydispersion and diameters ranging between 35 and 350 nm. This system also allowed the synthesis of DXM-loaded PLGA NPs in a one-step procedure [3].

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Poster

Glycosylated liposomes for targeting bacteria

Stefano Aiello,[a] Francesca Ceccacci,[b] Cecilia Bombelli,[b] Michela Picano,[a] Beatrice Simonis,[a] Livia Pagano,[a] Sara Ugolini,[a] Giovanna Mancini [c]

[a] Dipartimento di Chimica, Università di Roma "Sapienza", P. le Aldo Moro 5, 00185 Rome, Italy; [b] CNR-IMC Istituto di Metodologie Chimiche Sezione Meccanismi di Reazione, Università di Roma "Sapienza", P. le Aldo Moro 5, 00185 Rome, Italy; [c] CNR-IMC Istituto di Metodologie Chimiche, via Salaria km 29.300, 00016 Monterotondo Scalo RM, Italy.
E-mail of presenting author: stefano.aiello@uniroma1.it

Biofilm associated bacterial infections are one of the most relevant health problem due to the high antibiotic resistance of biofilm infections.[1] In fact, bacterial cells are embedded in a complex polymeric matrix that, from the one hand protects the cells from external agents and, from the other confers to bacteria new powerful tools to survive, such as for example the ability to communicate among them by "Quorum Sensing", a system of stimuli and responses correlated to their proliferation that exploits specific signal molecules[2,3]. Several molecules, such as ferulic acid, gallic acid and arbutin, resveratrol have been shown to inhibit Quorum Sensing in *in vitro* studies[4] but an optimal formulation for their targeted delivery in living organisms has not been developed yet.
We have designed and prepared cationic glycosylated liposomes for the encapsulation of some of the most promising Quorum Sensing inhibitors. Sugar moieties (glucose, mannose, galactose) should in fact interact with specific proteins expressed on bacterial surface, lectins, that bear binding sites for specific monosaccharides.

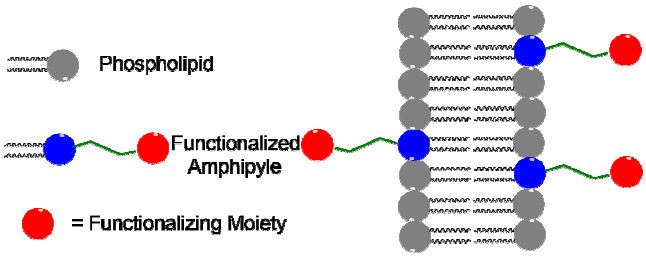

Figure 1: functionalized double layer

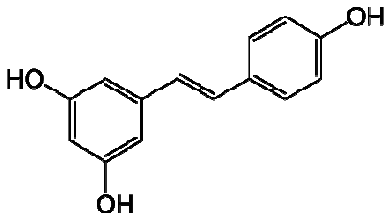

Figure 2: *trans*-resveratrol

Here we report on the synthesis of two new glycosylated lipids containing mannose and galactose moieties and on the agglutination of glycosilated liposomes mediated by Concanavalin A, a commercial plant lectin used as a model. Further, loading of resveratrol into the glycosylated liposomes has been explored.

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VP6-SUMO: a novel promising self assembling unit for drug-delivery

Adriana Amalfitano,[a] Francesca Bugli,[b] Cecilia Martini,[b] Giuseppina Nocca,[a,c] Alberto Vitali,[c] Marco De Spirito,[d] Massimiliano Papi,[d] Maurizio Sanguinetti,[b] Alessandro Arcovito[a]

[a] Institute of Biochemistry and clinical biochemistry, Catholic University of Sacred Heart, Rome; [b] Institute of Microbiology, Catholic University of Sacred Heart, Rome, Italy; [c] Centre of Molecular Recognition Chemistry, CNR, Rome, Italy; [d] Institute of Physics, Catholic University of Sacred Heart, Rome, Italy.
Email of presenting author: Adriana.amalfitano@unicatt.it

Introduction. Nanoparticles are acquiring even more interest in drug-delivery and are strongly studied to develop novel therapeutic strategies. Among them, we decided to study those derived by self-assembling protein subunits produced from viral capsid proteins, that are able to form the so called virus-like particles (VLPs). The low expression and purification yield during the production in *Escherichia coli* is, sometimes, a limit of this strategy. In order to overcome this problem, NPs formed by a highly conserved protein present in the middle of the human rotavirus' three-capsid structure called VP6 was fused with the small ubiquitin-like modifier (SUMO) at the N-terminal. The final adduct VP6-SUMO, is a very stable and soluble protein, still able to form trimers and to self-assemble in safe nanostructures such as nanospheres (NPs) and nanotubes (NTs), depending on the inducing pH used to activate this polymerization reaction [1,2]. The aim of this study was to entrap doxorubicin, an anticancer drug, into VP6-SUMO-NPs using them as carrier system to deliver the drug directly inside human hepatocellular carcinoma cell line (HepG2) and verify if the carrier can alter the anti-tumor capability of doxorubicin.

Experimental methods. After expression and production in *Escherichia coli* and purification by affinity chromatography, VP6-SUMO was put in presence of an acid buffer to induce the formation of the nanoparticle's quaternary spherical structure desired. During this step, it is possible to upload, in the forming NPs, drugs or bioactive molecules and then dialyze the final product in PBS before using it for in vitro and in vivo test. To evaluate the cellular uptake of NPs, the latter were loaded with a fluorescent probe (Calcein), incubated with HepG2 cell line and finally analyzed by confocal microscopy. The cytotoxicity on HepG2 cell line induced by NPs loaded with the doxorubicin (Dox-NPs) was also compared with doxorubicin directly added to the medium (Dox-free) by means of MTT. The cytotoxic effects caused by NPs empty were also evaluated.

Results and discussion. Transmission electron microscopy (TEM) results revealed that VP6-SUMO NPs obtained with this protocol are arranged in spheroidal structures with diameters between 100 and 200 nm. When these NPs were loaded with Calcein, they show a very fast uptake in HepG2 cell line yet after five minutes. The Doxorubicin entrapment efficiency was around 40% and cytotoxicity test showed a similar effect when the drug is administered as Dox-free and as Dox-NPs. Moreover, a small cytotoxic effect - provoked by carrier empty - was observed. **Conclusions.** From these experiments, we demonstrated that VP6-SUMO virus-like particles are able to entrap a bioactive molecule and deliver it into tumor cells. Future perspective will be to chemically engineer and/or genetically modify the surface of these VP6-SUMO NPs to deliver bioactive molecules in a more selective way.

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Solid lipid nanoparticles-mediated delivery of grape seed-derived proanthocyanidins to airway epithelial cells reduce oxidative stress

Stefano Castellani,[a] Adriana Trapani,[b] Anna Spagnoletta,[c] Lorena di Toma,[a] Sante Di Gioia,[a] Delia Mandracchia,[b] Thea Magrone,[d] Giuseppe Trapani,[b] Emilio Jirillo,[d] 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; [c] ENEA Research Centre Trisaia, Laboratory "BioProducts and BioProcesses", Rotondella (MT), Italy; [d] Department of Basic Medical Sciences, Neuroscience and Sensory Organs, University of Bari "Aldo Moro", 70125 Bari, Italy. Email of presenting author: sante.digioia@unifg.it

Objectives: Chronic respiratory diseases, which are still incurable and need novel therapeutic tools, are characterized by unsurmountable oxidative stress. Grape phenolic compounds, such as proanthocyanidins, are endowed with well-recognized anti-oxidant and anti-inflammatory activities. Given that these natural anti-oxidants have poor water solubility and oral bioavailability, we have developed a drug delivery system based on solid lipid nanoparticles (SLN), in order to encapsulate these therapeutic molecules. Our aim was to test their efficacy in reducing oxidative stress when used as carriers for molecules derived from grape seed extracts (GSE).

Methods: SLN containing GSE, formulated according to the melt-emulsification method (Trapani et al., *Biointerfaces*, 2015, *127*, 79) by using Gelucire® 50/13 as lipid, were characterized for their physicochemical parameters and the amount of incorporated GSE was determined by spectrophotometry. To evaluate the efficiency of anti-oxidant activity, airway epithelial cells (H441 line) were incubated for 24-72 h with SLN-GSE (or free GSE as control) at 2.5, 5 and 10 µM followed by treatment with 0.1 mM H_2O_2 for 24 h in order to induce oxidative stress. ROS production was evaluated by fluorimetry by using the H_2DCFDA probe, as previously described (D'Apolito et al., *Atherosclerosis*, 2015, *239*, 393), and flow-cytometry (Castellani et al., accepted in *Journal of Translational Medicine*).

Results: SLN-GSE exhibited a mean diameter of 243 nm and a zeta-potential of -14.5 mV. SLN entrapped an average of 0.059 mg proanthocyanidins/mg lipid, an amount which remained constant for up to 60 days. Pre-treatment of H441 cells with SLN-GSE (or free GSE) 24 h prior to the H_2O_2 treatment resulted in a significant reduction of ROS at the highest dose of SLN-GSE while free GSE was effective at the three doses used. Pretreatment for 48 and 72 h resulted in a significant reduction of ROS at the three doses of SLN-GSE used, while free GSE was no more effective at the lowest dose used.

Conclusions: GSE-loaded SLN exerted their effect as anti-oxidant agents for longer times than free GSE in airway epithelial cells, suggesting the controlled release of their payload. Overall, our results are promising for the development of novel natural substance-based pharmaceutics aimed at reducing the oxidative stress in chronic respiratory diseases.

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TiO_2-coated pSi microparticles for nanomedicine

Elena Chistè,[a] Ali Ghafarinazari,[a] Marta Donini,[b] Veronique Cremers,[c] Jolien Dendooven,[c] Christophe Detavernier,[c] Donatella Benati,[d] Marina Scarpa,[e] Stefano Dusi,[b] Nicola Daldosso [a]

[a] Department of Computer Science, Fluorescence Laboratory, University of Verona, Strada le Grazie 15, 37134, Verona, Italy; [b] Department of Medicine, Division of General Pathology, University of Verona, Strada le Grazie 8, 37134, Verona, Italy; [c] Department of Solid State Sciences, CoCooN Group, Ghent University, Krijgslaan 281/S1, 9000, Gent, Belgium; [d] Department of Neuroscience, Biomedicine and Movement, Anatomy and Histology Section, University of Verona, Strada le Grazie 8, 37134, Verona, Italy; [e] Department of Physics, Laboratory of Nanoscience, University of Trento, Via Sommarive 14, 38123, Trento, Italy.
Email of presenting author: elena.chiste@univr.it

Porous silicon (pSi) is a sponge-like material that, due to quantum confinement effect, is photoluminescent when excited by UV light. We developed a process to obtain mesoporous silicon microparticles by electrochemical etching and we proved them to be optically stable in biological buffers once opportunely coated. This material is a promising system for nanomedicine application, first, because it is biodegradable, biocompatible and not activator of the immune response, then, since the porosity and the photoluminescence make them suitable for drug delivery and bioimaging. The microparticles were coated by ALD (atomic layer deposition) in a rotary reactor to avoid optical and structural degradation in aqueous media. A thin and uniform TiO_2 layer was deposited on the pSi microparticles (pSi-TiO_2 microparticles), without occluding the pores. In-vitro tests were performed to control the effect of pSi-TiO_2 microparticles on human dendritic cells (DCs). We observed no decrease of cell viability after the internalization of the microparticles that maintained their PL, as can be seen in Figure 1, but an increase of the immune response (priming effect) in case of co-stimulation with an immune response activator.
These preliminary results, recently published [1], are promising for the pSi-TiO_2 microparticles application in nanomedicine, in particular, for drug delivery and bioimaging.

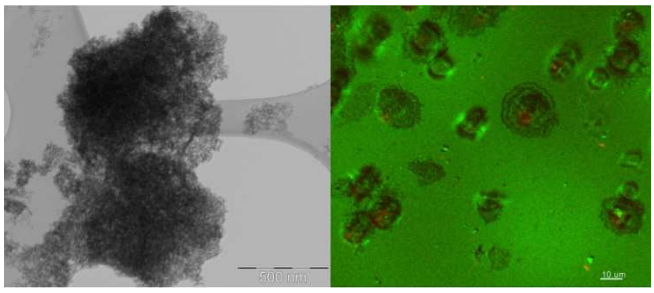

Figure 1. TEM images of pSi-TiO_2 microparticles (left) and their PL preservation after internalization in human DCs (right)

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Curcumin-loaded Poly (d,l-lactide-co-glycolide) nanovesicles induce antinociceptive effects after local administration in mice

Laura Ciarlo, Francesca Marzoli, Paola Minosi, Amalia Di Giannuario and Stefano Pieretti

National Center for Drug Research and Evaluation, Istituto Superiore di Sanità, Viale Regina Elena 299, 00166, Rome, Italy.
Email of presenting author: laura.ciarlo@iss.it

Both acute and chronic pain are the most widespread medical issue strongly affecting people in terms of health and quality of life. Unlike acute pain, chronic pain is a pathophysiological state arising from the alteration of the peripheral and/or central nervous systems. It is frequently accompanied by the onset of hyperalgesia (increased sensitivity to pain) and allodynia (painful sensation in response to usually innocuous stimuli). Pain is currently treated with two major groups of analgesic drugs, namely non-steroidal anti-inflammatory drugs (NSAIDs) and opioids; their use is associated with important side effects, which include gastrointestinal lesions [1] and nephrotoxicity [2]; in the case of NSAIDs, respiratory depression, tolerance and physical dependence for opioids [3]. For this reason, there is growing interest for the identification of alternative therapeutic strategies. Curcumin (1,7-Bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione) is a yellow polyphenol, diferuloylmethane, extracted from the rhizomes of turmeric (Curcuma longa)[4]. The therapeutic properties of curcumin are well known indeed possesses low intrinsic toxicity along with a wide range of pharmacological activities that include antitumor, anti-amyloid, antioxidant and anti-inflammatory capacities [5]. Antinociceptive properties of curcumin have also been reported in preclinical studies [6], but its poor bioavaibility limits clinical use as analgesic. Polymeric nanoparticle-based drug delivery is being increasingly investigated as a delivery route able to overcome many obstacles associated with the delivery of free drugs. Recently, we investigated the effects of curcumin-loaded PLGA nanovesicles (PLGA-CUR) administered via intravenous (i.v.) or intrathecal (i.t.) routes in several experimental models of pain [7]. We found that i.v. or i.t. routes of administration of PLGA-CUR nanoformulations were effective in reducing the nociception induced by chemical stimuli or after the ligation of the sciatic nerve in mice [7]. In the present study, putative antinociceptive effects induced by CUR and PLGA-CUR after local subcutaneous administration was investigated in two animal models of pain i.e. the formalin test and the hyperalgesia induced by zymosan. We found PLGA-CUR vesicles able to reduce nociception induced by chemical stimuli, whereas CUR alone induced only a transient but not significant antinociceptive effects. These results obtained after acute subcutaneous local PLGA-CUR vesicles administration, further suggest that PLGA-CUR formulation should be developed as a new potential drug in the treatment of pain in humans.

