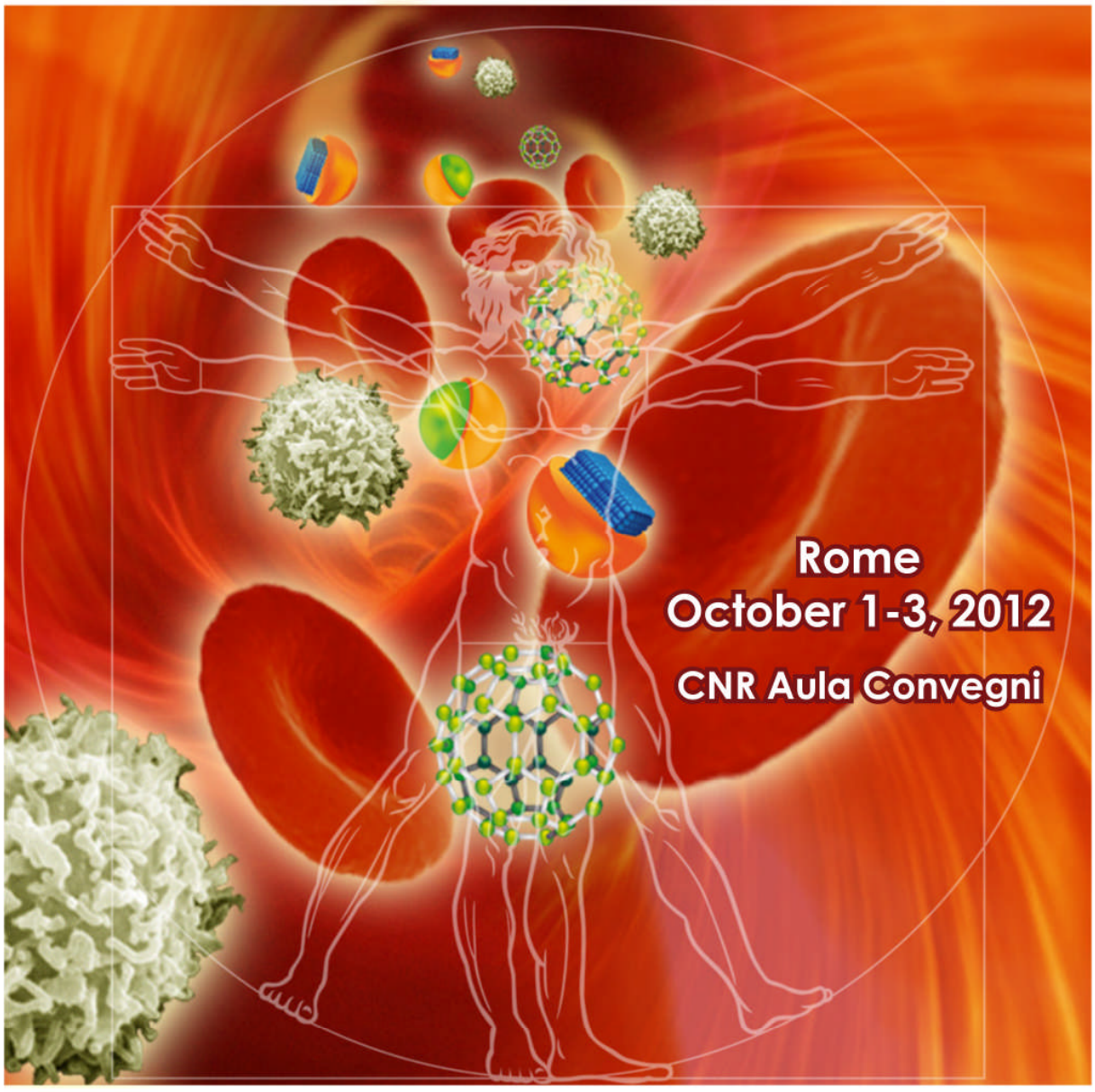
ABSTRACT BOOK

NANOMEDICINE: FROM MOLECULES TO DIAGNOSIS AND THERAPY



Rome
October 1-3, 2012

CNR Aula Convegni

Consiglio Nazionale delle Ricerche

Società Chimica Italiana

Istituto Superiore di Sanità

Abstract Book

NANOMEDICINE:
FROM MOLECULES TO DIAGNOSIS AND THERAPY

Rome

CONSIGLIO NAZIONALE DELLE RICERCHE

October, 1 - 3, 2012

The NANOMEDICINE: from Molecules to Diagnosis and Therapy Conference is organized under the sponsorship of

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CONFERENCE PROGRAM

Monday October 1

8.40 Registration

9.40 Opening Ceremony

Enrico Garaci,
President Istituto Superiore di Sanità, Rome

Giancarlo Angelini,
Director Institute of Chemical Methodologies, CNR, Rome

Velio Macellari,
Director Department of Technology and Health, ISS, Rome

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

Giovanna Mancini,
Institute of Chemical Methodologies, CNR, Rome

NANOPARTICLES FOR DIAGNOSIS

Chairpersons:

Nicolas Beziere, Davide Prosperi

Invited lectures

10.10 ***Yourii K. Gun'ko***
"Nanoparticles for biomedical applications"

10.50 ***Alberto Diaspro***
"How optical nanoscopy and super resolution microscopy can fit to nanomedicine?"

11.30 Coffee break

Selected lectures

11.50 ***Antonio Toppino***
"Synthesis and biological evaluation of new dual agents for MRI/BNCT applications"

12.10 ***Chiara Brioschi***
"Paramagnetic and fluorescent solid lipid nanoparticles targeting atherosclerotic plaques"

12.30 ***Francesca Costantini***
"Glucose level determination with enzymatic glass chip"

Industry Report

12.50 ***Massimo Placidi***
"Multiplexed label-free bio-affinity analysis and MALDI-MS characterisation of bound analyte"

13.10 *Lunch and poster session*

Chairpersons:

Yourii K.Gun'ko, Jean-Christophe Leroux

Invited lecture

14.30 ***Nicolas Beziere***
"Opto-acoustic imaging methods and applications; a powerful new approach to nano-particle imaging"

Selected lectures

15.10 ***Rouhollah Khodadust***
"Synthesis of dendrimeric magnetic nanoparticles and imaging studies using IgG-FITC"

15.30 ***Miriam Colombo***
"Site-specific conjugation of scfvs antibodies to nanoparticles by bioorthogonal strain-promoted alkyne–nitrone cycloaddition"

15.50 *Coffee break*

Invited lectures

16.10 ***Davide Prosperi***
"Nanodiagnostics: controlling the surface functionalization of nanoparticles to optimize the detection of cancer cells"

Selected lectures

16.50 ***Carlotta Marianeccci***
"MNPs in non ionic surfactant vesicles as smart delivery system for theranostic applications"

17.10 ***Lucia Pasquato***
"Toward a new generation of nanoparticles for therapy and diagnosis"

Tuesday October 2

TISSUE ENGINEERING

Chairpersons:

Pieter Cullis, Mauro Grigioni

Invited lectures

9.00 ***Beat H. Walpoth***
"Nanostructured Materials for Vascular Tissue Engineering"

Selected lectures

9.40 ***Gianni Ciofani***
"Myoblast behaviour on human recombinant elastin-like coatings"

10.00 ***Alessia Cedola***
"Early stage mineralization in tissue engineering mapped by high resolution X-ray microdiffraction and tomography"

10.20 ***Vittoria Raffa***
"Magnetic nanoparticles and magnetic fields direct neurite outgrowth: implication in nerve regeneration"

10.40 *Coffee break*

Invited lecture

11.00 ***Ruth Duncan***
"Polymer therapeutics as nanomedicines: from anticancer agents to tissue repair"

Selected lectures

11.40 **Valentina Corvaglia**
"Peptidic nucleic acid (PNA) assisted cellular migration along engineered surfaces"

12.00 ***Giada Graziana Genchi***
"Enhancement of neurite outgrowth and alignment in PC12 neuron-like cells on nanofibrous poly(3-hydroxybutyrate) substrates"

Invited lecture

12.20 ***Maurizio Prato***
"Nanomedicine with functionalized carbon nanotubes"

13.00 *Lunch and poster session*

Chairpersons:

Maurizio Prato, Velio Macellari

Selected lectures

14.50 ***Mauro Grigioni***
"From technological innovations to medical device"

15.10 **Antonio Amodeo**
"Cardiac cell therapy: a strategy for treatment of heart diseas"

Invited lecture

15.30 ***Gino Gerosa***
"Engineering of biomaterial properties at molecular level: the 3D nanoscale systems of self-assembling peptides in self-seeding heart valve design"

16.10 *Coffee break and poster session*

17.30 *Guided Tour at Palazzo Massimo*

20.00 *Social Dinner*

Wednesday October 3

NANOPARTICLES FOR THERAPY

Chairpersons:

Giovanna Mancini, Alberto Abraham Gabizon

Invited lecture

9.00 ***Jean-Christophe Leroux***
"Nanopharmaceutics as sequestering agents"

Selected lectures

9.40 ***Paolo Scrimin***
"Immune response elicited by saccharide-functionalized Gold nanoparticles"

10.00 ***Katayoun Derakhshandeh***
"The surface modified nanocarrier: anticancer efficacy, tissue distribution and blood pharmacokinetics of loaded anticancer drug"

10.20 ***Francesco Sansone***
"Guanidinium and arginine clustering on calixarene macrocyclic scaffolds as a novel strategy for improved cell transfection"

10.40 *Coffee break*

Invited lecture

11.00 ***Pieter Cullis***
"Lipid nanoparticle systems for in vivo delivery of siRNA: from basic science to clinical applications"

Selected lectures

11.40 ***Stefano Bellucci***
"Cytotoxic effects on human breast adenocarcinoma MCF-7 cell line induced by multi-walled carbon nanotubes"

12.00 ***Giancarlo Morelli***
"Bombesin labelled liposomes as target selective delivery system for Doxorubicin: in vitro and in vivo studies."