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Thiocolchicine-diphenylbutenyl aniline conjugates for the obtainment of self-assembled nanoparticles

Eleonora Colombo, Pierfausto Seneci, Daniele Passarella

Department of Chemistry, University of Milan, Via Golgi 19, 20133 Milano, Italy.
Email: Eleonora.colombo@unimi.it

Our continuous interest in the field of chemical approaches to target cancer cells moved us to study the preparation of novel classes of conjugate compounds using anticancer drugs as building blocks. In previous efforts we used squalene tail as self-assembling (S-A) inducer [1] and a disulphide containing linker to secure the release of the drugs after cell internalization [2]. Subsequently we demonstrated the possibility to generate hetero and fluorescent nanoparticles by mixing a paclitaxel-squalene conjugate and fluorescein-squalene conjugate [3]. In the light of facing the high demanding issue of resistance we studied the formation of cyclopamine-paclitaxel containing nanoparticles and we detected the internalization by confocal microscopy and super-resolution [4]. More recently we reported doxorubicin-cyclopamine hetero-nanoparticles that are able to reduce tumour growth and to decrease the toxicity of chemotherapy in mice [5]. We further demonstrated that heteronanoparticles consisting of conjugates characterized by a squalene tail linked to doxorubicin and ecdysteroid derivatives showed the overcoming of resistance [6].
In this work our efforts are focused on the combination of a new self-assembling inducer, which itself presents an antiproliferative activity [7], and a self-immolative linker, able to guarantee a better release of the active moieties in the presence of a lipase.

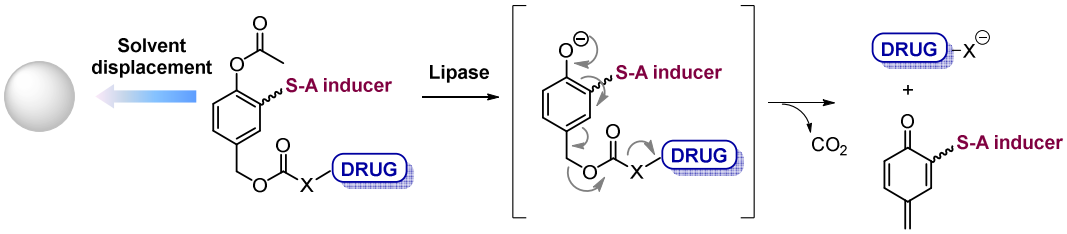

Figure 1. Structure of the conjugate able to self-assemble into NPs.

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Design and development of biosafety devices with therapeutic activities

Marisa Colone,[a] Letizia Angiolella,[b] Alberto Vitali,[c] Stefano Serra,[d] Alessandro Gori,[d] Annarica Calcabrini,[a] Annarita Stringaro [a]

[a] Centro Nazionale per la Ricerca e la Valutazione Pre-clinica e Clinica dei Farmaci, Istituto Superiore di Sanità, 00161, Rome, Italy; [b] Dipartimento di Sanità Pubblica e Malattie Infettive, Sapienza Università di Roma, 00185, Rome, Italy; [c] ICRM-CNRc/o Istituto di Biochimica e Biochimica Clinica, Università Cattolica di Roma, 00168, Rome, Italy, [d] ICRM-CNR, 20131, Milan, Italy.
Email of presenting author: marisa.colone@iss.it

The possibility to realize injectable reservoirs for controlled and specific release may provide a strategy for the administration of chemical drugs with poor solubility. The low oral bioavailability of hydrophobic drugs, their broad therapeutic windows, the necessity for a low daily dose and long-time treatment of disease are the items that support the requirement of new micro/nanodevices. Numerous studies are in progress to evaluate different devices (cationic liposomes, lysozyme-shelled hollow nano/microbubbles and nano/microcapsules) in order to increase the antifungal and antitumor efficacy of different drugs [1].
At present, biocompatible and biodegradable nanoparticles (NPs) are widely studied as an effective drug delivery device [2]. Among candidates for a drug carrier system, chitosan represents a kind of natural cationic polymer showing nontoxic, biocompatible, biodegradable features. Moreover, thanks to the specificity of short sequences recognized by cell receptors, recently many peptides have been used to improve the specific targeting of biologically active substances such as iRNAs, DNA, peptides and organic molecules [3]. We are designing peptide sequences able to recognize, transport and release in a highly specific manner biologically active natural compounds to specifically kill cancer and/or microbial cells. The aim of our work is to design peptide decorated-chitosan nanoparticles in which active elements, such as natural products with anticancer properties and antifungal characteristics, are associated to them.

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Alkaloid Voacamine encapsulated in cationic liposomes increases doxorubicin effect on osteosarcoma resistant cells

Maria Condello,[a] Luisa Giansanti,[b] Evelin Pellegrini,[a] Giovanna Mancini,[c] Stefania Meschini[a]

[a]National Center for Drug Research and Evaluation, National Institute of Health, Viale Regina Elena 299, 00161, Rome, Italy; [b]Department of Physical and Chemical Sciences, University of L'Aquila, Via Giovanni Falcone 25, 67100 L'Aquila, Italy; [c]Institute of Chemical Methodologies, National Research Council, P. le A. Moro 5, 00185 Rome, Italy.
Email of presenting author: maria.condello@iss.it

In vitro studies demonstrated that voacamine (VOA), a bisindolic alkaloid isolated from *Peschiera fuchsifoliae* plant, enhanced cytotoxic effect of doxorubicin (DOX) on resistant osteosarcoma and melanoma cells [1,2].The chemosensitizing effect of VOA on osteosarcoma cells was due to DOX accumulation increase by P-gp inhibition, the main efflux pump responsible of drug resistance [3]. Moreover, the dose of VOA used to sensitize tumor cells to DOX was not toxic to normal cells [2]. These properties and the insolubility of VOA in aqueous medium suggested the development of targeting liposomal formulations for the delivery of VOA.
Flow cytometric analyses of DOX intracellular uptake showed that VOA included into DOPC (1,2-dioleoyl-sn-glycero-3-phosphocholine) or DPPC (1,2-dipalmitoyl-phosphatidylcholine) liposomes did not increase DOX uptake with respect to free VOA. The addition of cholesterol to the formulations (DOPC/CHOL or DPPC/CHOL liposomes with VOA) did not improve DOX accumulation either. However, when VOA was included into liposomes composed of DOPC or DPPC, a gemini surfactant (SS), and cholesterol (DOPC/SS/CHOL and DPPC/SS/CHOL), DOX retention increased with respect to free VOA. MTT cell viability assay demonstrated that the most efficient liposome formulation was DOPC/SS/CHOL. Optical microscopic evaluations, 24 h after recovery in drug free medium, showed that osteosarcoma cells pre-treated with DOPC/SS/CHOL liposomes-VOA before DOX administration were dead compared to cells treated with free-VOA plus DOX. UIC2 flow cytometry shift assay showed that liposomes did not alter P-gp functionality, and that VOA loaded into liposomes exerted the inhibitory effect against P-gp as well as free VOA.
These results showed that the encapsulation of the VOA into liposomes improved its delivery in resistant tumor cells and its chemosensitizing efficacy. Further studies are aimed at clarifying if VOA loaded liposomes and free VOA have different molecular targets.

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New strategies to evaluate the efficacy *in vivo* of a novel benzophenone-based antibiotic against *S. aureus* lung infections in Cystic Fibrosis

Gabriella Costabile, Olivia Merkel

Department of Pharmacy, Ludwig-Maximilians-University, Butenandtstrasse 5, 81377, Munich, Germany.
Email of presenting author: gabriella.costabile@cup.uni-muenchen.de

Antimicrobial resistance (AMR) within a wide range of infectious agents is a growing public health threat of broad concern to countries and multiple sectors. As the 2014 WHO report says "A post- antibiotic era—in which common infections and minor injuries can kill" is "far from being an apocalyptic fantasy," but "instead a very real possibility for the 21st century". The AMR issue becomes even more pressing in pathologies such as Cystic Fibrosis (CF) [1]. CF is a complex multisystem disease caused by the defect in a single gene encoding an epithelial ion channel known as transmembrane conductance regulator (CFTR); a defective CFTR, results in thickened mucus secretions which cannot be easily cleared and predispose the lung to persistent and chronical bacterial infections that, with the AMR phenomena, cannot be well controlled anymore [2]. The general aim of this project is the design and the development of engineered nanoparticles for pulmonary delivery of a novel benzophenone-based antibiotic (SV7) against *S. aureus* chronic infection in CF therapy [3, 4]. *S. aureus*, in the treatment of CF, is an ideal target. In fact, it has been demonstrated that *S. aureus* is one of the first bacteria able to colonize the airways while producing the conditions for later infections for instance by *P. aeruginosa*. Thus, the eradication of S. aureus infection may be helpful in order to retard the chronicization of the infection and then the worsening of the lung injury. However, the individualization of new antibiotics is necessary but it is not enough against the induction of AMR phenomena. As a matter of fact, to exploit this important feature, only the reduction of patients' exposure to antibiotics may be really helpful in order to delay eventual AMR phenomena. In this sense, the local administration of novel API via inhalation route is considered as a route of choice for the treatment of pulmonary infections [5]. Lastly, since the evaluation of inhaled drug behavior through most reliable *in vivo* experimental models appears crucial for the translation to the clinics, a new *G. mellonella* model is involved as preliminary *in vivo* model in the attempt to solve the ethical issues related to the use of mammalian models [6-8].

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Polysaccharide-based nanohydrogels as drug carriers

Chiara Di Meo, Elita Montanari, Nicole Zoratto, Tommasina Coviello, Pietro Matricardi

Department of Drug Chemistry and Technologies, Sapienza University of Rome, P.le Aldo Moro 5, 00185, Rome, Italy.
Email of presenting author: chiara.dimeo@uniroma1.it

Natural and biocompatible polysaccharides, hyaluronic acid (HA) and gellan gum (Ge), were derivatized with hydrophobic moieties (cholesterol or riboflavin) in order to obtain amphiphilic polymers able to self-assemble in water leading to the formation of nanosized structures, named nanohydrogels [1-3] (NHs) (Fig. 1).

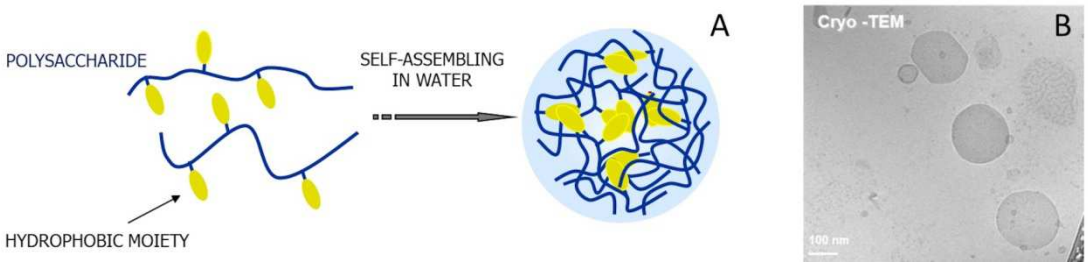

Figure 1. Self-assembling of derivatized polysaccharides leading to the formation of NHs (A); Cryo-TEM images of HA-based NHs (B).

These systems couple the features of both nanoparticles, as nano-dimensions, and hydrogels, such as soft consistency, high amount of water, and biocompatibility. Moreover, all developed systems showed a high capability to load model drugs, both hydrophilic [4] and hydrophobic ones [5], and also proteins [2], without losing their activity. In the case of hydrophobic molecules, NHs act as solubility enhancers, increasing the apparent water solubility and allowing to obtain water-based formulations.
Several derivatives were synthesized and characterized, and a new method to obtain simultaneously the formation, the sterilization and the drug loading into NHs was developed and patented [6].
All NHs were tested for their cytocompatibility on a series of cell lines, showing a complete safety up to 0.5 mg/ml. Several NHs formulations obtained with model drugs, such as antibiotics [4], anti-inflammatory [3] and anticancer drugs [5], were prepared and optimized; their activities were tested *in vitro* and *in vivo*, and in all cases an improvement of the therapeutic activity was observed.

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Levetiracetam loaded microemulsions for percutaneous drug delivery: physicochemical considerations

Ljiljana Djekic, Marija Primorac

Department of Pharmaceutical Technology and Cosmetology, University of Belgrade-Faculty of Pharmacy, Vojvode Stepe 450, 11221, Belgrade, Serbia.
Email of presenting author: ljiljanadjek@gmail.com

Levetiracetam (LEV), an antiepileptic drug, has been shown to have local peripheral antihyperalgesic and antiedematous effects in a rat model of localized inflammation. This significant observation suggests the potential of LEV for treating inflammatory pain conditions with a lower incidence of systemic side effects and drug interactions [1]. The delivery of highly hydrosoluble LEV (logP -0.6) requires a carrier having a high potential for percutaneous penetration inhancement, such as microemulsion systems [2]. The purpose of the study was formulation and physicochemical characterization of LEV-loaded microemulsions. The designed carrier (M), comprising Labrasol®/polisorbate 20/Kolliphor® RH 40/isopropyl myristate/water, was loaded with 5% (MLEV5%) and 10% (MLEV10%) of LEV into the carrier (up to 100%). Characterization of the samples was performed after storage at ambient conditions for 48 h and 1 month, and included: centrifugation test (3000 rpm for 30 min), measurement of pH and conductivity (δ), droplet size analysis by photon correlation spectroscopy, and rheological behavior characterization. The obtained results indicated that MLEV5% and MLEV10% were transparent, colourless (Fig. 1a), physically stable, oil-in-water (δ≥67.4 μS/cm) monodisperse nanodispersions (microemulsions) with average droplet size (Zave) near 13,5 nm (PdI<0.25) (Fig. 1b). Although acidic (pKa<-2), LEV did not significantly affect pH; the values were 4.19 (M), 4.29 (MLEV5%), and 4.36 (MLEV10%), thus acceptable for topical administration. The drug-free and LEV-loaded samples were Newtonian liquids (Fig. 1c) of low viscosity (η) with the value decreased as the drug level increased (866.3 mPas (M); 744.2 (MLEV5%); 599.7 mPas (MLEV10%), likely due to relaxation of the hydrogen bonds at the oil-water interface caused by drug dissolving in water phase. The formulated LEV-loaded microemulsions are prospective nanodrug delivery systems.

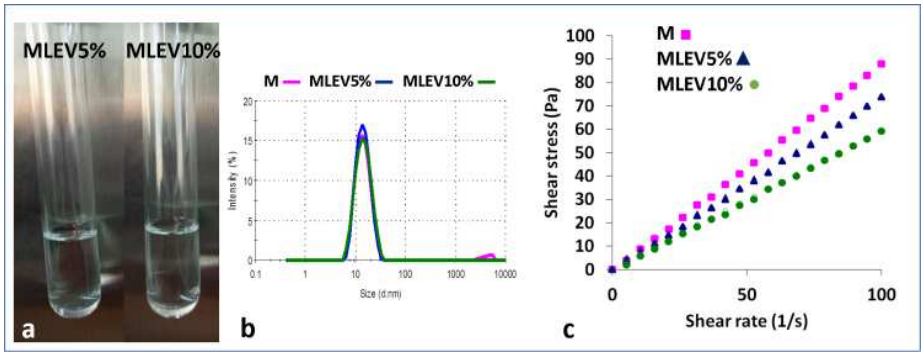

Figure 1. Appearance (a), droplet size distribution (b), and flow curves (c) of the drug-free (M) and LEV-loaded microemulsions (MLEV5% and MLEV10%).

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Hydrogels with escin β-sitosterol phytosomes – formulation approach

Ljiljana Djekic, Aleksandra Krstić, Jovana Lazić, Marija Primorac

Department of Pharmaceutical Technology and Cosmetology, University of Belgrade-Faculty of Pharmacy, Vojvode Stepe 450, 11221, Belgrade, Serbia.
Email of presenting author: ljiljanadjek@gmail.com

Complexing escin, a natural mixture of triterpene saponins from *Aesculus hippocastanum* seed, with phospholipids, produces escin β-sitosterol phytosome (ES) with enhanced selfassotiation ability and cell membrane permeability. With dermal use, escine can be released from the complex and achieve antiinflammatory and antiedematous effects. ES shows increased safety compared to escin and does not cause skin irritation or allergic reactions [1, 2]. The study aimed to formulate ES-loaded hydrogels for cutaneous application. The hydrogel prepared from Carbopol® 934 (1%), triethanolamine (1%), water (63%), propylene glycol (10%), and isopropanol (25%), was loaded with commercially available Escin β-Sitosterol Phytosome® (Indena, Italy) 1% (ES1%) and 5% (ES5%). Physicochemical properties (consistency, pH, flow behaviour, minimal apparent viscosity (η_{min}), maximal apparent viscosity (η_{max}), hysteresis area (A)) of the ES-loaded samples were compared with the escin-loaded samples prepared with the equivalent content of the active substance (E1% and E5%). ES1% and ES5% were opalescent light yellow and yellow semisolids, respectively, in contrast to the clear colorless soft semisolid gel E1% and the viscous liquid E5%. The more significant change in the escin-samples consistency was related with the higer pH decrease (Tab. 1) and weakening of the hydrogel network. For all samples was observed pseudoplastic flow behavior with thixotropy. The considered rheological parameters (Tab. 1) were higher for ES-loaded samples, thus the hydrogel structure was significantly less affected by ES in comparison with the pure escin. Additionally, ligh microscopic analysis of ES5% revealed presence of oval aggregates (<50 μm) likely formed bay ES selfaggregation. The results of the study indicate the superior performances of ES over pure escin in concentration range 1-5%, for development of the carbomer hydrogel formulations.

Sample	pH	η_{max} (Pas)	η_{min} (Pas)	A (Pa/s)
ES1	6.3	31.7	3.59	1315.83
ES5	5.78	13.9	1.56	928.67
E1	6.25	24.2	2.91	191.15
E5	4.95	0.98	0.20	136.46

Table 1. pH values, and rheological parameters (minimal apparent viscosity (η_{min}), maximal apparent viscosity (η_{max}), hysteresis area (A)) of the investigated samples.

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Acknowledgments: The study is supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia within the project III 46010.

Spectroscopic evaluation on the interaction of poorly soluble drug ibuprofen with cetyltrimethylammonium bromide micelles

Danina Krajišnik, Bojan Čalija, Ljiljana Djekic

Department of Pharmaceutical Technology and Cosmetology, University of Belgrade-Faculty of Pharmacy, Vojvode Stepe 450, 11221, Belgrade, Serbia.
Email of presenting author: ljiljanadjek@gmail.com

Interactions of cationic surfactants with negatively charged natural zeolites are excellent adsorbents for various poorly absorbable drugs, contributing their functionality as drug carriers [1]. The purpose of the study was evaluation of interaction of ibuprofen (I) with cationic surfactant cetyltrimethylammonium bromide (CTAB) micelles in purified water by photon correlation spectroscopy (PCS) (ZetasizerNano ZS90, Malvern Instruments, UK) *via* determination of average size, zeta potential (ZP) and conductivity (δ), and UV spectroscopy (Evolution 300 UV-VIS spectrophotometer, Thermo Fisher Scientific, UK), as a function of CTAB or equimolar I/CTAB concentration ranging from 0.11-33.33 $mmoll^{-1}$ at 40±0.1°C. CTAB without I at 1.11-11.11 $mmoll^{-1}$ formed micelles (average size ~40-100 nm) with a corresponding peak of high intensity (>80%). At higher concentrations of CTAB (22.22 $mmoll^{-1}$ and 33.33 $mmoll^{-1}$), an additional peak at 5560 nm was related with enhanced mutual interactions of micelles as their number increases. In size distribution curves of all samples with, low intensity peaks in a wide range of sizes were present, likely due to disturbation of the micelles and formation of hydrosoluble I/CTAB complexes [2] and their associates. ZP of CTAB micelles without I was comparable at low and high concentrations (Tab. 1). However, ZP and conductivity were increased as a function of the number of micelles or I/CTAB complex concentration (Tab. 1). The absence of chemical interactions between I and CTAB, confirmed by UV spectroscopy, indicate most likely hydrophobic interactions, which are more intensive in comparison to those between CTAB molecules in micelles. I/CTAB complexes could be suitable for adsorption onto negatively charged substrates improving their properties as potential drug carriers.

Table 1. Zeta potential (ZP) and conductivity (δ) of the investigated samples with CTAB without or with ibuprofen (I)

I/CTAB ($mmoll^{-1}$)	ZP (mV)	δ ($mScm^{-1}$)	I/CTAB ($mmoll^{-1}$)	ZP (mV)	δ ($mScm^{-1}$)
11.11/0	47.83±4.74	0.45±0.01	1.11/0	20.23±2.90	0.13±0.01
22.22/0	31.57±2.83	0.68±0.02	2.22/0	49.57±2.89	0.17±0.01
33.33/0	53.50±4.53	1.11±0.05	3.33/0	58.88±2.82	0.21±0.01
11.11/11.11	83.00±2.79	1.40±0.04	1.11/1.11	52.97±0.64	0.25±0.01
22.22/22.22	89.43±7.07	2.35±0.10	2.22/2.22	61.23±2.82	0.40±0.01
33.33/33.33	98.40±6.32	2.85±0.14	3.33/3.33	83.40±2.17	0.65±0.01

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The role of the monosialoganglioside-GM1 in the interaction between model membranes and unstructured metastable amyloid oligomers of salmon calcitonin

Marco Diociaiuti,[a] Laura Zanetti-Polzi,[b] Raoul Fioravanti,[a,c] Cecilia Bombelli,[d] Ilaria Fratoddi,[c] Cristiano Giordani [e]

[a] *Centro Nazionale Malattie Rare, Istituto Superiore di Sanità, I-00161 Roma, Italy;* [b] *Dipartimento di Fisica e Scienze Chimiche, Università dell'Aquila, via Vetoio (Coppito 1), 67010 L'Aquila, Italy;* [c] *Dipartimento di Chimica, Università degli Studi di Roma "Sapienza", I-00185 Roma, Italy;* [d] *CNR, Istituto di Metodologie Chimiche, Sezione Meccanismi di Reazione, c/o Dipartimento di Chimica, Università degli Studi di Roma "Sapienza", I-00185 Roma, Italy;* [e] *Instituto de Física, Universidad de Antioquia, Calle 70 No. 52-21, Medellín, Colombia.*
Email of presenting author: raoul.fioravanti@uniroma1.it

In this work, the interaction of Dipalmitoyl Phosphatidylcholine (DPPC) liposomes and salmon calcitonin (sCT) aggregates is examined. Specifically, we studied sCT monomers, prefibrillar oligomers (PFOs), proto- and mature-fibers (PF, MF respectively) interacting with DPPC/Chol/GM1 and simple DPPC liposomes investigated by Circular Dichroism (CD) spectroscopy and Transmission Electron Microscopy (TEM). Data clearly show the interaction of GM1 with all the sCT species examined and its role as aggregating agent for amyloid proteins favouring β-structure formation. Moreover, TEM data show that PFOs can modify the lipid bilayer by the formation of pore-like structures. These structures are very similar to that proposed by Molecular Dynamic simulations for Aβ based on experimental data obtained in model membranes supporting CD profiles of our system. We suppose that the negative charge on the sialic head group of GM1 drives the initial interaction with PFOs, exhibiting positive charges, whereas hydrophobic interactions could drive the amyloid insertion into the lipid bilayer.
The next step is the study of the interaction of sCT aggregates with liposomes of increasing complexity and biological similarity to better understand the evolution of amyloid diseases with focus on its beginning. The final application would be the synthesis of functionalized metal nanoparticles as drug delivers for Alzheimer disease.

Synergistic effect of the combination of chemotherapy with DTX and PDT with TPCS2a co-delivered by layer-by-layer nanoparticles

Elisa Gaio,[a] Claudia Conte,[b] Diletta Esposito,[b] Giovanni Miotto,[c] Fabiana Quaglia,[b] Francesca Moret,[a] Elena Reddi [a]

[a] *Cell Biology Unit, Department of Biology, University of Padova, Via Ugo Bassi 58/B, 35131, Padova, Italy;* [b] *Drug Delivery Laboratory, Department of Pharmacy, University of Napoli Federico II, Via Domenico Montesano 49, 80131, Napoli, Italy;* [c] *Department of Molecular Medicine, University of Padova, Via Ugo Bassi 58/B, 35131, Padova, Italy.*

Email of presenting author: elisa.gaio@studenti.unipd.it

Combinations of two or more drugs/treatment modalities are considered a valid tool to increase efficacy and reduce side effects of anticancer therapies by exploiting synergic effects and the consequent possibility of drug dose reduction while preserving therapeutic activity [1]. Nevertheless, the optimization of the drug ratio in order to obtain the highest extent of synergism between the two treatment modalities remains difficult to control when the drugs are administered in the standard formulations. To solve this issue, here, we propose hyaluronic acid (HA)-targeted layer-by-layer NPs co-loaded with docetaxel (DTX) and meso-tetraphenyl chlorin disulphonate (TPCS2a) at a fixed concentration ratio in order to obtain synergistic cytotoxic effect in DTX-sensitive as well as in DTX-resistant cell lines. Thus, calculating the Combination Index (CI), using the dedicated Compusyn software, we observed the highest synergism when drugs are co-delivered in the same NP (DTX/TPCS2a NP) in comparison with the other formulations tested (drugs in standard solvent, drugs in separate NPs) [2]. Interestingly, this result is maintained in the DTX-resistant cell line where, as indicated by the calculation of the Dose Reduction Index (DRI), the dose of chemotherapeutic can be reduced more than 100 times when delivered by DTX/TPCS2a NP. Based on these impressive results, we demonstrated the advantage of using HA-targeted layer-by-layer NPs as carriers of DTX and TPCS2a in order to finely control the drug ratio inside the NPs and to precisely deliver the payloads in cancer cells.

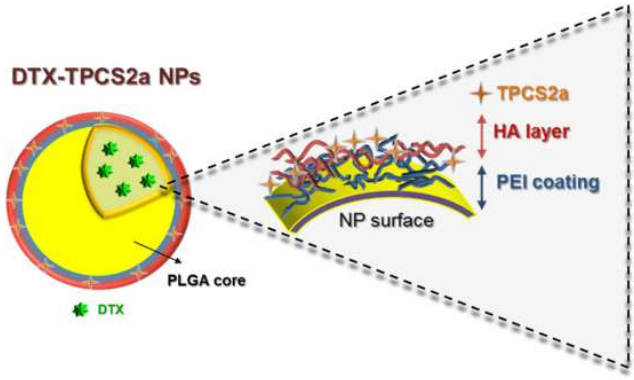

Figure 1. Schematic representation of DTX/TPCS2a NPs.