12.20 ***Claudia Conte***
"Biomimetic nanoparticles with sustained release: from conventional chemotherapy to combined strategies in treating cancer"

12.40 ***Giancarlo Masci***
"Polysaccharide nanogels by template chemical cross-linking in polyion complex micelle nanoreactors"

13.00 *Lunch and poster session*

Chairpersons:

Agnese Molinari, Ruth Duncan

Invited lecture

14.30 ***Livio Pagano***
"The clinical use of nanoparticles in patients with hematological malignacies"

15.10 ***Michele Caraglia***
"New nanotechnological tools for the delivery of zoledronic acids in human tumours: let's cross the brain!"

Selected lecture

15.50 ***Enzo Agostinelli***
"Endocannabinoids alone and in association with catalytically active bovine serum amine oxidase bound to magnetically drivable nanoparticles as a new anticancer therapy"

16.10 *Coffee break*

Selected lecture

16.30 ***Elena Simona Bacaita***
"Multiscale type behaviours of drug loaded polymeric micro/nanoparticles in the in-vitro release process."

Key note lecture

16.50 ***Alberto Abraham Gabizon***
"Liposomes as nanomedicine platform in cancer therapy"

17.30 *Closing remarks*

Keynote Lecture

Liposomes as Nanomedicine Platform in Cancer Therapy

Alberto Gabizon [a]

[a] *Department of Oncology, Shaare Zedek MC and Hebrew University-School of Medicine, Jerusalem, Israel.*

Email of presenting author: alberto.gabizon@gmail.com.

Many of the currently used chemotherapeutic agents in cancer have problematic toxicities compromising efficacy, and often resulting in life-threatening events. Nanomedical devices such as liposomes and other nanoparticles can provide effective control of the release rate and of the tissue distribution of these agents. The ensuing pharmacokinetic changes often have a major pharmacodynamic impact with attenuation of toxic effects, resulting in a substantial enhancement of the therapeutic index of the delivered drugs. Polyethylene-glycol (PEG) coating of liposomes results in inhibition of liposome uptake by the reticulo-endothelial system and significant prolongation of liposome residence time in the blood stream. A hallmark of these long-circulating liposomal drug carriers is their enhanced accumulation in tumors by a passive targeting mechanism known as enhanced permeability and retention effect. Currently, the main achievements of nanomedicine relevant to oncology practice are the controlled delivery of chemotherapeutic agents to improve their therapeutic index. An example of nanomedicine with demonstrated clinical added value in cancer therapy is PEG-liposomal doxorubicin, which has demonstrated clinically a favorable safety profile with an impressive reduction in cardiac toxicity and proven efficacy against various malignancies and can be considered as the first anti-cancer nanomedicine approved for clinical use[1]. Other forms of nanomedicine in clinical use include a cremophor-free albumin-based nanoparticle formulation of paclitaxel, and a mutivesicular lipid-based formulation of cytosine arabinoside for intrathecal administration[2]. Based on preclinical and early clinical studies, other formulations such as liposomal vincristine[3], and pegylated liposomal irinotecan[4] hold promise to offer an important clinical edge in cancer chemotherapy. Another approach applicable to liposomal drug delivery combines the concept design of a stable and long-circulating liposome with chemical modification of a drug to form a lipophilic prodrug with strong association to the liposomal bilayer. This is the case of a prodrug of mitomycin-C activated by thiolytic cleavage. PEG-liposomal mitomycin-C prodrug is more effective and less toxic than conventional chemotherapy in the treatment of various animals and human tumor models[5]. Co-encapsulation of synergistic combinations of agents in the same liposome is another potentially valuable approach in liposome delivery[6,7]. One example of this approach is a PEG-liposome formulation of alendronate, an amino-bisphosphonate with immuno-boosting properties, and doxorubicin[8]. Further to the passive targeting effect, the liposome drug delivery platform offers the possibility of grafting tumor-specific ligands on the liposome membrane for active targeting to tumor cells and/or tumor endothelial cells[9]. Ligand-specific targeting may facilitate intracellular drug delivery bypassing drug permeability barriers. Liposomes and other nanomedicines offer a unique platform for a variety of manipulations that can further enhance the value of the delivered drugs, and, importantly, provide real-time imaging of drug biodistribution using the nanocarrier as a theranostic platform[10].

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3. D. Thomas et al, *Cancer*, **2009**, *115*, 5490; **4.** D. Drummond et al, *Cancer Res*, **2006**, *66*, 3271.
5. A. Gabizon et al, *J Control Release*, **2012**, *160*, 245.
6. E. Feldman et al, *J Clin Oncol*, **2011**, *29*, 979; **7.** D. Zucker et al, *J Control Release*, **2012**, *160*, 281.
8. A. Gabizon et al, *unpublished*; **9.** W. Cheng, T. Allen, *Expert Opin Drug Deliv*, **2010**, *7*, 461.
10. A. Petersen et al., *Biomaterials*, **2011**, *32*, 2334.

Invited Lectures

Nanoparticles for biomedical applications

Yurii K. Gun'ko

School of Chemistry and CRANN, Trinity College Dublin, Dublin 2, Ireland

Email of presenting author: igounko@tcd.ie.

Recent advances and progress in nanobiotechnology have demonstrated that nanomaterials have a great potential as novel drug delivery vehicles, therapeutic agents, contrast agents and luminescent biological labels for bioimaging. The utilization of new nanosized materials in biology and medicine is a new fast developing research area. This mostly involves the application of nanoparticulate contrast agents and assays for biomedical imaging and diagnostics. Magnetic nanoparticles are of particular importance. For example magnetic particles can be utilized as drug delivery agents, which can be localized in the body at a site of interest using an external magnetic field [1]. Magnetic fluids based on aqueous dispersions of small size superparamagnetic nanoparticles have also been utilized as contrast agents for magnetic resonance imaging (MRI). Our research resulted in the development of new contrast agents for magnetic resonance imaging based on one dimensional linear assemblies of magnetic nanoparticles (Figure 1) [2, 3]. We have shown the potential use of these materials as contrast agents by measuring their MR responses in live rats. The new magnetic fluids have shown good biocompatibility and potential for *in vivo* MRI diagnostics. Another aspect of our research involves the utilisation of fluorescent nanomaterials such as multimodal fluorescent-magnetic nanoparticles and quantum dots (semiconducting nanoparticles) for cell imaging, biosensing and drug delivery [4, 5]. As result of the work we have developed new approaches for specific intracellular bio-labelling and targeted drug delivery using these nanocomposites. These nanosystems are also potentially applicable for controlled modification of structural and functional properties of extracellular components and tissue constituents.

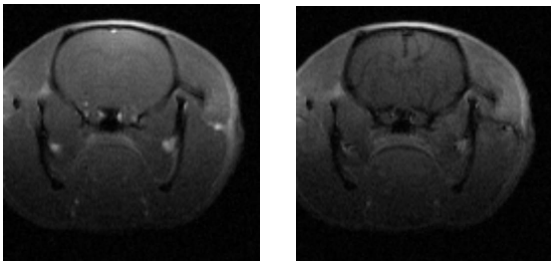

Figure 1. Fast Low Angle Shot image of mouse brain before (left) and after nanoparticulate contrast agent passes through (right).

1. Magnetic Nanomaterials, Ed. C. Kumar, book series "Nanotechnologies for the Life Sciences" WILEY-VCH, 2009.
2. S. A. Corr, S. J. Byrne, R. Tekoriute, C. J. Meledandri, D. F. Brougham, M. Lynch, C. Kerskens, L. O'Dwyer and Y.K. Gun'ko, *J. Amer. Chem. Soc*., **2008**, *130*, 4214.
3. G.-L. Davies, S. A. Corr, C. J. Meledandri, L. Briode, D. F. Brougham and Y.K. Gun'ko, *ChemPhysChem*, **2011**, *12*, 772.
4. J S. A. Corr, A. O' Byrne, Y. K. Gun'ko, S. Ghosh, D. F. Brougham, S. Mitchell, Y. Volkov and A. Prina-Mello, *Chem. Comm*., **2006**, *43*, 4474.
5. E. Govan, E. Jan, A. Querejeta, N. A. Kotov and Y. K. Gun'ko, Chiral luminescent CdS nano-tetrapods, *Chem. Comm*, **2010**, *46*, 6072.

How Optical Nanoscopy and Super resolution microscopy can fit to Nanomedicine?

Alberto Diaspro

Department of Nanophysics, Istituto Italiano di Tecnologia, Genova, Italy.
Department of Physics, Università degli Studi di Genova, Italy.

Email of presenting author: diaspro@fisica.unige.it

Several methodologies have been developed over the past several years for optical nanoscopy and super-resolution fluorescence microscopy. Among them stimulated emission depletion microscopy (STED), photoactivated localization microscopy (PALM), fluorescence photo activation localization microscopy (FPALM), and stochastic optical reconstruction microscopy (STORM). They have shown great promise for biological and medical research even if having some individual strengths and weaknesses. At the IIT headquarter in Genoa, we have recently developed original approaches aiming to 3D imaging of large scattering objects (F.Cella Zanacchi et al., Nature Methods (2011); P. Bianchini et al. PNAS, (2012)) and to achieve an
important flexibility (Galiani et al., Optics Express (2012). As well, we applied super resolution methods to direct laser writing on nanocomposite materials (B. Harke et al., ChemPhysChem (2012) and to atomic and force spectroscopy (B.Harke et al, Optical Nanoscopy (2012)). This lecture will describe the basic principles for
achieving super resolution, demonstrate some applications in biology and medicine. In particular, it will be outlined how such methods can fit to Nanomedicine: from nanocasules tracking to moleculecular effects of drugs, from confined active photointeractions to 3D super resolution imaging in vivo. A special kind of problems that can be attacked using such methods is the one related to study the fate and effect of biofunctionalization of nanoparticles for drug delivery in nanomedicine. Since both fields are comparatively new, scientific discussion would help in focusing on new outstanding goals.