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Brij® niosomes and vegetal oil nanoemulsions for topical delivery: a comparative study

Anna Imbriano,[a] Patrizia Nadia Hanieh,[a] Federica Rinaldi,[b] Daniele Passeri,[c] Livia Angeloni,[c] Marco Rossi,[c,d] Elena Del bavero,[e] Laura Cantù, [e] Carlotta Marianecci,[a] Maria Carafa [a]

[a] Department of Drug Chemistry and Technologies, Sapienza University of Rome, P.zzle A. Moro 5, 00185, Rome, Italy; [b] Center for Life Nano Science@Sapienza, Istituto Italiano di Tecnologia (ITT), Viale R. Elena 291, 00185, Rome, Italy; [c] Department of Basic and Applied Sciences for Engineering, Sapienza University of Rome, Via A. Scarpa 14, 00161 Rome, Italy; [d] Center for Nanotechnology for Engineering (CNIS), Sapienza University of Rome, P.le A. Moro 5, 00185, Rome, Italy; [e] Department of Medical Biotechnologies and Traslational Medicine, University of Milan, Via F.lli Cervi 93, 20090, Segrate (MI), Italy.
Email of presenting author: anna.imbriano@uniroma1.it

The aim of the present work is the comparison between different nanocarriers, such as nanoemulsions (NEs) and the niosomes by Brij®72 (Polyoxyethylene Stearyl Ether), for the topical delivery of active compounds useful for the treatment of many skin diseases. Brij®72 seem to be able to enhance the skin permeation of drugs and increase their residence time in the stratum corneum (SC), limiting transdermal permeation [1]. In order to evaluate the drug loading capacity an hydrophobic model drug was used. All formulations were characterized in terms of hydrodynamic diameter, ζ-potential and drug entrapment efficiency [2, 3]. Furtheremore stability studies were carried out over time at two different storage temperatures (4°C and 25°C). The shape and the surface morphology of all samples were also investigated using Atomic Force Microscopy (AFM) and Small-Angle X-ray Scattering (SAXS). Differences in terms of fluidity, microviscosity and polarity between niosomes and nanoemulsions were investigated. Finally, the release profile of drug was assessed using the synthetic membrane Strat-M™. According to the obtained preliminary results, all formulations seem to be promising delivery systems of therapeutic agents for the treatment of skin diseases.

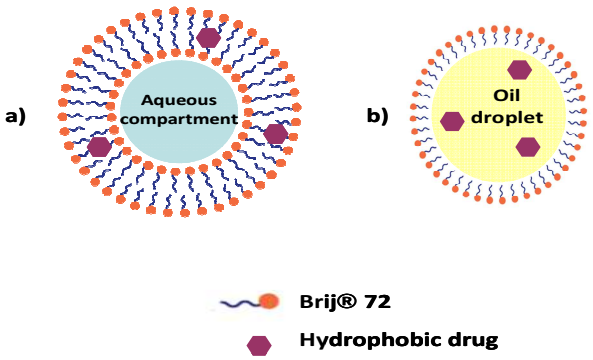

Figure 1. Schematic representation of a) niosomes and b) nanoemulsions.

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Chitosan glutamate-coated niosomes: a proposal for nose to brain delivery of pentamidine

Patrizia Nadia Hanieh,[a] Anna Imbriano,[a] Federica Rinaldi,[b] Livia Angeloni,[c] Daniele Passeri,[c] Marco Rossi,[c,d] Elena Del Favero,[e] Laura Cantù,[e] Carlotta Marianecci,[a] Maria Carafa [a]

[a] Department of Drug Chemistry and Technologies, Sapienza University of Rome, P.zzle A. Moro 5, 00185, Rome, Italy; [b] Center for Life Nano Science@Sapienza, Istituto Italiano di Tecnologia (ITT), Viale R. Elena 291, 00185, Rome, Italy; [c] Department of Basic and Applied Sciences for Engineering, Sapienza University of Rome, Via A. Scarpa 14, 00161 Rome, Italy; [d] Center for Nanotechnology for Engineering (CNIS), Sapienza University of Rome, P.le A. Moro 5, 00185, Rome, Italy; [e] Department of Medical Biotechnology and Translational Medicine, University of Milan, Via F.lli Cervi 93, 20090, Segrate (MI), Italy.
Email of presenting author: anna.imbriano@uniroma1.it

The limiting factor in the brain delivery is the presence of the blood-brain barrier (BBB).
In the last years, intranasal drug delivery has emerged as a non-invasive route to reach directly the brain, circumvent the BBB, from the nose along the olfactory and trigeminal nerve pathways [1]. The aim of this *in vitro* study has been to prepare and characterize pentamidine loaded niosomes (Pentasomes) coated with chitosan glutamate useful for intranasal drug delivery [2]. Changes in hydrodynamic diameter and ζ-potential of coated and uncoated Pentasomes were assessed by dynamic light scattering (DLS), while morphology were also studied by atomic force microscopy (AFM) and small angle x-ray scattering (SAXS). Bilayer properties and mucoadhesive behavior were investigated by fluorescence analyses. Finally, mucoadhesive studies were evaluated in terms of changes in vesicle size and ζ-potential values after the addition of chitosan glutamate to Pentasomes and after the contact with mucin solution. The characteristics of the proposed systems, such as pentamidine entrapment and mucin interaction, have shown promising results to deliver pentamidine to the brain via nasal administration.

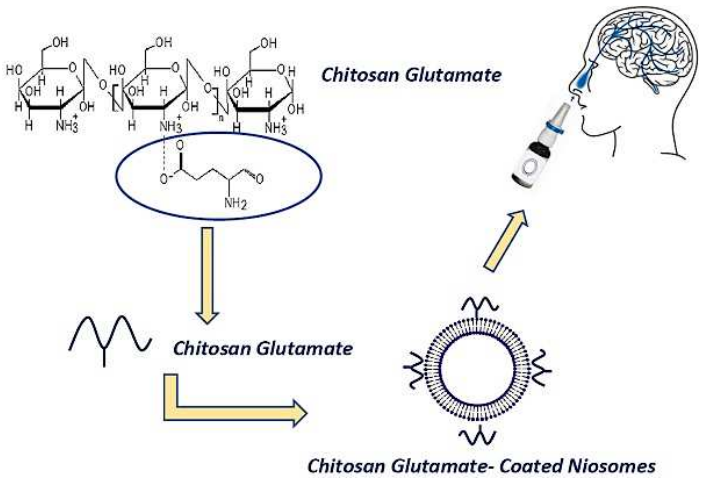

Figure 1. Graphic representation of Pentasomes after chitosan glutamate coating.

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Sub-5nm silica-coated magnetic iron oxide fluorescent nanoparticles for potential biomedical applications: biocompatibility study in human CaCo-2 cellular model

Mario Ledda,[a*] Daniela Fioretti,[a*] Maria Grazia Lolli,[a*] Sabrina Foglia,[b] Massimiliano Papi,[c] Giovanna Iucci,[d] Giovanni Capellini,[d] Monica Rinaldi,[a] and Antonella Lisi [a]

[a]*Department of Biomedical Sciences, Institute of Translational Pharmacology (IFT), National Research Council (CNR), via del Fosso del Cavaliere 100, 00133, Rome, Italy;* [b]*Department of Engineering, ICT and technologies for energy and transportation, Institute of Materials for Electronics and Magnetism (IMEM), National Research Council (CNR), Parma, Italy;* [c]*Department of Science, University Roma Tre, Rome, Italy;* [d]*Institute of Physics, Catholic University of the Sacred Heart, Rome, Italy.*
Email of presenting author: mariagrazia.lolli@ift.cnr.it

[*]*Authors sharing first co-authorship*

Magnetic iron oxide nanoparticles (IONPs), for their intriguing properties, have attracted a great interest as they can be employed in many different biomedical applications. In this work, mild condition, and inexpensive method, was used to synthesize ultrafine 3 nm superparamagnetic water-dispersible nanoparticles. They were obtained at room temperature and dispersed in alkaline aqueous solution. The nude ultrafine 3 nm superparamagnetic nanoparticles were covered with silica and functionalized with fluorescein isothiocyanate (FITC) dye through a new one pot synthetic strategy modifying procedures already reported. The obtained sub-5nm silica-coated magnetic iron oxide fluorescent (sub-5 SIO-Fl) nanoparticles were assayed for cellular uptake, biocompatibility and cytotoxicity in a human colon cancer (CaCo-2) cellular model. By confocal microscopy analysis we demonstrated that nanoparticles as-synthesized are internalized and, even at the highest concentration used, are biocompatible, non-toxic, do not interfere with the CaCo-2 cell cytoskeletal organization nor with their cellular adhesion. We further demonstrated at molecular level that these nanoparticles do not interfere with the expression of key differentiation markers and do not affect pro-inflammatory cytokines response in Caco-2 cells. Overall, these results showed the in vitro biocompatibility of the sub-5 SIO-Fl nanoparticles promising their safe employ for diagnostic and therapeutic biomedical applications.

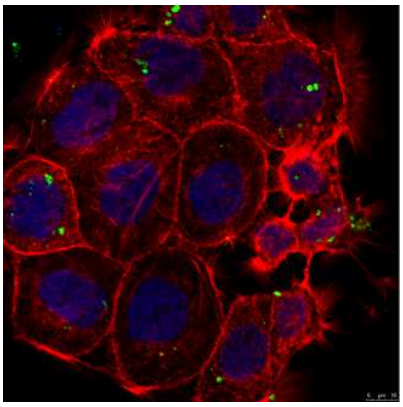

Figure 1. Confocal image of CaCo-2 cells after 48h incubation with 50µg/ml sub-5 SIO-Fl nanoparticles. Photographs were taken at a magnification of 40X. (Foglia S. et al. Sci. Rep. 2017).

Enhanced antinociceptive and anti-inflammatory effects of ibuprofen encapsulated in niosomal vesicles

Francesca Marzoli,[a] Paola Minosi,[a] Laura Ciarlo,[a] Amalia Di Giannuario,[a] Maria Carafa,[b] Carlotta Marianecci,[b] Stefano Pieretti [a]

[a] National Centre for Drug Research and Evaluation, Italian National Institute of Health, Viale Regina Elena 299, 00161 Roma. Italy; [b] Department of Drug Chemistry and Technology, University of Rome "Sapienza", Rome, Italy. Email of presenting author: francesca.marzoli@iss.it

The non-steroidal anti-inflammatory (NSAID) drug Ibuprofen (α-methyl-4-(2-methylpropyl)-benzeneacetic acid), is widely used in the treatment of pain, fever and inflammatory diseases. As far as its analgesic actions, ibuprofen inhibits the production of prostanoids, which mediate peripheral and central pain sensation, reducing the threshold to stimulation of nociceptors and increasing their terminal membrane excitability [1]. Niosomes, are unilamellar or multilamellar non-ionic surfactant vesicles. The main characteristic of these vesicles is their capability of encapsulating both lipophilic and hydrophilic drugs; hydrophilic drugs are encapsulated in the core of vesicles, while lipophilic drugs can be encapsulated into the lipophilic domain of the lipid bilayer [2]. In this work, we evaluated the analgesic activity of subcutaneous injection (*s.c*) of ibuprofen loaded TW20Gly niosomes (N-IBU), in comparison with free ibuprofen (IBU) in acute and chronic pain animal models.

In vivo anti nociceptive activities of Ibuprofen-loaded vesicles were preliminarily tested by performing the writing and capsaicin screening assays. In writhing test, acetic acid as peripheral pain inducer was utilized. Analgesic activity was determined by entering the reduction of the number of writhes after acetic acid injection. In the early tested mice, a statistically significant reduction of writhes was observed only in IBU branch, whereas in the late tested mice the strongest reduction was observed in N[IBU] treated mice. In capsaicin experiments, in which mice were injected under the hind paw with capsacin, nociceptive activity was evaluated by measuring the time spent by mice in licking the injected paw. N-IBU significantly decreased capsaicin-induced paw licking, while IBU was ineffective. N-IBU also induced the strongest antinociceptive effects in Zymosan-induced hyperalgesia test. N-IBU increased pain threshold also in a model of neuropathic pain i.e. chronic sciatic nerve ligation, reducing hypealgesia and allodyia 2h after the treatment up to 4h. In the same conditions IBU did not gave any significant effect.

We can conclude that the encapsulation of the drug into the niosomes significantly increases IBU analgesic activity, promoting a long lasting action of this drug. Thus, we propose TW20Gly niosomes as a new and more effective strategy to vehicle IBU to treat acute and chronic pain conditions.

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Prolongation of local pain insensitivity by anesthetic lidocaine loaded pH-TW20 Gly niosomes: effects on nociception in murine models of pain

Paola Minosi,[a] Francesca Marzoli,[a] Laura Ciarlo,[a] Amalia Di Giannuario,[a] Maria Carafa,[b] Ferderica Rinaldi,[b] Stefano Pieretti [a]

[a] *National Center for Drug Research and Evaluation, Istituto Superiore di Sanità , Rome, Italy;* [b] *Department of Drug Chemistry and Technology , University of Rome "Sapienza", Rome, Italy.*
Email of presenting author: paola.minosi@iss.it

Current drugs treating neuropathic pain fail in up to 40-50% of the patients, because they have limited efficacy and are associated with dose related unwanted adverse effects [1]. One of the most extensively studied agents for neuropathic pain in animals and humans is lidocaine, a local anesthetic with a short duration of action [2]. The great interest in lidocaine delivery systems is increased in the last years. The final purpose is to prolong the effective time of lidocaine and to reduce the frequency of administration. Particularly, pH-sensitive molecules to niosome formulation represents an effective and promising delivery strategy [3]. pH-sensitive nonionic surfactant vesicles (niosomes) by polysorbate-20 derivatized by glycine (added as pH sensitive agent), were developed to deliver Lidocaine (LID). Lidocaine (5%) were chosen into niosome (N[LID]) (TW20-GLY LIDO 5%) [3]. Experiments to assess the *in vivo* efficacy of lidocaine loaded pH-TW20 GLY niosomes were carried out in murine models to evaluate the potential advantages of stimuli responsive nanocarriers, loaded with lidocaine in pain treatments. The data related to these tests and obtained from lidocaine loaded pH-TW20 Gly niosomes were compared with those obtained from free lidocaine, in order to highlight the overlap with the data. The following models of pain were used: formalin test, zymosan-induced hyperalgesia, Tail flick test and sciatic nerve ligation inducing neuropathic allodynia and hyperalgesia. The subcutaneous administration of N[LID] in the dorsal surface of mice paw 10 min or 180 min before formalin in a volume of 40 µL/paw and 1h after zymosan A in a same volume was able to reduce the response to nociceptive stimuli in the formalin test and hyperalgesia induced by zymosan. The already high effects of free lidocaine were improved in terms of higher duration of its action over time. The results obtained by Tail flick test confirmed that N[LID] has a longer analgesic effect than free lidocaine, especially in terms of longer duration of action. Experience to date suggests that 40 µL/paw s.c. administration of N[LID] significantly reduced allodynia and hyperalgesia produced by sciatic nerve ligation. Niosome represents an effective and promising delivery strategy, which may greatly increase the utility of niosomes as a targeted delivery vehicle, which is degraded only in the target area, where the drug will be released and accumulated. In our opinion, N[LID] should be developed as a new potential drug in the treatment of pain in humans.