Opto-acoustic imaging methods and applications: a powerful new approach to nano-particle imaging.

Nicolas Bézière[a], Vasilis Ntziachristos[a]

[a]*Institute for Biological and Medical Imaging, Technische Universität München and Helmholtz Zentrum München, Ingolstädter Landstraße 1, D-85764 Neuherberg, Germany.*

Email of presenting author: nicolas.beziere@helmholtz-muenchen.de.

Optical imaging is a powerful modality in imaging, in particular biological samples, providing extremely high resolution images. However, *in vivo*, this modality becomes less useful due to its very limited penetration depth due to photon scattering. While several solutions have been developed to try to circumvent the naked eye limitations, they remain unsatisfactory when it comes to observing whole organisms. Opto-acoustic imaging, relying on the optoacoustic effect (the emission of sonic waves by photoabsorbers caused by transient illumination by laser pulses) allows bypassing of the usual optical imaging depth barrier. This hybrid modality allows for the unique mapping of light absorption in a complete living animal with a resolution that can reach the micrometer range. As light can be absorbed by both endogenous components (blood, melanin…) and exogenous absorbers (fluorophores, nanoparticles…) it provides material scientists and life science specialists an innovative tool to analyze samples in a powerful way.

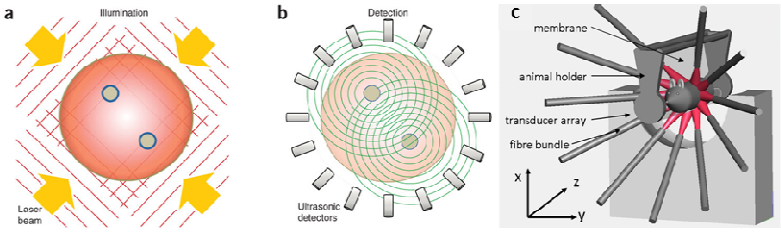

Figure 1. Opto-acoustic principle with illumination (a) and ultrasonic emission (b)[1] from discrete samples and set-up for in vivo experiments (c) [2].

While hardware and software development are still ongoing, the attention of optoacoustic research is slowly shifting toward the different possible biological applications of the modality. In particular, drug tracking can be and has been performed *in vivo* using different types of nanoparticles. Notably, recent development on gold nanoparticle contrast agents for opto-acoustic imaging, as well as the tracking of liposomes in living animals will be presented.

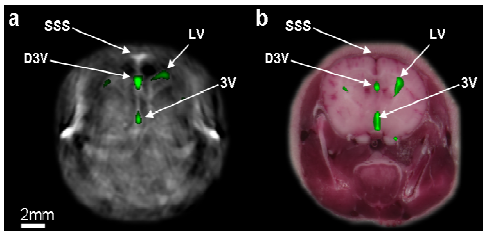

Figure 2. Imaging of fluorescent liposomes loaded with gold nanorods in the ventricles of a mouse brain using opto-acoustic (a) and classical cryo-sectioning (b)[3].

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2. A. Buehler et al. *Opt. Lett.*, **2010**, *35*, 2475.
3. N. Lozano et al. *JACS*, **2012**, epub ahead of print.

Nanodiagnostics: controlling the surface functionalization of nanoparticles to optimize the detection of cancer cells

Davide Prosperi

Department of Biotechnology and Bioscience, University of Milano-Bicocca, Piazza della Scienza 2, 20126, Milano, Italy;

Email of presenting author:davide.prosperi@unimib.it.

Magnetic resonance imaging (MRI) is a broadly utilized noninvasive clinical imaging method for prevention and treatment of malignant diseases. However, MRI suffers from low sensitivity; therefore, great efforts have been made to develop efficient target-oriented contrast agents. In this context, multifunctional nanoparticles (MFN), which combine multiple properties, including fluorescence emission and optical, magnetic and electronic unique characteristics, have been envisaged as promising multimodal tracers for noninvasive diagnosis of cancer.[1] The design of ideal targeted MFN needs careful optimization of fundamental features including uniform size and shape, surface charge, optical and magnetic properties, and efficient functionalization with suitable homing ligands to improve the signal amplification and target selectivity toward malignant cells. One of the greatest challenges in designing MFN functionalized with proteins resides in the possibility to control the ligand orientation on the nanoparticle surface.[2] One strategy involves the use of fusion proteins containing a low-molecular weight enzyme that recognizes a suicide inhibitor anchored to the solid surface resulting in a covalent immobilization.[3] An alternative route makes use of a bioactive protein shell consisting in a newly designed recombinant single-domain protein A fragment, which mediates an orderly Fc site-specific antibody immobilization on MFN resulting in a target-directed Fab presentation.[4,5] As a case study for the development of tumor-targeting nanoprobes, we focus on a anti-HER2 monoclonal antibody, which can bind the HER2 receptor in metastasizing breast cancer cells. This novel targeted nanoparticle model is assessed by fluorescence analysis, MRI and ultrastuctural investigation, both in vitro and in vivo.[6]

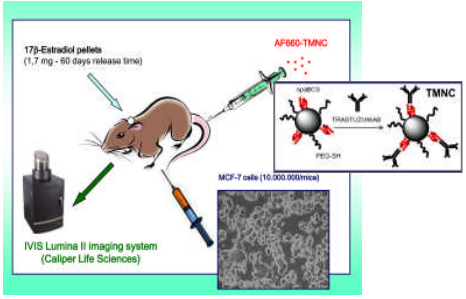

Figure 1. Schematic representation of an experimental setup using targeted nanoparticles to localize HER2-positive MCF7 cell in mice.

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2. J. B. Haun, et al., *Nat. Nanotech.*, **2010**, *5*, 660.
3. M. Colombo, et al., *Small*, **2012**, *8*, 1492.
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6. F. Corsi, et al., *ACS Nano*, **2011**, *5*, 6383.

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Nanostructured Materials for Vascular Tissue Engineering

Beat H. Walpoth, MD, FAHA

Cardiovascular Research, Department of Surgery, Geneva University Hospital, Faculty of Medicine, Geneva, Switzerland

Email of presenting author: Beat.Walpoth@hcuge.ch

Background: Long-term patency of conventional synthetic grafts is unsatisfactory below 6mm of internal diameter (ID). Poly(ε-caprolactone) (PCL) is a promising biodegradable polymer with longer degradation time. We aimed to evaluate in vivo healing and degradation characteristics of small-diameter vascular grafts made of PCL nanofibers in comparison with expanded polytetrafluoroethylene (ePTFE) grafts.

Methods and Results: 2-mm ID grafts were prepared by electrospinning using PCL (Mn=80,000 g/mol). Either PCL (n=15) or ePTFE (n=15) grafts were implanted into 30 rats. Rats were followed up to 24 weeks. At conclusion of the follow-up period, patency and structural integrity were evaluated by digital subtraction angiography (DSA). The abdominal aorta including the graft was harvested, and investigated under light microscopy. Endothelial coverage, neointima formation and transmural cellular in-growth were measured by computed histo-morphometry. All animals survived until the end of follow-up, and all grafts were patent in both groups. DSA revealed no stenosis in the PCL group, but stenotic lesions in one graft at 18 weeks (40%) and in another one at 24 weeks (50%) in the ePTFE group. None of the grafts showed aneurismal dilatation. Endothelial coverage was significantly better in the PCL group. Neointima formation was comparable between the two groups. Macrophage and fibroblast in-growth with extracellular matrix formation, and neoangiogenesis were better in the PCL group. After 12 weeks, foci of chondroid metaplasia located in the neointima of PCL grafts were observed in all samples.

Conclusions: Nano-structured, small-diameter PCL grafts represent a promising alternative for the future regarding their better healing characteristics compared to ePTFE grafts. Faster endothelialization and extracellular matrix formation accompanied with degradation of graft fibers seem to be the major advantages. Further evaluation of degradation and graft healing characteristics may potentially lead to clinical use of such grafts for revascularization procedures.

Polymer Therapeutics as Nanomedicines: From Anticancer Agents to Tissue Repair

Ruth Duncan

Cardiff, UK and [c/o]Polymer Therapeutics Lab., Centro de Investigación Príncipe Felipe, Valencia, Spain

Email of presenting author: profruthduncan@btinternet.com; http://www.ruthduncan.co.uk

Over recent decades a growing number first generation nanomedicine(s) (> 40) have entered routine clinical use as nanopharmaceuticals and imaging agents (reviewed in [1]). There are currently >70 products in clinical trials just as anticancer therapies. Much has been learnt regarding the critical product attributes (in relation to safety and efficacy) of such complex, multicomponent systems, and this is providing an exciting platform for to the introduction of next generation nanotechnologies. With the convergence scientific disciplines seeking to apply advances in nanoscience and new nanomaterials to healthcare applications it is important to understand the difference between 'nice science', and those nanotechnologies that have real potential to contribute clinically useful products. The key to success is an understanding of translational needs, and the regulatory path governing the journey from Lab to Clinic.

An increasing number of polymer therapeutics have gained market authorisation and this family may be considered amongst the most successful nanomedicines to date (reviewed in [2-4]). Preclinical and clinical experience has brought new insights regarding the most appropriate preclinical models for lead candidate optimisation, and clinically important biomarkers that may enable selection of the most suitable patients for nanomedicine therapy [1,5]. Early polymer therapeutics were developed as anticancer agents, but treatments for other target diseases, together with various routes of administration, have followed [4]. Most polymer-protein and polymer-aptamer conjugates involve PEG. We used N-(2-hydroxypropyl)methacrylamide (HPMA) copolymers to create the first synthetic polymer-based anticancer polymer-drug conjugates [2,3]. Although are so far well-tolerated clinically, they are non-biodegradable. Even if lower molecular weight polymers are used, following endocytic capture there is inevitably be a potential risk of lysosomal accumulation, this could be a particular problem with chronic administration. Recently we reported a new approach for protein conjugation called polymer-masking–unmasking protein therapy (PUMPT) [4]. This uses a multi-functional, biodegradable polymer (dextrin or hyaluronic acid) for conjugation The polymer can envelope a protein thus masking its bioactivity. Locally triggered polymer degradation allows time-dependant protein ‘unmasking’, resulting in local, controlled reinstatement of bioactivity. Proof of concept has been demonstrated using dextrin conjugates of phospholipase A2 an anticancer agent, and of human recombinant epidermal growth factor (rhEGF) for topical administration as a putative treatment for chronic wounds [6-8].

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2. R. Duncan, *Nature Rev. Drug Discov* **(2003)** 2(5), 347.
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Nanomedicine with Functionalized Carbon Nanotubes

Maurizio Prato

Dipartimento di Scienze Chimiche e Farmaceutiche, Università degli Studi di Trieste, Piazzale Europa 1, 34127 Trieste, Italy

Email of presenting author: prato@units.it

Nanomedicine represents a novel research topics which is receiving a lot of attention because of its high potential. Among the wide range of novel nanometer scale structures available, single-wall carbon nanotubes (SWNT) and multi-wall carbon nanotubes (MWNT) stand as unique materials for fundamental research and potential applications in nanomedicine.
Our group has been involved in the organic functionalization of various types of nanocarbons, including carbon nanotubes, nanohorns, fullerenes and nanoonions. The organic functionalization offers the great advantage of producing soluble and easy-to-handle CNTs. As a consequence, since biocompatibility of CNT is expected to improve, many functionalized carbon nanotubes may find useful applications in the field of nanomedicine. Their use as drug delivery agents and scaffolds for vaccines has already been demonstrated. CNT functionalized with bioactive moieties are particularly suited for targeted drug delivery. In fact, not only they exhibit reduced toxicity but also possess a high propensity to cross cell membranes.
The use of carbon nanotubes as active substrates for neuronal growth has given so far very exciting results. Nanotubes are compatible with neurons, but especially they play a very interesting role in interneuron communication. Improved synaptic plasticity is just one example.
During this talk, we will discuss the use of CNTs for applications in drug delivery and regenerative medicine.

Engineering of biomaterial properties at molecular level: the 3D nanoscale systems of self-assembling peptides in self-seeding heart valve design

G. Gerosa, M. Dettin

Dpt. Cardiac Thoracic and Vascular Sciences and Dpt. of Industrial Engineering – University of Padova, Italy

Email of presenting author: gino.gerosa@unipd.it

Abstract — Biological prostheses generally have good hemodynamic characteristics and avoid long-term pharmacological therapies. Their treatment with glutaraldehyde determines progressive tissue deterioration with consequent necessity of new surgery. Furthermore, all commercially available tissue valve substitutes are nonviable and consequently their utility in surgery for children is limited.
The goal of this project is to engineer self-seeding heart valves that, allowing cell repopulation, mature quickly *in vivo* and have a shorter preparation time. For this purpose, self-assembling peptides hydrogels could be used as filler to add beneficial properties to decellularized scaffolds.
These synthetic peptides have a high propensity to form beta-sheets that in PBS spontaneously assemble into nanofibrous scaffolds. The resulting hydrogels, resistant to proteolytic digestion and completely atoxic, are considered very promising scaffolds for several cell types (neuronal cells, cardiomyocytes, chondrocites, osteoblasts, mesenchymal stem cells).
In this project, the decellularized pericardium is the 3D scaffolding whereas the SAP hydrogel creates a fluffy nanofibrous environment able to call and house cells inside the natural decellularized scaffold. Here we demonstrate the capacity of SAP to penetrate and to remain inside the structure obtained simply by pre-incubation of decellularized pericardium in a peptide solution, that is a very novel and smart approach. The presence of peptides induces pericardium hydration and increase in thickness. Biological assays using mesenchymal stem cells are in progress.

Nanopharmaceutics as sequestering agents

Jean-Christophe Leroux

Institute of Pharmaceutical Sciences, Department of Chemistry and Applied Biosciences, ETH Zürich, Wolfgang Pauli Str. 10, 8093 Zürich, Switzerland.

Email of presenting author: jleroux@ethz.ch

Traditionally, colloids such as liposomes, nanoparticles and polymers have been used in pharmaceutical sciences as delivery systems for drugs and imaging agents. There are, however, cases where they can treat diseases or intoxications by removing endogenous or exogenous toxic compounds from the body. Well known examples are activated charcoal and the polymeric binder sevelamer, which are indicated in the treatment of drug overdose and hyperphosphatemia, respectively. In the past few years, the field of biodetoxification has indeed experienced a rapid growth with the emergence of highly active sequestering colloids, and their evaluation in novel indications (*e.g.* infections, inflammation)[1]. In this presentation, we will cover two applications, namely the treatment of calcium channel blocker intoxications and celiac disease (*i.e.* autoimmune enteropathy triggered by gluten in genetically sensitive individuals) where liposomes and polymeric binders can function as effective sequestrants. Transmembrane pH-gradient liposomes were optimized to extract from serum the calcium channel blockers diltiazem and verapamil. When the liposomes were intravenously injected to rats one hour after an oral dose of verapamil, the plasmatic drug levels increased dramatically indicating a redistribution of verapamil in the blood compartment. This resulted in an attenuation of the pharmacological activity of the drug and a faster recovery of cardiovascular parameters after an overdose[2]. In the case of celiac disease, a polymeric binder was designed to sequester the immunogenic protein fraction of gluten in the gastrointestinal tract. In a transgenic mouse model of gluten sensitivity, the oral administration of the polymeric binder prior to gluten intake reduced the immunogenic response and the deleterious effects of gluten on the intestinal mucosa. Moreover, the polymer was not absorbed and excreted in the feces[3]. This approach could represent a potential adjuvant therapy to a gluten free diet in unresponsive patients.

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Lipid Nanoparticle Systems for In Vivo Delivery of siRNA: From Basic Science to Clinical Applications

Pieter Cullis

The University of British Columbia

Email of presenting author: pieterc@mail.ubc.ca

RNAi-based drugs such as siRNA require sophisticated delivery systems in order to achieve therapeutic benefits. These delivery systems must protect encapsulated siRNA from degradation in the circulation, promote accumulation in target tissue and facilitate intracellular delivery into target cells. In order to be suitable for clinical use, these delivery systems must also be relatively non-toxic and must encapsulate siRNA efficiently into well-defined, reproducible nanomedicines using a scalable manufacturing process. Lipid nanoparticles (LNP) are currently the leading delivery systems for satisfying these demands. Efficient loading into LNP can be achieved using ionizable cationic lipids that are relatively non-toxic and can be optimized to achieve maximum intracellular delivery of siRNA following uptake into target cells. With regard to manufacture of LNP siRNA systems, formulation processes require rapid mixing of an aqueous stream, containing siRNA, with an enthanolic solution containing cationic lipid and PEG-lipid. We have devised scalable microfluidic mixing technology that results in the formation of LNP siRNA systems over the size range 20-100 nm with siRNA encapsulation efficiencies approaching 100%. It is shown that LNP siRNA systems containing optimized ionizable cationic lipids are highly potent and relatively non-toxic agents for silencing hepatocyte target genes following i.v. injection, achieving 50% or greater target gene silencing of 10 μg siRNA/kg body weight with therapeutic indices of 1000 or higher. This is currently the world-leading “gold standard” for the potency of siRNA-based therapeutics in vivo.

The clinical use of nanoparticles in patients with hematological malignancies

Livio Pagano

Cattedra di Ematologia, Università Cattolica S. Cuore, Roma

Email of presenting author:lpagano@rm.unicatt.it

Current cancer treatments include surgical intervention, radiation and chemotherapeutic drugs, which often also kill healthy cells and cause toxicity to the patient. It would therefore be desirable to develop drugs that can either passively or actively target neoplastic cells, without causing damage to the normal cells. The use of targeting nanocarriers in this scenario, above all based on liposomes and polymer–protein conjugates, are approved for wider use.

Nano drugs are currently utilized in patients with hematological malignancies both for the treatment of underlying malignancies and for the treatment of side effects due to the chemotherapy.

Among the drugs frequently employed, a relevant role is played by liposomal compounds. In fact liposomal doxorubicin and daunorubicin were already tested in acute leukemia and lymphoma patients with good results. The major advantage of these compounds is in particular the lower cardiotoxicity. The oldest and better known drugs utilized in hematology among liposomal compounds are liposomal Amphotericin B (L-AmB). In fact due to the neutropenia that followed chemotherapy above all acute leukemia patients can develop infectious complications and the most dangerous, among these, are fungal infections. These infectious diseases are characterized by a high mortality rate. Until some years ago the only antifungal agent at our disposition was deoxycolate AmB, however this compound was characterized by relevant side effects that reduced the efficacy. From 1997 L-AmB is available and clinical trials showed a better tolerance and a higher efficacy so that at present it is the drug of choice for these complications.

Another category of nano drugs widely utilized are monoclonal antibodies conjugated with toxin (i.e. Gentuzumab = anti-CD33 plus calicheamicin) or radio-conjugated (i.e. Ibritumomab = anti CD20 plus 90Y). The first showed a high efficacy in the treatment of acute myeloid leukemia particularly in elderly patients, while the second is indicated in refractory/resistant non-Hodgkin's Lymphomas.

Also other drugs as Peg-asparaginase or Peg-G-CSF are currently utilized. The first in the treatment of acute lymphoblastic leukemia, while the second that is growth factors that stimulate the production of neutrophils after chemotherapy is usually administered in order to prevent febrile neutropenia, maintain the time scheduling of chemotherapies and to collect CD34 cells for autologous transplantations. However the real advantage of these last drugs is not completely clear.

New nanotechnological tools for the delivery of zoledronic acids in human tumours: let's cross the brain!