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Development of size-controlled PLGA-PEG nanoparticles for targeted drug delivery in inflammatory Bowel disease

Lauren J. Mohan,[a,b] Jacqueline S. Daly,[a] Zebunnissa Ramtoola [b]

[a] *Division of Biology, Department of Anatomy, School of Medicine,* [b] *School of Pharmacy, Royal College of Surgeons in Ireland, Dublin 2, Ireland.*
Email of presenting author: laurenmohan@rcsi.ie

Nanoparticle (NP)-based drug delivery vehicles represent an innovative strategy for targeted drug delivery in inflammatory bowel disease (IBD). Evidence in animal models of IBD has shown that orally administered NPs exhibit preferential deposition in inflamed intestinal tissue over healthy, facilitating drug delivery to the site of disease [1]. Size is a major determinant affecting NP transport across the intestinal barrier and therefore is a key consideration in the design of delivery vehicles. To investigate the effect of NP size on transport it is essential to develop NPs of reproducible and uniform sizes. In this study we aimed to generate and characterise poly(lactic-co-glycolic acid)-polyethylene glycol (PLGA-PEG) NPs of three defined sizes, produced using a solvent dispersion method [2]. Herein we describe the production of NPs of different sizes through variation of PLGA-PEG concentration. Physicochemical characterisation of NPs was performed by dynamic light scattering, Laser Doppler Electrophoresis and transmission electron microscopy. Size-distribution analysis was carried out by differential centrifugation. The suitability of lyophilisation for the recovery of NPs was explored [3]. In addition the stability of lyophilised NPs and NPs stored as aqueous dispersions, at 4°C, was determined over a 12 week time period. Increasing PLGA-PEG concentration resulted in an increase in NP size, generating NPs of ~100, ~300 and ~600 nm in size. Statistical analysis of NP size for batches formulated at each polymer concentration revealed the reproducibility of the solvent dispersion method. Differential centrifugation of NPs was found to facilitate a reduction in NP sample polydispersity. All NPs displayed zeta potential values >-22 mV, indicating colloidal stability. Storage of NPs at 4°C as an aqueous dispersion was superior for maintenance of physical stability for up to 12 weeks for further applications. The results of this study suggest that solvent dispersion is an attractive and reproducible method for the preparation of NPs of defined sizes. NP uniformity and stability are critical to ensure consistent parameters for definitive *in vitro* analysis. Future work will focus on transport studies of different NP sizes in *in vitro* disease models of the intestinal barrier. Understanding the impact of size on the passive targeting ability of NPs is fundamental to the design and development of oral nanomedicines for IBD.

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An insight to interface properties of low-energy nanoemulsions for curcumin dermal delivery using electron paramagnetic resonance spectroscopy - a link with release kinetics and biological activity

Ines Nikolić,[a] Evgenia Mitsou,[b] Bojan Markovic,[c] Vassiliki Papadimitriou,[b] Aristotelis Xenakis,[b] Snezana Savic [a]

[a] *Department of Pharmaceutical Technology and Cosmetology, University Belgrade, Vojvode Stepe 450, 11221, Belgrade, Serbia;* [b] *Institute for Biology, Medicinal Chemistry and Biotechnology, National Hellenic Research Foundation, 48 Vassileos Constantinou Ave, 11635, Athens, Greece; Department of Pharmaceutical Chemistry, University of Belgrade, Vojvode Stepe 450, 11221 Belgrade, Serbia.*

Email of presenting author: ines.nikolic@pharmacy.bg.ac.rs

Introduction: Low-energy nanoemulsions (LE-NEs) represent promising, but poorly investigated nanocarriers with several physicochemical and biological advantages compared to conventional vehicles, capable of meeting highly imposed expectations for demanding drug molecules [1]. Curcumin (CU) is a polyphenol of natural origin, famous not only for its powerful pleiotropic effects, but also for difficulties in terms of stability and biopharmaceutical performances [2].

Aims: Characterization of interface properties of developed LE-NEs was performed using electron paramagnetic resonance (EPR), with a view to capturing potential correlation between interface fluidity/rigidity with release kinetics of CU (in 3 different concentrations: 0.1, 0.2 and 0.3 % *m/m*) and its reflection to antioxidant activity (also assessed by EPR).

Results: Blank LE-NE (F), consisting of medium-chain triglycerides, as the oil phase, and a combination of polysorbate 80 and soybean lecithin (9:1) as stabilizers, with *surfactants-to-oil ratio* 1, exhibited very small droplet size (110 nm ± 0.21 nm) and narrow size distribution. Release kinetics of CU showed that all formulations followed Higuchi diffusion model. In each case the total amount of released CU was ≈ 10 % *m/m*. Antioxidant activity test (Fig. 1) revealed that CU incorporation in the LE-NEs was followed by a prolonged, but less intense activity compared to free curcumin. This could be explained through EPR results - even though CU interacted with the interface, its rigidity made it difficult for CU to move and pass through it, resulting in lower release amounts and suppressed activity.

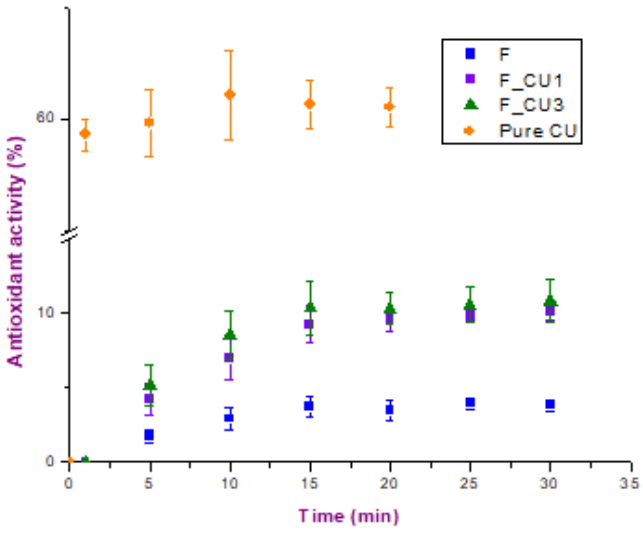

Figure 1. Antioxidant activity obtained by EPR

Conclusion: EPR appeared to be a multifunctional tool, enabling to link physicochemical properties of carrier's inner structure with biopharmaceutical performances, and valuable method in antioxidant activity evaluation.

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Polymeric and Solid Lipid Nanoparticles for nose-to-brain delivery of geraniol-ursodeoxycholic acid conjugate: development and characterization studies

Edilson Oliveira Junior,[a] Eleonora Truzzi,[b] Cecilia Rustichelli,[b] Eleonora Maretti,[b] Eliana Lima,[a] Marco Fogagnolo,[c] Alessandro Dalpiaz,[c] Eliana Leo [b]

[a] *Laboratory of Pharmaceutical Technology - FarmaTec, Faculty of Pharmacy, Federal University of Goiás, University Square nº 1166, 74605 Goiânia, Brazil;* [b] *Department of Life Sciences, University of Modena and Reggio Emilia, via G. Campi 103, 41125 Modena, Italy;* [c]*Department of Chemical and Pharmaceutical Sciences, University of Ferrara, Via Fossato di Mortara 19, 44121 Ferrara, Italy.*
Email of presenting author: edilsonrojunior@gmail.com

Neurodegenerative disorder treatment is a challenge mainly due to the difficulty of drug transport across the blood-brain barrier [1]. Intranasal administration of nanoparticles as carrier system may increase drug concentration into the brain [2]. Geraniol (GER) has demonstrated antioxidant and neuroprotective activities in Parkinson's disease animal models [3]. However, due to its volatility, GER is hardly incorporated into freeze-drying nanoparticles. On the other hand, GER-ursodeoxycholic acid conjugate (GER-UDCA) is a non-volatile derivative with high potentiality to be incorporated into nanocarriers. Therefore, in this work GER-UDCA-loaded Solid Lipid Nanoparticles (SLNs) and PLGA nanoparticles (NPs) intended for nose-to-brain delivery were developed and characterized. SLNs were prepared by emulsion/solvent evaporation method and NPs by nanoprecipitation method. Briefly, formulations were optimized considering various processing variables and nanoparticle characterization was performed in terms of morphology, size, surface charge, drug loading (DL%), encapsulation efficiency (EE%) and *in vitro* drug release. Finally, the stability of free and encapsulated GER-UDCA was evaluated in enzymatic medium from rat liver homogenates. GER-UDCA-SLN and GER-UDCA-NPs showed spherical shape, mean size of 120/180 nm with polydispersity index < 0.2, and zeta potential around −22/-26 mV, respectively. After freeze-drying, the DL% was 6% for SLN and 12% for NPs with EE% values of 89.3% and 60.1%, respectively. Preliminary data regarding *in vitro* release of GER-UDCA from the nanoparticles evidenced a higher dissolution rate than the free drug, probably due to the increase of surface contact. Results in liver rat homogenate suggested a contribution of the nanoparticles in the stability of the prodrug in physiologic environments. In conclusion, these GER-UDCA-loaded nanocarriers demonstrated a possible application in further *in vivo* studies of nose-to-brain drug delivery.

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DoE optimization of ketoconazole-loaded Nanostructured Lipid Carriers (NLC) based on Amazon fat (*Virola surinamensis*) Ucuuba

Rayanne R. Pereira,[a,b] Matteo Testi,[b] Roseane M. R. Costa,[a] Josè O. C. Silva Junior,[a] Cristina Padula,[b] Fabio Sonvico[b]

[a] *Pharmaceutical Sciences Faculty, Federal University of Para, Belem, Brazil;* [b] *Food and Drug Department, University Of Parma, Parma, PR, Italy.*
E-mail of presenting author: pereirarayanne52@gmail.com

Virola surinamensis, know like Ucuuba, is an oleaginous plant species found in Central and South America, especially in Amazonian region of Brazil. Ucuuba seeds are rich in fat (60-70%), already in use for the production of soaps, shampoos and moisturizers [1]. In the era of nanotechnology, nanostructured lipid carriers (NLC) present several advantages in relation to polymer nanoparticles, liposomes, nanoemulsions, microemulsions and traditional emulsions. In this context the aim of this work was the optimization of NLC produced with Ucuuba fat (NCL-ucuuba), for topical application, using ketoconazole as a model drug. The NLC-ucuuba were prepared by High Pressure Homogenization (HPH), (Panda Plus 2000, GEA Niro Soavi, Parma, Italy), using pegylated vitamin E (TPGS) as surfactant, and propylene glycol monocaprylate (Capryol ™ 90, Gattefossé) as liquid lipid. A Box-Behnken experimental design was performed, where the percentage of surfactant (3-6% w/v), that of liquid lipid (2-3% w/v) and of solid lipid (ucuuba fat 7 to 8% w/v) were the independent variables. In total 17 formulations were produced by varying factor concentration to minimize particle size, minimize polydispersity index (PDI) and maximize the entrapment efficiency of ketoconazole in NLC-ucuuba. Nanoparticles of 23.9 nm to 90.1 nm were obtained, the particle size was observed to decrease with increasing Vit E TPGS concentration ($p<0.0001$) while percentage of liquid lipid was not significant ($p> 0.05$) on particle size. Ucuuba fat concentration directly affected the size of NLC-ucuuba. The PDI ranged from 0.248 to 0.558. Counterintuitively, increasing concentrations of surfactant in the formulation contributed to an increase of the PDI (p-value 0.0041), while neither solid nor liquid lipid concentration showed significant effects on particle sized distribution. The entrapment efficiency ranged from 93.9 to 99.7, not presenting statistical significance. The manufactured optimized formulation produced according to the model obtained through the DoE showed particle size of 32.8 nm, encapsulation efficiency of 98.2 % and PDI of 0.207. In conclusion, NLC-ucuuba can be classified as having the necessary characteristics for topical application and studies are ongoing to explore their biopharmaceutical properties.

Folate-functionalized DNA nanocages for targeted delivery of Doxorubicin to cancer cells

Sofia Raniolo,[a] Valeria Unida,[b] Alessio Ottaviani, [b] Alessandro Desideri, [b] and Silvia Biocca [a]

[a] *Department of Systems Medicine, University of Rome Tor Vergata, Via Montpellier 1, 00133, Rome, Italy;* [b] *Department of Biology, University of Rome Tor Vergata, Via della Ricerca Scientifica 1, 00133, Rome, Italy.*

Email of presenting author: sofiaraniolo@gmail.com

DNA possesses intrinsic properties of high stability and biocompatibility that make it an extremely suitable polymer for the generation of nanoparticles. DNA nanostructures can be functionalized with different molecules, such as small ligands or antibodies, in order to achieve specific cellular targeting to be used for a wide variety of applications, including drug delivery. Here, we have assembled covalently bound truncated DNA octahedral nanocages modified with a biotin molecule, which allows the detection through streptavidin-biotin reaction, and folic acid for targeting cancer cells overexpressing the alpha isoform of the folate receptor (αFR). HeLa cells, a human αFR overexpressing tumor cell line, bind and internalize folate-DNA cages with an efficiency at least 40 times higher than that observed in cells not expressing this receptor. Folate-modified DNA cages are internalized in endosomes through αFR-mediated pathway and accumulate inside cells in a time-dependent way with high intracellular stability (>48 hours). We have loaded DNA cages with Doxorubicin (DOX), an anticancer drug that intercalates the double helices of DNA. DOX-loaded folate-DNA cages divert the DOX natural traffic and accumulate in the cytoplasm where DOX is released inducing a toxic mechanism that degrades DNA cages, avoiding the problem of nanostructures accumulation in vivo. DOX, delivered through folate-DNA cages, shows a cytotoxic effect at a lower concentration compared to DOX delivered in the free state that selectively kills αFR-positive cancer cells. Taken together these results indicate that functionalization with folate can be pursued to selectively target αFR overexpressing cells in cancer therapy since the use of nanostructures as drug vehicles can potentially improve the *in vivo* pharmacological effects of drugs, reducing their dosage and minimizing unwanted side-effects.