Michele Caraglia[1], Manuela Porru[2], Silvia Zappavigna[1], Amalia Luce[1], Alida Panico[1], Giuseppina Salzano[3], Sara Lusa[3], Giuseppe De Rosa[3], Carlo Leonetti[2]

[1]Department of Biochemistry, Biophysics and General Pathology, Second University of Naples; [2]Experimental Chemotherapy Laboratory, Regina Elena Cancer Institute, Rome; [3]Department of Chemistry and Pharmaceutical Chemistry Toxicology, University "Federico II" of Naples.

Email of presenting author: michele.caraglia@alice.it

The emergence of nanotechnology could allow the optimization of the delivery of anti-cancer agents in tumour tissues and are also useful for the crossing of physiological barriers such as blood-brain-barrier (BBB)[1]. On this light, zoledronic acic (ZOL) is a drug used for the treatment of bone metastases and recent data report that beneficial effect of ZOL may result from a direct anti-tumour activity. One of the most important limits of ZOL is its limited delivery in tumour tissues and excessive accumulation in the bone[2]. In the light of these considerations, there is a need to develop new ZOL formulations with a lower affinity for bone and a longer half-life in the circulation that would result in increased probability to affect peripheral tumours. We have developed pegylated liposomes encapsulating ZOL (Lipo-ZOL) that has a stronger anti-cancer activity both *in vitro* and *in vivo* on several cancer cell lines of different histogenesis. In details, they were able to potentiate both the apoptotic and anti-angiogenic effects of ZOL in prostate tumour tissues xenografted in nude mice[3]. However, the low encapsulation efficiency of ZOL in liposomes and their size were technical limitations to be overcome. Therefore, we developed ZOL-containing self-assembly PEGylated nanoparticles (ZOL-NPs) based on ZOL complexes with calcium phosphate NPs (CaPZ NPs) and cationic liposomes. They were based on a ready-to-use preparation technology and were characterized by a high ZOL loading efficiency, optimized sizes and good size distribution. Moreover, they were more potent than ZOL in inducing cell growth inhibition on several cancer cell lines of different histogenesis. We have compared the anti-cancer properties of either ZOL-NPs or LIPO-ZOL. The anti-cancer activity of ZOL-NPs in nude mice xenografted with prostate cancer PC3 cells was higher than that one induced by LIPO-ZOL. In addition, ZOL-NPs induced the complete remission of tumour xenografts and an increase of survival time higher than that one observed with LIPO-ZOL. It has also to be considered that PC3 tumour xenografts were almost completely resistant to the anti-cancer effects induced by free ZOL. Both nanotechnological products did not induce toxic effects not affecting the mice weight nor inducing deaths. Moreover, the histological examination of some vital organs such as liver, kidney and spleen did not find any changes in terms of necrotic effects or modifications in the inflammatory infiltrate. On the other hand, ZOL-NPs but not LIPO-ZOL caused a statistically significant reduction of the tumour associated macrophages (TAM) in tumour xenografts. This effect was paralleled by a significant increase of both necrotic and apoptotic indexes. The effects of the NPs were also higher in terms of neo-angiogenesis inhibition[4].

Another challenge for nanomedicine is the treatment of central nervous system (CNS) disorders. In fact, the presence of the blood-brain barrier (BBB), formed by a complex interplay of endothelial cells, astrocyte and pericytes, strongly affects the crossing of therapeutic moieties towards CNS. Therefore, we have functionalized these nanoparticles with transferrin (TRF) in order to allow their crossing through BBB with the aim to treat glioblastomas.

The encapsulation in NPs resulted in higher in vitro cytotoxic activity than free ZOL on Ln229, U373MG and U87MG glioblastoma cells. However, the potentiation of anti-proliferative activity of TRF conjugated NPs was less than that one induced by naked NPs and correlated with TRF receptor expression on tumour cells. On the other hand, TRF-NPs-ZOL showed a higher antitumor efficacy if compared with that one caused by naked NPs-ZOL in mice-bearing. In conclusion, a new dawn in the treatment of glioblastoma can rise with the use of these modified nano-tools.

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Selected Lectures

Synthesis and biological evaluation of new dual agents for MRI/BNCT applications

Antonio Toppino, Annamaria Deagostino, Simonetta Geninatti, Diego Alberti, Paolo Venturello, and Silvio Aime.

Dipartimento di Chimica, Università degli Studi di Torino, Via Pietro Giuria, 7, 10125, Torino, Italy

Email of presenting author: antonio.toppino@unito.it

BNCT (Boron Neutron Capture Therapy) is a binary therapy for the treatment of cancer based on the selective uptake of the stable ^{10}B isotope by tumor cells, followed by irradiation with low energy thermal neutrons. In order to be effective BNCT requires 15–30 μg of ^{10}B per g of tumor, therefore, *in vivo* visualization of ^{10}B distribution is important.[1] Thanks to its superb spatial resolution MRI appears to be one of the most appropriate technique to tackle this task. In this work the synthesis and the *in vitro* and *in vivo* biological evaluation of a panel of new dual agents for MRI/BNCT applications is reported. Those agents are obtained starting from a versatile dicarba-*closo*-dodecaborane intermediate, which assures a high payload of ^{10}B atoms and can be functionalized with different biological vectors and different MRI probes. On one side the carborane cage has been functionalized with lipophilic moieties, like palmityl chains[2] (AT101) or double-tailed moiety (AT102) or cholesterol (AT103) in order to bind the carborane cage to the nanosized vector represented by LDL or liposomes (the real biological vectors). On the other side the carborane has been funtionalized with a Gd-DOTA complex, which allows the boron concentration in cell by means of MRI detection to be quantified. The uptake of AT101 from tumor cell was assessed on cell cultures of human hepatoma (HepG2), murine melanoma (B16) and human glioblastoma (U87). MRI measurements were undertaken *in vivo* on mice bearing tumors in which B16 tumor cells were inoculated at the base of the neck and the amount of boron taken up in the tumor region was above the threshold required for successful NCT treatment. After neutron irradiation, tumor growth was followed for 20 days by MRI. The group of treated mice showed markedly lower tumor growth with respect to the control group [3] (Figure 1). Biological and irradiation experiments using AT102 and AT103 are currently in progress.

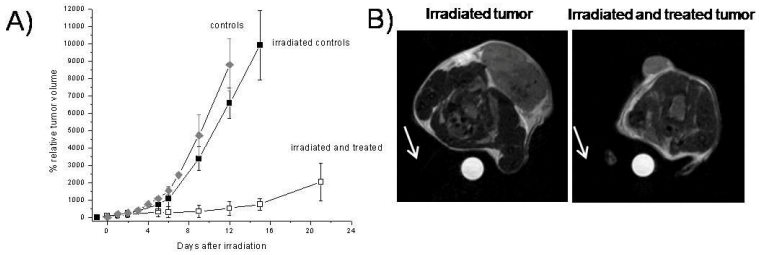

Figure 1 Tumor-growth evaluation performed after neutron irradiation. A) Growth of B16 tumours after injection in vivo of AT101–LDL(□), radiation control (■), and untreated control (◆). B) T2- weighted RARE images acquired on tumour-bearing mice irradiated with neutrons without (left) or after (right) administration of B atoms. The images were acquired 12 days after neutron irradiation.

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Paramagnetic and fluorescent Solid Lipid Nanoparticles targeting atherosclerotic plaques.

Chiara Brioschi[a], Silvia Salina[a], Claudia Cabella[a], Simona Ghiani[a], Luigi Miragoli[a], Giovanni Valbusa[a], Federico Maisano[a], Alessandro Maiocchi[a].

[a] *Bracco Research Centre, Bracco Imaging S.p.a., via Ribes 5, 10010 Colleretto Giacosa (TO), Italy*

Email of presenting author: Chiara.Brioschi@bracco.com

Despite the therapeutic advances over the last 20 years, cardiovascular disease is still the leading cause of death worldwide. This is mainly related to the increasing prevalence of atherosclerosis, owing to the ageing population, together with the widespread under-recognition and under-treatment of individuals with risk factors for atherosclerosis. Even though the degree of stenosis is the most used imaging criterion indicative for an intervention , almost 90% of ischemic strokes are caused by thrombosis due to plaque rupture that can occurs in vessels with moderate stenosis.

In order to provide a new class of MRI contrast agents with enhanced paramagnetic properties and improved plaque affinity, we designed a new technological platform based on Solid Lipid Nanoparticles (SLNs). SLNs are considered excellent vehicles for the targeted delivery of diagnostic and therapeutic agents, since they combine good tolerability, high bioavailability, low toxicity and large-scale production processes. The bioavailability of SLNs at pathological sites like atherosclerotic plaques is strictly related to the permeability of the lesion, due to an induced endothelium leakage triggered by several inflammation factors. Indeed the assessment of the inflammation state of both the plaque and the surrounding tissues is a key factor suitable for the identification of unstable plaques.

After intravenous administration of fluorescent paramagnetic SLNs in ApoE -/- mice it was possible to discriminate among different states of inflammation in the atherosclerotic plaque progression following the MRI signal of the arterial wall. Furthermore the *in vivo* MRI findings were validated by *ex vivo* histological analysis and fluorescence microscopy to identify the preferred accumulation sites of SLNs within plaques having different biological composition.

Moreover microscopy was used to characterize the morphological parameters associated with the vulnerability conditions, and the possibility to target vulnerable lesions using SLNs.

The results clearly demonstrate that using fluorescent paramagnetic SLNs it becomes possible to quantify both the dynamics of macrophage accumulation and the permeability of the fibrous cap covering the atherosclerotic lesions, hopefully providing an additional tool to stratify patients having higher risk of plaque rupture.