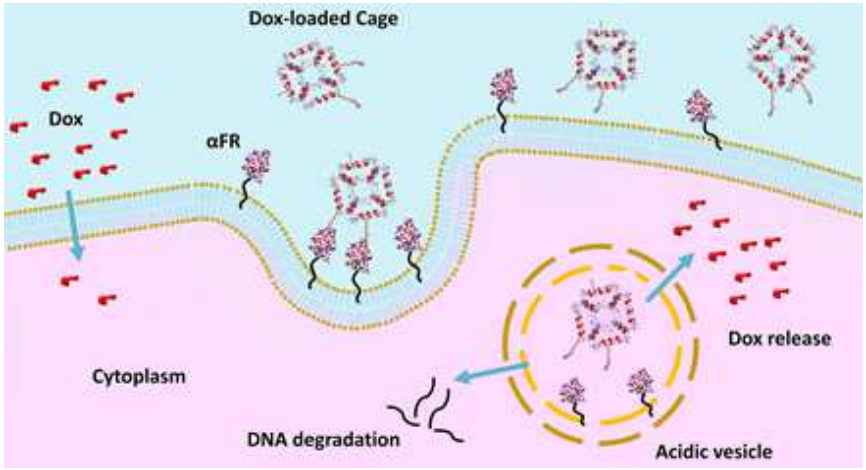

Figure 1. Schematic representation of the uptake and intracellular fate of DOX-loaded folate-DNA cages in αFR overexpressing cells.

In vitro characterization of drug loaded niosomes for brain delivery

Federica Rinaldi,[a] Maria Carafa,[b] Simone De Panfilis,[a] Laura Di Magno,[a] Carlotta Marianecci,[b] Giovanna Peruzzi [a]

[a] *Center for LifeNano Science IIT@Sapienza, Istituto Italiano di Tecnologia, Viale Regina Elena, 00161, Rome, Italy;* [b] *Department of Drug Chemistry and Technology, University of Rome "Sapienza", Piazzale A. Moro, 00185, Rome, Italy.*
Email of presenting author: federica.rinaldi@uniroma1.it

The complexity of human diseases may lead to poorly effective drug treatments. The development of nanocarriers able to deliver active compounds to target specific organs and tissues recently acquired high impact in the scientific community.
Indeed, properly designed nanocarriers are potentially able to reach the CNS (central nervous system) through different strategies [1].
To this aim, we designed specific vesicular systems, niosomes (non-ionic surfactant vesicles), and we characterized these nanovectors in terms of size, ζ-potential and stability in different media.
We used brain tumor cell lines as *in vitro* model systems (DAOY medulloblastoma cells and SKN neuroblastoma cells) that were treated with lipophilic fluorescent molecules loaded niosomes (by Span or Tween surfactants). As shown in Fig. 1, curcumin and Nile red where efficiently internalized in both cellular models, demonstrating the interaction of non-ionic surfactant vesicles with brain tumor cells.

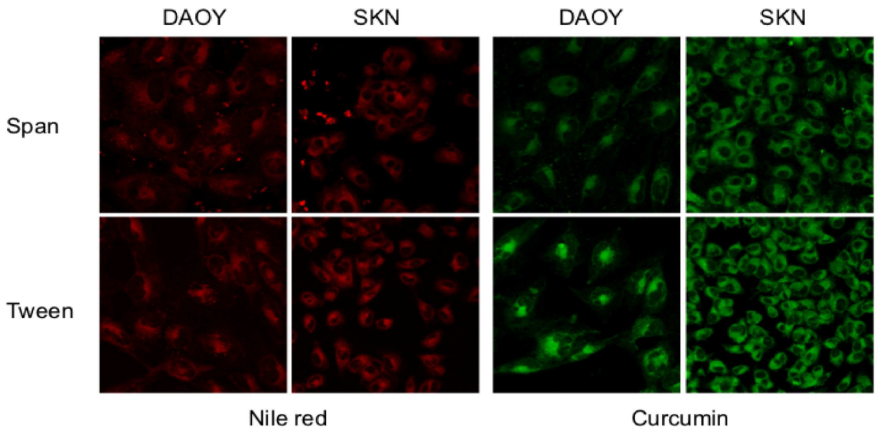

Figure 1. Fluorescence confocal images of tumoral cell lines after interaction with Nile red / curcumin loaded niosomes.

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Polyion-induced liposomal aggregates for antitubercular drug delivery

Francesca Sciolla,[a] Domenico Truzzolillo,[b] Federica Rinaldi,[c] Patrizia Nadia Hanieh,[d] Maria Carafa,[d] Federico Bordi,[a] Simona Sennato [a]

[a]Institute for Complex Systems (ISC)–CNR and Physics Department Sapienza University of Rome, Italy; [b]Laboratoire Charles Coulomb CNRS-Université de Montpellier, Montpellier, France; [c] Center for Life Nano Science@Sapienza, Istituto Italiano di Tecnologia, Rome, Italy; [d]Department of Drug Chemistry and Technology, Sapienza University of Rome, Italy.
Email of presenting author: francesca.sciolla@gmail.com

The self-assembly of charged liposomal particles with oppositely charged polyions gives rise to long-lived finite-size aggregates with intriguing properties [1,2].This new cluster phase is formed by intact vesicles "glued together" by the oppositely charged polyions, the structure resembling a sort of blackberry formed by individual compartments (vesicles) [3,4]. Stability and size of the aggregates can be properly tuned by varying the ratio of the concentrations of liposomes and polyions (known as re-entrant condensation behavior). These nano-structured clusters have a high potential for pharmaceutical applications, particularly when the primary colloidal particles are bio-compatible lipid vesicles as phospholipid liposomes [5].
The present study aims to develop novel liposomal aggregates with an efficient loading of isoniazid (INH), a first line anti-Mycobacterium tuberculosis-drugs. Considering the selective uptake the macrophages, due to the large size of the aggregates, this system is particularly suitable for exploring therapeutic strategies against this pathogen, intracellularly located in macrophages. In this investigation, anionic unilamellar liposomes were prepared using different amounts of a charged lipid (DPPG) mixed with the zwitterionic HSPC (Hydrogenated Soybean Phosphatidylcholine), the composition being adjusted to optimize stability and drug encapsulation efficiency. The biocompatible short polyion, the ε-Polylisine (PLL), has been used. Both liposomal and aggregate samples have been studied by dynamic light scattering, electrophoresis and transmission electron microscopy. Differential Scanning Calorimetry coupled to Static Light Scattering were used to better understand the parameters governing drug loading and the drug-lipid interaction. Stable and unilamellar ISH-liposomes and PLL-liposomal aggregates have been obtained with size and effective charge tuned by the PLL content. Moreover, we have proven that the interaction with ISH is able to modify the phase transition temperature, the thickness and ordering of liposomal membrane and dependent on the liposomal composition.

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Novel nanomicelles-loaded gelling ocular inserts for the delivery of cyclosporine-A: technological characterization

Eleonora Terreni,[a] Daniela Monti,[a] Patrizia Chetoni,[a] Susi Burgalassi,[a] Silvia Tampucci,[a] Edwin Chipala,[b] Raid G. Alany,[b,c] Ali AT. Al-Kinani [b]

[a] Department of Pharmacy, University of Pisa, Via Bonanno 33, I-56126, Pisa, Italy; [b] School of Life Sciences, Pharmacy and Chemistry, Kingston University London, Penrhyn Road, Surrey KT1 2EE, Kingston upon a Themes, London (UK); [c] School of Pharmacy, The University of Auckland, Auckland, New Zealand.
Email of presenting author: eleonora.terreni@farm.unipi.it

Cyclosporine A (CyA) is a cyclic undecapeptide with an immunosuppressive activity approved for the topical treatment of several immune-mediated ocular surface disorders such as the sever Dry Eye Syndrome (DES). Its high molecular weight, low water solubility and high lipophilicity makes the ocular bioavailability and the development of an ophthalmic formulation of CyA a real challenge. The only approved European marketed formulation for a "once a day" administration for treatment of DES is Ikervis®, a nanoemulsion containing 1mg/mL of CyA [1]. Nanoemulsions could present some disadvantages related to the stability, ocular irritation, and pharmacokinetics issues [2]. To improve CyA solubility, nanomicelles consisted of non-ionic surfactants mixture of Vitamin E-TPGS and Octoxynol-40 (Nano-CyA) was prepared to obtain an aqueous clear solution suitable for ocular administration. CyA-loaded (0.1%w/v) nanomicelles have shown promising results in terms of size, drug entrapment, loading capacity, and ocular tolerability. In addition, to improve CyA ocular resident time and bioavailability Nano-CyA was incorporated into a solid insert constituted by different mucoadhesive polymers (PVA, CMC, XG, ALG, CAR) able to form a gel system when in contact with the ocular tear fluid and to increase Nano-CyA interaction with the ocular tissues. The inserts were prepared by casting of aqueous polymeric dispersions containing Nano-CyA, previously analyzed for drug entrapment, loading capacity and size distribution. After measurement of the weight, thickness, and diameter, the final inserts were analyzed by physico-chemical point of view using FTIR and DSC analysis. Furthermore, the mechanical properties by tensile strength measurement, folding endurance and surface morphology by SEM analysis were determined. Finally, the CyA content in the solid inserts were evaluated by HPLC.
The more promising formulations will be subjected to further *in vivo* studies to evaluate ocular tolerability and the pharmacokinetic behavior.

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Small-angle neutron scattering characterization of liposomes for anti-tuberculosis inhaled therapy

Eleonora Truzzi,[a] Angela Capocefalo,[b] Carlo Castellano,[c] Fabio Domenici,[d] Fiorella Meneghetti,[e] Eleonora Maretti,[a] Luca Costantino,[a] Valentina Iannuccelli,[a] Eliana Leo [a]

[a] Department of Life Sciences, University of Modena and Reggio Emilia, via Campi 103, 41125, Modena, Italy; [b] Department of Physics, Sapienza University of Rome, P.le Aldo Moro 5, 00185, Roma, Italy; [c] Department of Chemistry, University of Milan, via Golgi 19, 20133, Milano, Italy; [d] Department of Chemical Science and Technology, University of Rome Tor Vergata, via della Ricerca Scientifica, 00133, Roma, Italy; [e] Department of Pharmaceutical Science, University of Milan, Via L. Mangiagalli 25, 20133 Milano, Italy.
Email of presenting author: eleonora.truzzi@unimore.it

The present investigation studied the effects of two first-line anti-tuberculosis (TB) drugs, rifampicin (RIF) and isoniazid (INH), on the structure of multilamellar liposomes. Liposomes have been shown to be a promising system for inhaled therapy [1]. The study of liposome-drug interaction is essential, and small-angle neutron scattering (SANS) technique provides valuable and unique data about steric bilayer thickness, particle dispersion, number of lamellae and drug localization under physiological conditions [2]. Unloaded, single drug-loaded and co-loaded liposomes were prepared using different amounts of drugs by reverse phase evaporation method. Liposomal suspensions were prepared using D_2O, in order to emphasize the contrast between the aqueous and the lipid/drug phases. The samples were characterized by dynamic light scattering, atomic force microscopy and finally by SANS technique (Rutherford Appleton Laboratory, U.K.). Neutron scattering curves were analyzed using a multi-shell spherical model of the fitting routine SASView 2.2.0. Liposomes have been shown to be physicochemically stable during the experiments, efficiently drug-loaded, and able to control drug release. Dimensional analysis demonstrated that particle sizes are in the range of SANS dimensional detection. SANS curves exhibited Bragg peaks for all samples, confirming the multilamellar liposome structure. By fitting the data, significant differences among the samples have been highlighted. RIF-liposomes were less ordered than unloaded liposomes. Indeed, a reduction of the lamellae number was observed and the periodicity of the lipid bilayers slightly increased with the increment of the drug loading. This is probably due to RIF interaction with phospholipid tails, which can destabilize liposome lamellarity, since RIF is a hydrophobic drug. In INH-liposomes, the drug payloads did not change vesicle structure, because INH is a hydrophilic drug. However, INH induced a change in the inter-bilayer periodical spacing, which could be compatible with the formation of drug-liposome interactions at the water-lipid interface. Finally, the RIF-INH co-loaded liposomes exhibited the same characteristics of unloaded liposomes, suggesting that INH and RIF together have a stabilizing effect on the structure. In fact, no destabilization and no changing in inter-bilayer periodical spacing were observed. In conclusion, SANS analysis provides fundamental information about drug-liposome interactions to comprehend the relation between system structure behaviour and its biological activity. Moreover, data suggest that the co-encapsulation of the two anti-TB drugs may have a synergic effect on liposome stability.

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Study on anti-fibrotic effects of phospholipid-based formulations in human hepatic stellate cells

Gina Valentino, Cristina Zivko, Lorine Brülisauer, Paola Luciani

Department of Pharmaceutical Technology, Institute of Pharmacy, Friedrich-Schiller-University Jena, Germany. E mail of presenting author: gina.valentino@uni-jena.de

During liver fibrogenesis, myofibroblastic transdifferentiation (or "activation") of hepatic stellate cells (HSC) has been identified as a key event [1]. Activated HSC are characterized by loss of lipid droplets and dysregulated scar proteins production. Essential phospholipids have shown to exert antioxidative and antifibrotic effects due to their polyenylphosphatidylcholine-rich (PPCs) fraction but mechanism of action and impact on HSC are still unclear [2]. The aim of this project is to develop novel PPC-based formulations and identify potential antifibrotic candidates by means of an *in vitro* model based on the differentiation of human immortalized HSC, LX-2.

Methods: Liposomes of soy phosphatidylcholine (SPC)**,** 1,2-dilinoleoyl-*sn*-glycero-3-phosphocholine (DLPC), 2-dioleoyl-*sn*-glycero-3-phosphocholine (DOPC) and DOPC/DLPC (53/47 mol %) were produced by extrusion method *and characterized by dynamic light scattering (DLS).* LX-2 cells, in their standard activated state, were incubated with liposomal formulations (05-20 mM) for 24 h, or further activated for 24 h with TGF-β (10 ng/mL) and then treated with liposomes (0.5-5 mM). Retinol and palmitic acid (PA) (10 mM retinol; 300 mM PA) were used a control to inactivate cells. LX-2 lipid droplets were stained with Oil Red O (ORO) while the scar production was followed by Sirius Red and Fast Green. Changes in membrane fluidity were studied by means of fluorescence anisotropy of the probe diphenylhexatriene (DPH) (8 µM) on adherent cells.

Results and Discussion: All liposomes had a hydrodynamic diameter *of < 200 nm (*polydispersity index < 0.15). *LX-2 exposed to DLPC, SPC, DOPC/DLPC treatments revealed an increase in cytoplasmic lipid droplets coincident with an intense ORO staining, indicating cell deactivation, visible also upon retinol and PA treatment.* In contrast, DOPC liposomes had only a minor effect on lipid droplets. Collagen appeared upon treatment with DOPC liposomes. On the contrary, DLPC, SPC and DOPC/DLPC liposomes treatments showed a lower content of collagen and more abundant non-collagenous proteins. Moreover, a decrease of LX-2 anisotropy upon treatment with all the liposomes and especially with DLPC was observed.

Conclusions: The abundance of lipid droplets, the deficiency of collagen and the rise in membrane fluidity in LX-2 exposed to SPC, DLPC and DOPC/DLPC suggest a reversion of HSC differentiation which occur in a minor extent upon treatment with DOPC. In order to select the most efficient excipients for novel phospholipid-based antifibrotic therapies and shed light on their mechanisms on liver fibrosis, an in-depth investigation of single PPCs components is ongoing.

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In vitro cytotoxicity evaluation of 5- Fluorouracil (5-FU) loaded PHB coated magnetic nanoparticles on breast cancer SKBR-3 cell line

Serap Yalcin,[a] Ufuk Gündüz[b]

[a] *Department of Molecular Biology and Genetics, Ahi Evran University,40100, Kırsehir, Turkey;* [b] *Department of Biology, Middle East Technical University,06800, Ankara, Turkey.*
Email of presenting author: serapyalcin1982@gmail.com

This study investigated the preparation, characterization and anti-proliferative effect of 5- Fluorouracil loaded PHB coated magnetic nanoparticles for breast cancer therapy. In this study, we developed a new method that enabled for the first time in situ and PHB coated MNPs, involving the coprecipitation of iron salts in the presence of PHB. The 5- Fluorouracil loaded PHB-MNPs was confirmed by FTIR analysis. In vitro cytotoxicity effects of 5- Fluorouracil-loaded PHB-MNPs on SKBR-3 cells were evaluated by means of the XTT Cell Proliferation Kit (Biological Industries, Israel) according to manufacturer's instructions. SKBR-3 cells were seeded into 96-well microtiter plates (Greiner) at a concentration of $5.0X10^4$. The cytotoxic effect of 5- Fluorouracil -loaded PHB-MNPs on SKBR-3 cells was investigated by XTT cell proliferation assay, and IC_{50} values were calculated. In this study, empty PHB-MNPs were found not significantly cytotoxic up to 500 mg/mL. The IC_{50} value of 600 mg/mL 5- Fluorouracil loaded PHB-MNPs were found as 12,5 µM on SKBR-3 cells. IC50 value of free 5- Fluorouracil as 23,7 µM for SKBR-3 cells. From the IC_{50} values, it was seen that the loading of 5- Fluorouracil onto PHB-MNPs increased its efficiency nearly up to 3- to 3.5-fold. The results indicated that the synthesized magnetic nanoparticles have a high potential to be used as a 5- Fluorouracil delivery system, which can be targeted to tumor cells under a magnetic field. The magnetic targeting will prevent the side effects of chemotherapy, while the effective release from the nanoparticles may reduce the amount of 5- Fluorouracil required, and the frequency of drug administration.

Synthesis and characterization of *Annona Muricata* extract loaded magnetic nanoparticles for cancer therapy

Serap Yalcin,[a] Rana Kavurmacı[b]

[a] *Department of Molecular Biology and Genetics, Ahi Evran University, 40100, Kırsehir, Turkey;* [b] *Department of Advanced Technologies, Ahi Evran University, 40100, Kırsehir, Turkey.*
Email of presenting author: serapyalcin1982@gmail.com

Annona muricata, also known as soursop, graviola, or guanabana, has traditionally been used to treat various diseases. Plant extracts include effectively toxins, alkaloids and acetogenins. Several studies have been investigated the in vitro effects of extracts on various cancer cell lines. However, plant extracts are also known toxic on neurons and have contributed to Parkinson's disease-like syndromes [1]. The magnetic nanoparticles can be widely used for cancer treatment which increases anti-cancer agents accumulation in the targeted tumor cells and healthy cells are not damaged. In this study, we investigated the preparation, characterization and anti-proliferative effect of Annona muricata extract loaded magnetic nanoparticles on triple negative cancer cell. we synthesed PHB coated MNPs, involving the coprecipitation of iron salts in the presence of PHB. The structural properties, functional groups, size distribution and magnetic properties of the synthesized nanoparticles were characterized by FT-IR, TEM, VSM, DLS analyses. The extract loaded PHB-MNPs was confirmed by FTIR analysis. Antiproliferative effects of extract loaded PHB-MNPs on MDA-MB-231 cells were evaluated by means of the XTT Cell Proliferation Kit (Biological Industries, Israel) according to manufacturer's instructions. As a results, IC_{50} value of free extract and extract-loaded magnetic nanoparticles were identified as 330µg/ml and 42 µM on MDA-MB-231 cells, respectively. In conclusion, the novel extract-loaded magnetic nanoparticles may constitute a biodegradable and biocompatible delivery system for targeted delivery to tumors without healthy cells damage.

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Overcoming drug resistance by cisplatin loaded magnetic nanoparticles on cisplatin resistant model cell line

Serap Yalcin,[a] Ufuk Gunduz[b]

[a] *Department of Molecular Biology and Genetics, Ahi Evran University,40100, Kırsehir, Turkey;* [b] *Department of Biology, Middle East Technical University,06800, Ankara, Turkey.*
Email of presenting author: serapyalcin1982@gmail.com

In this study, we investigated anti-proliferative effect of Cisplatin loaded PHB coated magnetic nanoparticles on Cisplatin resistance model cell line for breast cancer therapy. In this study, Cisplatin loaded PHB-MNPs was confirmed by FTIR analysis. Drug loading and release characteristics, and stability of the nanoparticles were investigated. Anti-proliferative effects of Cisplatin-loaded PHB-MNPs on Cisplatin resistance MDA-MB231 cells (1000nM) were evaluated by means of the XTT Cell Proliferation Kit (Biological Industries, Israel) according to manufacturer's instructions. Cisplatin was loaded onto PHB-MNPs, and the release efficiencies at different pHs were studied under in vitro conditions. The most efficient drug loading concentration was found about 80% at room temperature in phosphate-buffered saline (pH 7.4). The Cisplatin loaded MNPs were stable up to 2 months in neutral pH for mimicking physiological conditions. The cytotoxic effect of cisplatin-loaded PHB-MNPs on Cisplatin resistance cells was investigated by XTT cell proliferation assay, and IC_{50} values were calculated. In this study, empty PHB-MNPs were found not significantly cytotoxic up to 500 mg/ml. Cisplatin-loaded PHB-MNPs were about 2.5-fold more cytotoxic as compared with free drug on cisplatin resistance MDA-MB-231 cell line. The results indicated that the synthesized magnetic nanoparticles have a high potential to be used as a Cisplatin delivery system, which can be targeted to Cisplatin resistance tumor cells. The magnetic targeting will prevent the side effects of chemotherapy, while the effective release from the nanoparticles may reduce the amount of Cisplatin required in tumor.

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Delivery of miR-29 using nanoparticles suppress triple negative breast cancer cell growth

Serap Yalcin

Department of Molecular Biology and Genetics, Ahi Evran University,40100, Kırsehir.
Email: serapyalcin1982@gmail.com

Breast cancer is the most frequently diagnosed cancer in women worldwide. Molecular mechanisms play important roles in cancer progression and aggressivity. In the last years, micro-RNAs have been associated in molecular pathways of cancer and other diseases. Mir-29 has inhibitory role in tumorigenesis. Studies showed that miR-29 is downregulated in breast cancer [1,2]. In this study, to restore normal miR-29 levels in triple negative breast cancer cell line we developed an optimisation method. In this method, dextran nanoparticles conjugated with miR-29 in a delivery system that specifically targets triple negative cell line. Results indicated that the presence of miR-29 conjugated nanoparticles enhanced the selective delivery of miR-29. Further, the upregulation of miR-29 by nanoparticles resulted in the inhibition of cells growth in triple negative breast cancer cell line.

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Does length matter? Suitability of halloysite nanotubes in drug delivery applications

F. Tardani[a], S. Casciardi[b], B. Ruzicka[a], S. Sennato[a]

[a] CNR-ISC, Department of Physics, La Sapienza, Piazzale Aldo Moro 5, 00185 Roma, Italy; [b2]INAIL Research Institute, Department of Occupational and Environmental Medicine, Epidemiology and Hygene, Roma, Italy.
Email of presenting author: simona.sennato@roma1.infn.it

In last decades, a large number of different colloidal systems are under consideration as innovative carriers of biologically active compounds as alternative to viral vectors. The ideal nanocarrier would require high target specificity, high loading capacity, good stability, slow and controlled cargo release. Liposomes, vesicles and nanoparticles have been effectively shown as nanocarriers [1]. Even if quite promising, these systems are not always suitable for large-scale applications. In this context, recently the halloysite nanotubes (HNT) have been considered as an effective alternative for a number of reasons [2]. Firstly, HNTs are hydrophilic aluminium-silicate tubes with a nanometer-sized cavity (*lumen*) which can easily filled with the desired drug. It is widely reported that the loading process can be easily performed in water. Moreover, as other nanoclays, HNTs are naturally abundant, cheap and biocompatible. Despite the numerous studies about HNTs, some simple questions are still to be solved. The high density of HNTs ($2 g/cm^3$) produce annoying settling problems. Sedimentation can produce severe accumulation of HNTs in biological tissues [3]. In addition, commercial halloysite nanoclays are very polydisperse in length and diameter. As already observed for carbon nanotubes, cytotoxic effects are expected also for very long HNTs.

In order to improve their actual usability, a deeper knowledge of their behaviour in water dispersions is required. In this investigation, we used HNTs of different sources and purity. Aqueous dispersions were prepared with or without addition of salt and additives to monitor stability against aggregation and sedimentation. A very stable fraction was identified though Dynamic Light Scattering and microscopy measurements. This fraction is mainly composed of short tubes, freely diffusing in the solvent with low aggregation degree. These short tubes could have potential application as nanocarriers with high biocompatibility and low accumulation problems.

Figure 1. Effect of tube length and instability on settling and accumulation of circulating nanotubes in blood vessels.

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List of Participants

1	Aiello Stefano	Dipartimento di Chimica, Università di Roma "Sapienza", Rome, Italy. *Email: stefano.aiello@uniroma1.it*	**P1**
2	Akbaba Hasan	Department of Pharmaceutical Biotechnology, University of Ege, İzmir, Turkey. *Email: hasan.akbaba@ege.edu.tr*	**SL26**
3	Alberti Diego	Department of Molecular Biotechology and Health Sciences, University of Torino, Italy. *Email: diego.alberti@unito.it*	**SL1, SL22**
4	Albrecht Volker	Biolitec research GmbH, Jena, Germany. *Email: volker.albrecht@biolitec.com*	**SL2**
5	Amalfitano Adriana	Catholic University of Sacred Heard. *Email: adriana.amalfitano@unicatt.it*	**SL16, SL31, P2**
6	Arancia Giuseppe	Istituto Superiore di Sanità. *Email: giuseppe.arancia@libero.it*	
7	Arcovito Alessandro	Institute of Biochemistry and Clinical Biochemistry, Catholic University of Sacred Heart,Rome, Italy. *Email: alessandro.arcovito@unicatt.it*	**SL16, SL31, P2**
8	Astone Chiara	Università degli Studi di Roma "La Sapienza". *Email: chiara.astone@gmail.com*	
9	Bertoni Serena	Drug Research Program, Division of Pharmaceutical Chemistry and Technology, Faculty of Pharmacy, University of Helsinki, Finland; PharmTechLab, Department of Pharmacy and Biotechnology, University of Bologna, Italy. *Email: serena.bertoni4@unibo.it*	**SL23**
10	Bombelli Cecilia	CNR-IMC Istituto di Metodologie Chimiche Sezione Meccanismi di Reazione. *Email: cecilia.bombelli@uniroma1.it*	**SL10, P1, P14**
11	Borocci Stefano	University of Tuscia. *Email: borocci@unitus.it*	
12	Bozzuto Giuseppina	National Center for Drug Research and Evaluation, Istituto Superiore di Sanità Rome, Italy. *Email: giuseppina.bozzuto@iss.it*	**SL9**
13	Brocco Monica	Core facilities - Istituto Superiore di Sanità. *Email: monica.brocco@iss.it*	
14	Calcabrini Annarica	National Centre for Drug Research and Evaluation - ISS. *Email: annarica.calcabrini@iss.it*	**P7**
15	Caselli Federica	Department of Civil Engineering and Computer Science, University of Rome Tor Vergata, Rome, Italy. *Email: caselli@ing.uniroma2.it*	**SL8**
16	Ceccacci Francesca	CNR-IMC Istituto di Metodologie Chimiche Sezione Meccanismi di Reazione. *Email: francesca.ceccacci@uniroma1.it*	**P1**
17	Ceci Pierpaolo	Institute of Molecular Biology and Pathology, CNR , Rome, Italy. *Email: pierpaolo.ceci@cnr.it*	**SL16**
18	Celenza Giuseppe	Department of Biotechnological and Applied Clinical Sciences, University of l'Aquila, Italy. *Email: celenza@univaq.it*	**SL30**
19	Chistè Elena	Department of Computer Science, Fluorescence Laboratory, University of Verona, Italy. *Email: elena.chiste@univr.it*	**P4**
20	Chronopoulou Laura	Department of Chemistry, Sapienza University of Rome, , Italy. *Email: laura.chronopoulou@uniroma1.it*	**SL31**
21	Ciarlo Laura	National Center for Drug Research and Evaluation, Istituto Superiore di Sanità, Rome, Italy. *Email: laura.ciarlo@iss.it*	**P5, P19, P20**
22	Colombo Eleonora	Department of Chemistry, University of Milan, Italy. *Email: eleonora.colombo@unimi.it*	**P6**