Glucose level determination with enzymatic glass chip

Francesca Costantini[a], Roald Tiggelaar[b], Han Gardeniers[b] and Cesare Manetti[a]

[a]*Dipartimento di Chimica, Sapienza Università di Roma, P.le Aldo Moro 5 00136, Rome, Italy;*
[b]*Mesoscale Chemical Systems, MESA+ Institute for Nanotechnology, University of Twente, P.O. Box 217 7500 AE, Enschede, The Netherlands;*

Email of presenting author: Francesca.Costantini@uniroma1.it

Diabetes is a widespread chronic disease, which affects millions of people. Measuring the glucose level in blood (glycemia) is crucial for the dosage of insulin to be used by diabetic people. Currently available portable devices are based mostly on current detection, and exhibit a good sensitivity but suffer, however, a low selectivity due to biologically electroactive interferences.[1]
We have developed a portable microfluidic device by which glycemia can be measured with the same principle as applied in daily laboratory routines in hospitals. The glucose blood level is determined by carrying out a cascade enzymatic reaction in a microfluidic channel in a glass chip (Fig. 1a). The embedded channel has a length of 175 mm, and a (nearly) half-circular cross-section (110 µm width, 50 µm depth). The interior was functionalized with a layer of poly(2-hydroxyethyl methacrylate) (PHEMA). Subsequently PHEMA hydroxyl functional groups reacted with succinic anhydride and n-hydroxy-succinimide to obtain amino-ester groups. Glucose oxidase (GOX) and horseradish peroxidase (HRP) easily reacted with amino-ester groups[2], resulting in covalent attachment to the polymer film (Fig. 1b). Post to enzymatic functionalization, different concentrations of *β*-D-Glucose are flushed in the chip via inlet *X*, while a solution containing an excess of 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) is inserted via inlet *Y*. GOX oxidizes *β*-D-Glucose to *δ*-D-gluconol-actone and H_2O_2. Hydrogen peroxide activates HRP, which on its turn oxidizes ABTS, resulting in the formation of a colored solution (absorbance at 735 nm) that exits the chip via outlet *Z*. A calibration curve of *β*-D-Glucose was made by monitoring the oxidation of ABTS with a small-volume UV-Vis system in-line with the functionalized chip. The glucose concentration in human serum of a healthy donor was determined with the chip as well as with conventional equipment, and was found to be 65 mg/dl and 62 mg/dl, respectively. These results demonstrate that glucose determination in serum can be done fast and efficiently with this miniaturized analytical system, and evidence that glucose level determination with identical accuracy and selectivity as of commonly used large-scale equipment is feasible.

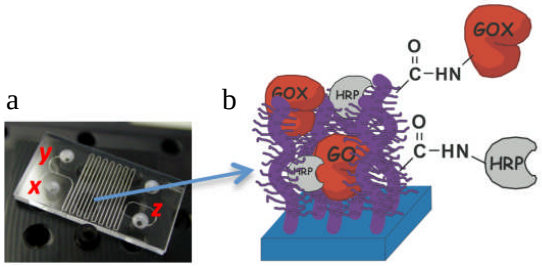

Figure 1. a) glass microfluidic device for determining glucose levels, b) GOX and HRP covalently linked to PHEMA polymer film.

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Synthesis of dendrimeric magnetic nanoparticles and imaging studies using IgG-FITC

Rouhollah Khodadust[1], Gozde Unsoy[1], Serap Yalcin[2], Pelin Mutlu[3], Ufuk Gunduz[1]

[1]*Department of Biotechnology, Middle East Technical University, Ankara 06800, Turkey*
[2]*Kaman Vocational High School, Ahi Evran University, Kırşehir 40100, Turkey*
[3]*Central Labratory Molecular Biology and Biotechnology R&D, Middle East Technical University, Ankara 06800, Turkey*

Email of presenting author: hodadust_r@yahoo.com

Magnetic particles are widely studied for their applications in biology and medicine. These magnetic particles are generally composed of magnetite (Fe3O4) core and a polymeric shell where the drugs, nucleic acids, and antibodies are bound. Dendrimer modified magnetic nanoparticles are suitable drug delivery system because of their functional groups, symmetry perfection, nanosize, and internal cavities. Polyamidoamine (PAMAM) dendrimers are hydrophilic, biocompatible, monodisperse, cascade-branched macromolecules with highly flexible surface chemistry that facilitates functionalization. Antibody conjugation to the nanoparticles gives rise to targeted drug delivery in cancer therapy. In this study, magnetic nanoparticles were synthesized and coated with PAMAM dendrimer layers. Their crystalline structures, magnetic characteristics, size, shape, surface chemistry and cytotoxicity were approved by different analysis methods. Cellular internalization of dendrimeric nanoparticles were observed by inverted light scattering microscopy. The results are promising due to the fact that, nanoparticles can be internalized into the cells even if they are applied at low concentrations and cell viability was not affected. Then, the IgG antibody was linked forming IgG conjugated fluorescent (FITC) dendrimeric nanoparticles which were applied onto the breast cancer (MCF 7) cell lines. Cellular internalization of IgG linked nanoparticles was detected by confocal microscopy. The results of this study demonstrated that the synthesized PAMAM coated iron oxide nanoparticles could be suitable as potent magnetic targeting and imaging systems when linked with IgG-FITC.

Site-specific conjugation of scfvs antibodies to nanoparticles by bioorthogonal strain-promoted alkyne–nitrone cycloaddition

Miriam Colombo,[a] Silvia Sommaruga,[a] Serena Mazzucchelli,[a] Laura Polito,[b] Paolo Verderio[a] and Davide Prosperi[a,b]*

[a] *Department of Biotecnology and Bioscience, University of Milano-Bicocca, Piazza della Scienza 2, 20126, Milano, Italy;* [b]*Institute of Molecular Science and Technology. CNR Via Fantoli 16/15, 20138 Milano, Italy.*

Email of presenting author: m.colombo140@campus.unimib.it.

We present the potential of a new method based on a single-step bioorthogonal reaction, namely the strain-promoted alkyne-nitrone cycloaddition (SPANC), as an efficient variant of the copper-free "click" reaction, for the reliable surface functionalization of magnetofluorescent nanoparticles with proteins. This approach has several advantages compared with other methods: 1) the reaction is fast and versatile, 2) a complete control on the site of conjugation of the protein (and consequently on the protein orientation on the nanoparticle) can be achieved with a precision of a single aminoacid, 3) the reaction works best in a biocompatible environment and 4) the reaction is byproduct-free, thus each step of purification is simple and efficient.
The method was validated using an scFv antibody fragment against HER2 tumor marker resulting in its prompt immobilization on multifunctional nanoparticles (MFN), leading to water-stable bioengineered targeted MFN, which exhibited a complete conservation of protein effectiveness in selectively targeting HER2 receptor in living cells. As the structural motif of scFv fragments is highly conserved, and other kinds of nanoparticles can be modified identically with the same polymer used herein, this approach is expected to be of general utility and may become a universal strategy for the development of a new generation of targeted nanoparticles. [1]

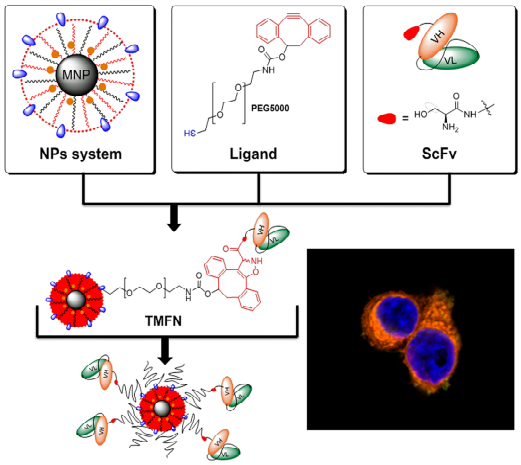

Figure 1. Site-specific conjugation of scFvs to nanoparticles by bioorthogonal strainpromoted alkyne-nitrone cycloaddition.

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MNPs in non ionic surfactant vesicles as smart delivery system for theranostic applications

Carlotta Marianecci[a],Federica Rinaldi[a], Giovanni Baldi[b], Daniele Passeri[c], Chunhua Dong[c, d], Angela Sorbo[e], Marco Rossi[c], Maria Carafa[a]

[a]*Department of Chemistry and Drug Technolgies, University of Rome "Sapienza", P.zzle A Moro 5 00185, Rome, Italy;*
[b]*Ce.Ri.Col, Colorobbia Italia, S.p.A. Via Pietramarina, 53, 50053, Sovigliana, (Fi) Italy;* [c]*Department of Basic and Applied Sciences for Engineering, University of Rome "Sapienza", via A. Scarpa 14, 00161, Rome, Italy;*
[d]*Department of Physics, University of "Sapienza", P.zzle A Moro 2 00185, Rome, Italy;* [e]*Department of Food Safety and Veterinary Public Health, Istituto Superiore di Sanità, viale Regina Elena 299, 00161, Rome, Italy.*

Email of presenting author: carlotta.marianecci@uniroma1.it

Nanomedicines are submicrometer-sized carrier materials designed to improve the biodistribution of systemically administered (chemo)therapeutic agents. Clinically relevant examples of nanomedicine formulations are vesicles, polymers, micelles, solid (lipid) nanoparticles, and antibodies. By delivering pharmacologically active agents more effectively and more selectively to the pathological site (site-specific drug delivery) and/or by guiding them away from potentially endangered healthy tissues (site-avoidance drug delivery), nanomedicines aim to improve the balance between the efficacy and the toxicity of systemic (chemo)therapeutic interventions.
Besides for drug targeting to pathological sites and for therapeutic purposes, nanomedicine formulations have also been more and more used for imaging applications as well as, in recent years, for theranostic approaches, that is, for systems and strategies in which disease diagnosis and therapy are combined.
To this end, on the one hand, "classical" drug delivery systems, such as vesicles, polymers, micelles, solid (lipid) nanoparticles, and antibodies, are being co-loaded both with drugs and with contrast agents. On the other hand, also nanomaterials with an intrinsic ability to be used for imaging purposes, such iron oxide-based nanoparticles (MNPs), are increasingly being loaded with drugs or alone and implemented for combining disease diagnosis and therapy [1]. Based on the advances made in, non ionic surfactant vesicles loaded with commercial and synthetic lipophilic and hydrophilic MNPs have been prepared. Non ionic surfactant vesicles have been chosen for their advantages over liposomes such as higher chemical and physical stability and lower costs of production.
Unloaded and loaded vesicles have been characterized in terms of dimensions (by dynamic light scattering and atomic force microscopy), zeta potential, serum stability, bilayer characteristics, calcein release and overall iron content (by inductively coupled plasma mass spectrometry).