23	Colone Marisa	National Centre for Drug Research and Evaluation - ISS. *Email: marisa.colone@iss.it*	**P7**
24	Condello Maria	National Centre for Drug Research and Evaluation - ISS. *Email: maria.condello@iss.it*	**SL9, P8**
25	Conte Claudia	Drug Delivery Laboratory, Department of Pharmacy, University of Napoli Federico II, Napoli, Italy. *Email: claudia.conte@unina.it*	**SL24, P15**
26	Costabile Gabriella	Department of Pharmacy, Ludwig-Maximilians-University, Munich, Germany. *Email: gabriella.costabile@cup.uni-muenchen.de*	**P9**
27	Couvreur Patrick	University of Paris-Sud, Université Paris-Saclay Institut Galien, UMR CNRS 8612, 5 rue J-B Clément F-92296 Chatenay-Malabry, France. *Email: patrick.couvreur@u-psud.fr*	**IL1**
28	D'Avenio Giuseppe	National Center for Technological Innovation in Public Health, Istituto Superiore di Sanità Rome, Italy. *Email: davenio@iss.it*	**SL9**
29	Decarolis Nadia	nordtest srl. *Email: ndecarolis@nordtest.it*	
30	Di Gioia Sante	Department of Medical and Surgical Sciences, University of Foggia, Italy. *Email: sante.digioia@unifg.it*	**SL13, P3**
31	Di Meo Chiara	Department of Drug Chemistry and Technologies, Sapienza University of Rome, Italy. *Email: chiara.dimeo@uniroma1.it*	**P10**
32	Diociaiuti Marco	Centro Nazionale Malattie Rare, Istituto Superiore di Sanità, Roma, Italy. *Email: marco.diociaiuti@iss.it*	**SL10, P14**
33	Djekic Ljiljana	Department of Pharmaceutical Technology and Cosmetology, University of Belgrade-Faculty of Pharmacy, Belgrade, Serbia. *Email: ljiljanadjek@gmail.com*	**P11, P12, P13**
34	Erel Akbaba Gülşah	Experimental Therapeutics and Molecular Imaging Lab, Department of Neurology, Neuro-Oncology Division, Massachusetts General Hospital, Boston, MA, USA; Department of Pharmaceutical Biotechnology, Faculty of Pharmacy, Izmir Katip Celebi University, Izmir, Turkey; Department of Pharmaceutical Biotechnology, Faculty of Pharmacy, Ege University, Izmir, Turkey. *Email: gulsaherel@gmail.com*	**SL21**
35	Fahr Alfred	Biolitec research GmbH, Jena, Germany. *Email: alfred.fahr@uni-jena.de*	**SL2**
36	Ferrari Angelo	CNR - Institute of Chemical Methodologies. *Email: angelo.ferrari@cnr.it*	
37	Ferroni Claudia	CNR - Institute of Organic Syntesis and Photoreactivity. *Email: claudia.ferroni@isof.cnr.it*	**SL6, SL17**
38	Fioravanti Raoul	Centro Nazionale Malattie Rare, Istituto Superiore di Sanità, Rome, Italy; *Email: raoul.fioravanti@uniroma1.it*	**SL10, P14**
39	Formisano Giuseppe	National Centre for Drug Research and Evaluation - ISS. *Email: giuseppe.formisano@iss.it*	
40	Fracassi Alessandro	Laboratorium für Organische Chemie, ETH Zürich, Switzerland. *Email: afracassi@org.chem.ethz.ch*	**SL29**
41	Gaio Elisa	Cell Biology Unit, Department of Biology, University of Padova. *Email: elisa.gaio@studenti.unipd.it*	**P15**
42	Galantini Luciano	Università La Sapienza. *Email: luciano.galantini@uniroma1.it*	
43	Garello Francesca	Department of Molecular Biotechnology and Health Sciences, University of Torino, Italy. *Email: francesca.garello@unito.it*	**SL22**

44	Gelain Fabrizio	ISBREMIT, IRCSS Casa Sollievo della Sofferenza, Opera di San Pio da Pietralcina, San Giovanni Rotondo, Foggia. Center for Nanomedicine and Tissue Engineering (CNTE), ASST Niguarda Ca Granda, Milan, Italy. *Email: f.gelain@css-mendel.it*	**IL8**
45	Giansanti Luisa	Department of Physical and Chemical Sciences, University of L'Aquila, Italy. *Email: luisa.giansanti@gmail.com*	**P8**
46	Giardini Giorgio	CNR - Institute of Chemical Methodologies. *Email: giorgio.giardini@cnr.it*	
47	Giordani Cristiano	Instituto de Física, Universidad de Antioquia, Medellín, Colombia. *Email: cristiano.giordani@udea.edu.co*	**SL10, P14**
48	Grimm Jan	Molecular Pharmacology Program & Department of Radiology - Memorial Sloan Kettering Cancer Center, New York, USA. Gerstner Sloan-Kettering Graduate School of Biomedical Sciences, New York, USA. Pharmacology, Radiology - Weill Cornell Medical College, New York, USA. Integrated Cancer Center - King's College London, UK. *Email: grimmj@mskcc.org*	**IL2**
49	Herrmann Inge Katrin	Swiss Federal Laboratories for Materials Science and Technology (Empa), St. Gallen, Switzerland. *Email: Inge.Herrmann@empa.ch*	**IL3**
50	Imbriano Anna	Department of Drug Chemistry and Technologies, Sapienza University of Rome, Italy. *Email: anna.imbriano@uniroma1.it*	**P16, P17**
51	Kovačević Anđelka	Institute of Pharmacy, Faculty of Biological Sciences, Friedrich Schiller University Jena, Germany. *Email: andelka.kovacevic@uni-jena.de*	**SL11**
52	Lolli Maria Grazia	National Research Council (CNR), Rome, Italy. *Email: mariagrazia.lolli@ift.cnr.it*	**P18**
53	Mancini Giovanna	Institute of Chemical Methodologies - CNR. *Email: giovanna.mancini@cnr.it*	**P1, P8**
54	Marianecci Carlotta	Department of Chemistry and Technology of Drugs, Sapienza University, Rome, Italy. *Email: carlotta.marianecci@uniroma1.it*	**IL7, P16,P17, P19, P26**
55	Markova Elena	Institute of pharmaceutical technology, Center of pharmaceutical nanotechnology, Faculty of pharmacy, Ss. Cyril & Methodius University, Skopje, R. Macedonia *Email: elena.markova1@yahoo.com*	**SL12**
56	Martini Cecilia	CNR - Institute of Organic Syntesis and Photoreactivity *Email: cecilia.martini@isof.cnr.it*	**SL16, SL17, P2**
57	Marzoli Francesca	National Center for Drug Research and Evaluation, Istituto Superiore di Sanità, Rome, Italy. *Email: francesca.marzoli@iss.it*	**P5, P19, P20**
58	Medard Nicolas	NANOLANE, Pole Novaxud, Le Mans, France. *Email: nicolas.medard@eolane.com*	**SL5**
59	Meschini Stefania	National Centre for Drug Research and Evaluation - ISS. *Email: stefania.meschini@iss.it*	**SL9, P8**
60	Minosi Paola	National Center for Drug Research and Evaluation, Istituto Superiore di Sanità, Rome, Italy. *Email: paola.minosi@iss.it*	**P5, P19, P20**
61	Mohan Lauren	Division of Biology, Department of Anatomy, School of Medicine; School of Pharmacy, Royal College of Surgeons in Ireland, Dublin 2, Ireland. *Email: laurenmohan@rcsi.ie*	**P21**
62	Molinari Agnese	National Centre for Drug Research and Evaluation - ISS. *Email: agnese.molinari@iss.it*	**SL9**
63	Murgia Sergio	Department of Chemical and Geological Sciences, University of Cagliari, Monserrato (CA), Italy. *Email: murgias@unica.it*	**SL4**

64	Nicoletti Isabella	CNR - Institute of Chemical Methodologies. *Email: isabella.nicoletti@cnr.it*	
65	Nikolić Ines	Department of Pharmaceutical Technology and Cosmetology, University Belgrade, Belgrade, Serbia. *Email: ines.nikolic@pharmacy.bg.ac.rs*	**P22**
66	Oliveira J. Miguel	3B's Research Group- Biomaterials, Biodegradable and Biomimetic, University of Minho, Headquarters of the European Institute of Excellence on Tissue Engineering and Regenerative Medicine, Avepark, 4805-017 Barco, Guimarães, Portugal; ICVS 3Bs PT Government Associate Lab, Braga, Guimarães, Portugal; The Discoveries Centre for Regenerative and Precision Medicine, Headquarters at University of Minho, Avepark, 4805-017 Barco, Guimarães, Portugal. *Email: miguel.oliveira@i3bs.uminho.pt*	**IL6**
67	Oliveira Junior Edilson	Laboratory of Pharmaceutical Technology - FarmaTec, Faculty of Pharmacy, Federal University of Goiás, Goiânia, Brazil. *Email: edilsonrojunior@gmail.com*	**P23**
68	Pellegrini Evelin	National Center for Drug Research and Evaluation, National Institute of Health, Rome, Italy. *Email: evelin.pellegrini@guest.iss.it*	**P8**
69	Pellegrino Chiara	AcZon S.r.l. *Email: chiara.pellegrino@aczonpharma.com*	
70	Pereira Rayanne Rocha	Pharmaceutical Sciences Faculty, Federal University of Para, Belem, Brazil; *Email: pereirarayanne52@gmail.com*	**P24**
71	Pieretti Stefano	National Center for Drug Research and Evaluation, Istituto Superiore di Sanità, Rome, Italy. *Email: stefano.pieretti@iss.it*	**P5, P19, P20**
72	Quici Massimo	CNR - Institute of Chemical Methodologies. *Email: massimo.quici@cnr.it*	
73	Raniolo Sofia	Department of Systems Medicine, University of Rome Tor Vergata, Rome, Italy. *Email: sofiaraniolo@gmail.com*	**P25**
74	Raucci Maria Grazia	Institute of Polymers, Composites and Biomaterials (IPCB) - CNR, Naples (Italy). *Email: mariagrazia.raucci@cnr.it*	**SL19**
75	Renault-Mahieux Morgane	AP-HP, Henri Mondor Hospital Group, Pharmacy Department, Créteil, France; UTCBS, INSERM U1022 UMR CNRS 8258, Paris Descartes University, Paris, France. *Email: morgane.renaultm@hotmail.com*	**SL15**
76	Rinaldi Federica	Center for Life Nano Science@Sapienza, Istituto Italiano di Tecnologia (ITT), Rome, Italy. *Email: federica.rinaldi@iit.it*	**P20, P26, P27**
77	Russo Krauss Irene	Department of Chemical Science, University of Naples Federico II, Naples, Italy; CSGI, Florence, Italy. *Email: IRENE.RUSSOKRAUSS@UNINA.IT*	**SL27**
78	Salvati Anna	Groningen Research Institute of Pharmacy, University of Groningen, The Netherlands. *Email: a.salvati@rug.nl*	**IL10**
79	Sansone Francesco	Dipartimento di Scienze Chimiche, della Vita e della Sostenibilità Ambientale, Università di Parma, Italy. *Email: francesco.sansone@unipr.it*	**SL7**
80	Santoliquido Roberto	ALFATEST s.r.l., Rome, Italy. *Email: roberto.santoliquido@alfatest.it*	**SL18**
81	Sarra Angelo	Department of Science, University of Roma Tre, Rome, Italy. *Email: angelo.sarra@uniroma3.it*	**SL25**

82	Sciolla Francesca	Istitute for Complex Systems (ISC) – CNR and Sapienza University of Rome. *Email: francesca.sciolla@gmail.com*	**P27**
83	Scipioni Anita	Università La Sapienza. *Email: anita.scipioni@uniroma1.it*	
84	Sennato Simona	Institute for Complex Systems ISC–CNR and Physics Department, Sapienza University of Rome, Italy. *Email: simona.sennato@roma1.infn.it*	**SL3, SL9,SL25, P27, P35**
85	Shalabalija Dushko	Institute of pharmaceutical technology, Center of pharmaceutical nanotechnology, Faculty of pharmacy, Ss. Cyril & Methodius University, Skopje, R. Macedonia. *Email: d_salabalija@hotmail.com*	**SL28, SL12**
86	Soleimanian Yasamin	Department of Food Science and Technology, Isfahan University of Technology, Isfahan, Iran. *Email: y.soleimanian@gmail.com*	**SL14**
87	Storm Gert	Dept. Pharmaceutics, Utrecht Institute for Pharmaceutical Sciences (UIPS), Utrecht University. University Medical Centre Utrecht (UMCU), Division Imaging, Utrecht. Dept. Biomaterials Science & Technology (BST), MIRA Institute for Biomedical Technology and Technical Medicine, University of Twente, Enschede, The Netherlands. *Email: G.Storm@uu.nl*	**IL9**
88	Stringaro Annarita	National Centre for Drug Research and Evaluation - ISS. *Email: annarita.stringaro@iss.it*	**P7**
89	Talamini Laura	IRCCS-Istituto di ricerche farmacologiche Mario Negri, Milano, Italy. *Email: laura.talamini@marionegri.it*	**SL20**
90	Tardiola Stefano	CNR - Institute of Chemical Methodologies. *Email: stefano.tardiola@cnr.it*	
91	Terreni Eleonora	Department of Pharmacy, University of Pisa, Italy. *Email: terreni.eleonora@gmail.com*	**P28**
92	Toccacieli Laura	National Centre for Drug Research and Evaluation - ISS. *Email: laura.toccacieli@iss.it*	
93	Truzzi Eleonora	Department of Life Sciences, University of Modena and Reggio Emilia, Modena, Italy. *Email: eleonora.truzzi@unimore.it*	**P23, P29**
94	Unida Valeria	Department of Biology, University of Rome Tor Vergata, Rome, Italy. *Email: valeria.unida@gmail.com*	**P25**
95	Valentino Gina	Department of Pharmaceutical Technology, Institute of Pharmacy, Friedrich-Schiller-University Jena, Germany. *Email: gina.valentino@uni-jena.de*	**P30**
96	Webster Thomas J.	The Art Zafiropoulo Chair and Department Chair Department of Chemical Engineering, Northeastern University, Boston, USA. *Email: th.webster@northeastern.edu*	**IL5**
97	Wurm Frederik	Max Planck Institute for Polymer Research, Mainz, Germany. *Email: wurm@mpip-mainz.mpg.de*	**IL4**
98	Yalcin Serap	Department of Molecular Biology and Genetics, Ahi Evran University, Kırsehir, Turkey. *Email: serapyalcin1982@gmail.com*	**P31, P32, P33, P34**

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