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Toward a new generation of nanoparticles for therapy and diagnosis

Lucia Pasquato, Silvia Bidoggia, Mariangela Boccalon, and Davide Porrelli.

Dipartimento di Scienze Chimiche e Farmaceutich e INSTM Unità di Trieste, Università di Trieste, via L. Giorgieri 1, Trieste, 34127, Italy.

Email of presenting author:: lpasquato@units.it.

Progress in utilizing inorganic nanoparticles for biomedical applications has advanced rapidly due to the extensive amount of work done in the synthesis and modification of the materials.[1] These nanosized materials provide a robust framework in which two or more components can be incorporated to give multifunctional capabilities. An example can be seen in gold nanomaterials.[2] Gold nanoparticles are bioinert, nontoxic, and readily synthesized and functionalized.[3] They also provide a multifunctional platform for both therapeutic and diagnostic purposes. Indeed, through proper functionalization, these particles can be engineered to accumulate at illness cells using targeting ligands providing a powerful tool, for example, for gene therapy.[4] The biophysico-chemical properties of the vehicle, such as size, charge, surface hydrophilicity, and the nature and density of the ligands on their surface, can all impact the circulating half-life of the particles as well as their biodistribution.

Innovation may be introduced by controlling the surface properties of the monolayer protecting the gold core. Indeed, recently it has been demonstrated that particles coated with a molecularly ordered ligand shell were able to enter cells directly through the membrane without perforating it basing on a novel physical chemistry phenomenon.[6] This property is ideal as it provides the particles with minimal if none genotoxicity. Mixed monolayers composed of mixtures of hydrogenated/fluorurated ligands favor the phase segregation and consequently the ordered morphology of the NP surface. [7] In addition, the introduction in the monolayer of perfluorocarbon ligands might enable, for example, the imaging by ^{19}F MRI techniques of the nanoparticles and, consequently, the tracking *in vivo* of cell fate.

In this communication we will discuss the approaches for the realization of such innovative nanoparticles easy to make, because obtained by self-assembly strategies, but with an unprecedented degree of complexity, with respect to nanotechnology platforms for drug delivery applications know to date, as far as their features is concerned.

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Myoblast behaviour on human recombinant elastin-like coatings

Gianni Ciofani[a], Giada Graziana Genchi[a,b], Virgilio Mattoli[a], Antonella Bandiera[c]

[a]Center for Micro-BioRobotics @SSSA, Istituto Italiano di Tecnologia, Viale Rinaldo Piaggio 34, 56025, Pontedera, Italy; [b]The BioRobotics Institute, Scuola Superiore Sant'Anna, Viale Rinaldo Piaggio 34, 56025, Pontedera, Italy; [c]Department DSV, University of Trieste, Via Giorgieri 1, 34127, Trieste, Italy.

Email of presenting author: gianni.ciofani@iit.it.

Recombinant proteins represent a new and promising class of polymeric materials in the field of biomaterials research. An important model for biomaterial design is elastin, the protein responsible for the elasticity of several tissues. Human elastin-like polypeptides (HELP) have been developed as recombinant versions of elastin with the purpose of enhancing some peculiar characteristics of the native protein, like that of self-assembling [1]. In this research, we have carried out a comparative study on cell response to the interaction with standard collagen and HELP coatings through the culture of H9c2 rat myoblasts.

Collagen and HELP coatings were performed by incubating standard cell culture poly-styrene substrates with 100 μg/ml of protein solution overnight at 4°C. Thereafter, substrates were rinsed with phosphate buffered saline solution and seeded with cells for proliferation (10,000 cell/cm^2) and differentiation assessment at confluence. Cell proliferation was evaluated with WST-1 metabolic test at 24 h and 72 h after seeding. Myotube formation was assayed at 6 days after differentiation induction by immunofluorescence staining of myosin heavy chain (MHC), TRITC-phalloidin f-actin staining, and DAPI nuclei staining.

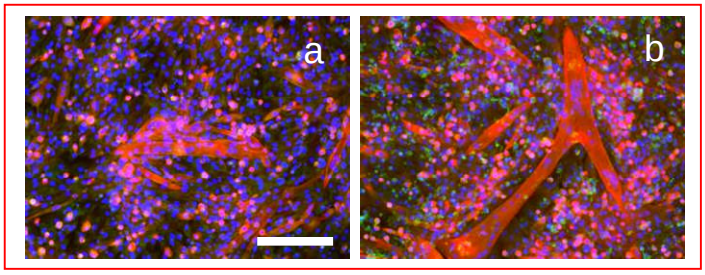

Figure 1. Immunofluorescence images of H9c2 cells differentiated on collagen-coated (a) and HELP-coated substrates (b). MHC in green, f-actin in red, nuclei in blue. Scale bar 100 μm.

WST-1 assay evidenced an excellent metabolic activity of H9c2 cells cultured on HELP-coated substrates, 15% higher with respect to the controls on collagen-coated substrates ($p < 0.05$). Differentiation was positively affected by HELP coating as well. Figure 1b clearly shows that the myotubes developed on HELP coating were significantly larger and longer with respect to those on the collagen coating, as a control (Figure 1a). Quantitative assessment of the myotube size confirmed an increment of the mean length and width of about 175% and 100%, respectively.
All these results strongly support the use of Human Elastin-Like Polypeptides as an excellent biomaterial for tissue engineering and regenerative medicine applications [2].

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Early stage mineralization in tissue engineering mapped by high resolution X-ray microdiffraction and tomography

A. Cedola[1],G. Campi[2], A. Ricci[2], A. Guagliardi[2], C. Giannini[2], M. Mastrogiacomo[3] , S. Lagomarsino[4], R. Cancedda[3]

[1]*Istituto di Fotonica e Nanotecnologie - CNR, via Cineto Romano 42, 00156 Roma, Italy.*
[2]*Istituto di Cristallografia, CNR-IC, via Amendola 122/O, 70126 Bari, Italy*
[3]*Dipartimento di Medicina Sperimentale dell'Università di Genova & AUO San Martino-IST Istituto Nazionale per la Ricerca sul Cancro, Largo R. Benzi 10, 16132, Genova, Italy*
[4]*IPCF-CNR –UOS Roma c/o Dip Fisica Universita' "Sapienza", P.le A. Moro, 2 Rome – Italy*

Email of presenting author: cedola@ifn.cnr.it

The biomineralization mechanism is here explored within a tissue engineering approach where bone is formed in porous ceramic constructs seeded with bone marrow stromal cells and implanted *in vivo*. Unlike previous studies, this model system reproduces the mammalian bone formation, investigated here with high temporal resolution: different mineralization stages were monitored at different distances from the scaffold interface so that their spatial analysis corresponded to a temporal monitoring of the bone growth and the mineralization processes. The spatial resolution achieved by our diffraction technique, assured high accuracy in the reconstruction of the different temporal mineralization steps and provided some hints to the challenging issue of the first-formed mineral deposit at the organic-mineral interface. Our results indicated that in the first stage of biomineralization, organic tissue provides bioavailable calcium and phosphate ions, ensuring a constant reservoir of amorphous calcium phosphate (ACP) during hydroxyapatite (HA) nanocrystals formation. In this regards, we offer a new contribute to the role of ACP in the HA formation, suggesting a *continuous* organic-mineral transition assisted by a *dynamic pool* of ACP. After HA nanocrystals formed, scaffold and collagen act as template for the nanocrystals arrangement at microscopic and nanometric scale respectively.
These results are also confirmed by the X-ray phase contrast micro-tomography of the implanted scaffolds which provides a 3D reconstruction of the different phases involved in the early stage of the biomineralization.

Magnetic nanoparticles and magnetic fields direct neurite outgrowth: implication in nerve regeneration

Cristina Riggio[a], M.P. Calatayud [b], B. Sanz [b], T.E. Torres [b], M. R. Ibarra [b], , G.F. Goya [b], V. and A. Cuschieri [a], Vittoria Raffa [b, c]

[a] *Institute of Life Science, Scuola Superiore Sant'Anna, Piazza Martiri della Libertà 33, 56127 Pisa, Italy;* [b] *Instituto de Nanociencia de Aragón, Universidad de Zaragoza, Mariano Esquillor 50018, Zaragoza, Spain;* [c] *Department of Biology, Universita' di Pisa, Via Luca Ghini 5, 56126 Pisa, Italy*

Email of presenting author: vraffa@biologia.unipi.it

Nerve regeneration and recovery of nerve function have been a major issue in neuroscience in regards to the treatment of injured neurons after accident or a degenerative disease. The regeneration of peripheral nerves is an example of plasticity within the nervous system. Following an injury to a peripheral nerve, axons distal to the injury site begin to degenerate as a result of protease activity. The regeneration process, which begins after the phagocytic clearance of the debris is completed, occurs at the proximal end and proceeds toward the distal stump. Functional re-innervation requires that axons continue to grow until they reach their distal target. In humans, axonal regeneration occurs at a rate of about 1 mm/day; thus major injuries (neurotmesis) can take many months to heal with recovery of nerve function [1]. Extensive research in bioengineering has been focused on the development of innovative strategies for reducing this prolonged recovery time. The general idea is to create physical or biochemical guidance cues to direct axonal re-growth across nerve lesion sites. One common approach is the so called guidance therapy, based on the use of scaffolds (autologous tissue grafts, non-autologous tissue graft, natural based materials, synthetic materials, etc) working as "nerve guides" or "nerve guidance channels" , i.e., they provide a conduit to guide the nerve regeneration. Here, we propose a novel minimally invasive methodology for physical guidance based on the use of magnetic nanoparticles (MNPs) and magnetic fields (M). We demonstrate that the application of a tensile force to a neuron or an axon can stimulate neurite initiation or axon elongation in the desired direction. We used MNPs to generate these tensile forces under the effect of an external M and to manipulate axons in order to elongate and to overcome inhibitory substrates. MNPs are largely employed in biomedicine and in clinics [2]. The particles used in this work are iron oxide nanoparticles, synthesized *ad hoc* by our team [3]. These particles offers a high saturation magnetization and a low cytotoxic profile. They were functionalised with NGF-β for cellular recognition and a fluorescent moiety for intracellular tracking. In PC12 cells cultured with the functionalised MNPs, the particles were found in the cell body but also in the cone growth of developing neuritis. We demonstrated that the application of a static magnetic fields cause neuritis of PC12 cells to grow in a specific direction thanks to the mechanical force exercised by the MNPs bound to the cells.
Such methodology hold the potentiality for clinical translation.

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Peptidic nucleic acid (PNA) assisted cellular migration along engineered surfaces

Valentina Corvaglia,[a] Riccardo Marega,[b] Rosaria Vulcano,[b] Davide Bonifazi[a,b]

[a] *Department of Chemical and Pharmaceutical Sciences, University of Trieste, P.le Europa 1, 34127, Trieste Italy;* [b] *Department of Chemistry, University of Namur (FUNDP), Rue de Bruxelles 61, 5000, Namur, Belgium.*

Email of presenting author: valecorvaglia@gmail.com

The strategies to pattern molecules with defined order onto solid supports have revolutionized the electronics, optics, and photovoltaics fields,[1] and are crucial for many biological applications including tissue engineering and the development of model substrates to investigate fundamental issues in cell growth, adhesion, and migration.[2] Central to the improvement of these technologies is the nanofabrication of DNA and proteins arrays. More specifically, surface-confined DNA arrays are important in the development of novel DNA sequencing and gene mapping techniques.[3] In this research project we will deal with chemical strategies to produce suitable surface modifications in order to induce multidirectional cellular migration along gold surfaces. To achieve this objective we want to use and characterize self-assembled monolayers (SAMs) of thiolated DNA chains (DNA-SH) adsorbed on gold surfaces through the hybridization with complementary modified single-stranded PNAs. PNA is a structural DNA mimic obtained by polymerization of N-(2-aminoethyl) glycine monomers that replace the ribose-phosphate backbone characteristic of natural nucleic acids.[4] It is an achiral, uncharged, and relatively rigid biopolymer of high biological and chemical stability, and it can bind complementary DNA strands with higher affinity than the corresponding DNA sequences.

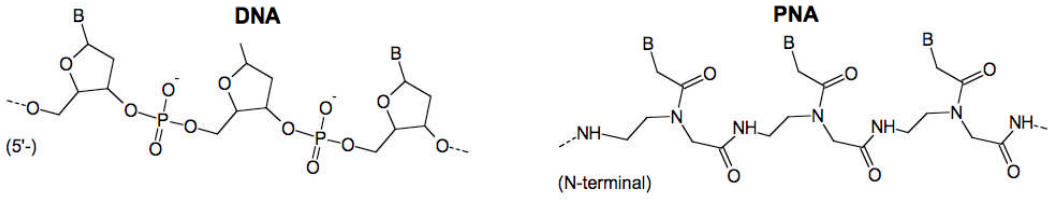

Figure 1. Schematic representation of DNA and PNA molecules.

For all these reasons we have chosen PNA as a key molecule to promote and assist the movement of cells. By producing a chemical gradient of DNA-SH along a gold surface in the presence of a chemotactic molecule it will be possible to obtain and control a directed cellular migration.

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Enhancement of neurite outgrowth and alignment in PC12 neuron-like cells on nanofibrous poly(3-hydroxybutyrate) substrates

Giada Graziana Genchi[a,b], Gianni Ciofani[b], Alessandro Polini[c], Arianna Menciassi[a,b], Virgilio Mattoli[b]

[a]*The BioRobotics Institute, Scuola Superiore Sant'Anna, Viale Rinaldo Piaggio 34, 56025, Pontedera, Italy;* [b]*Center for MicroBioRobotics @SSSA, Istituto Italiano di Tecnologia, Viale Rinaldo Piaggio 34, 56025, Pontedera, Italy;* [c]*National Nanotechnology Laboratory of CNR-Nanoscienze, Via Arnesano 16, 73100, Lecce, Italy.*

Email of presenting author: g.genchi@sssup.it.

In the latest decades, the application of polymeric conduits between transected nerve stumps has been proven as an effective method to assist peripheral nerve regeneration after injury. The recovery of structural and functional integrity in damaged nerves increases when conduits are topographically patterned on the micro- and nanoscale. For the first time in the literature, poly(3-hydroxybutyrate) fibers were electrospun with 200-400 nm diameters and with either a random or parallel orientation. Their use in neural tissue engineering was evaluated concerning the suitability to support neurite outgrowth and alignment. To the purpose, PC12 neuron-like cells were used, and their differentiation was assessed upon administration of nerve growth factor (six days of culture). Neurite orientation was found dependent on fiber orientation: neurites were randomly and parallel oriented on random and parallel fibers, respectively. Parallel fibers supported the outgrowth of longer neurites, with ~50% neurites occurring in the 100-200 μm range, whereas ~75% neurites occurred in the 20-80 μm range on random fibers. Coherently to other authors' findings [1,2], it is here suggested that anisotropic fibers with diameters in the 200-400 nm range may promote neurite guidance and faster growth over longer distances, all valuable conditions to a possible application of parallel fibers as nerve conduits for peripheral nervous system regeneration [3].

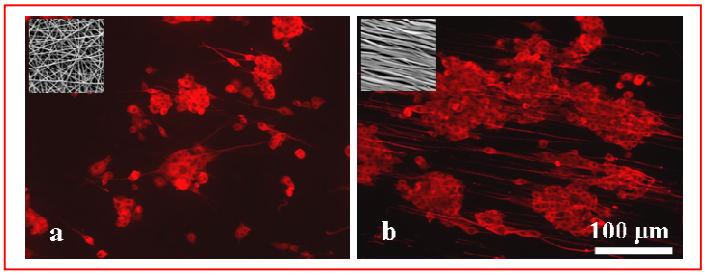

Figure 1. β3-tubulin immunofluorescent staining of PC12 cells differentiated on randomly (a) and parallel oriented (b) PHB fibers. Inlets: scanning electron microscopy images (7000X) of the fibers .

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From technological innovations to medical devices

Mauro Grigioni, Giuseppe D'Avenio, Carla Daniele, Silvia Tedesco, Giorgio De Angelis

Department of Technology and Health, Italian National Institute of Health (ISS), Viale Regina Elena 299, 00161, Rome, Italy.

Email of presenting author: mauro.grigioni@iss.it.

Nanomedicine employs molecular-scale systems to address medical problems, to maintain and improve human health using several technologies to implement complex functions. Tissue engineering and nanotechnologies have been used to manipulate or realize scaffolding or controlled drug delivery to provide innovative devices or systems. These definitions are linked to the concepts of medical device and drug, since these can represent the main roles of the engineered molecular systems.
A necessary stage to verify the quality of innovative knowledge is the assessment phase, supported by specific standards, possibly fostered by the regulatory domain. Finally, in order to gain market approval, the final product must be in compliance with applicable European directives.
The Italian National Institute of Health (ISS), in its function of advice and control actor in the national health system, develops research, methods and procedures to be used in the evaluation of medical devices. ISS biomechanics engineers' experience started in 90s with the study of an ideal heart valve substitute, obtained by the acellularised porcine aortic root, to be subsequently put in a suitable bioreactor. The environment provided by the latter was capable to mimic the shear rate values that endothelial cells experience in vivo, to impart pulsatile, steady or static or secondary flow conditions, while at the same time it allowed to test hydraulics and fluid dynamics of the valve sample. Thus, the tester was designed to be used directly by the surgeons for patient-specific device production.
An alternative way to produce cardiovascular implantable devices is electrospinning, developed to use a synthetic polymer as scaffold for heart valve substitutes, vessels or patches to be loaded with specific molecules or cells, with the properties of promoting the reabsorption of structural materials of the synthetic scaffold, while supporting native structures and cells (e.g. stem cells differentiation etc.).
The electrospinning technique was implemented at the ISS to optimize the fabrication of implantable devices, offering the possibility of fabricating tissue products with appropriate properties (e.g., mean porosity). For biomedical applications, porosity represents a relevant factor for cell attachment, mass transfer and tissue integration, but it is recognized also as a signal to stimulate differentiation. The ISS study used polycaprolactone (PCL) as basic polymer for heart valve and vessel substitutes to be reabsorbed in time, while promoting a biological structure close to the native one. The final valve substitute was tested in unseeded conditions in vitro, in the pulmonary position of a pulse duplicator used in ISS to test valve prostheses.
Assesment methods were also developed at ISS to test cells under biomechanical or biochemical stimuli, e.g. progressive shear stress flow chamber and culture devices for toxicity or viability studies based on the electrical cell impedance theory.
Today new research efforts regard the variables involved in spinnability of different polymers blends, to improve the design and development of scaffolds with at the simultaneous use of polymer and active molecules in the production process. These are the main current activities of the group, together with the preparation of risk analisys tools for the certification of innovative technologies, such as machines and motors at nanoscale, which could ultimately lead to actual nanorobots.

Cardiac cell therapy: a strategy for treatment of heart disease

Antonio Amodeo[1], Domenico D'Amario[2], Elisa Messina[3].

[1]Department of Pediatric Cardiac Surgery, Bambino Gesù Hospital, piazza Sant'Onofrio, 4 , 00165 Rome, Italy; [2]Department of Cardiology, Università Cattolica del Sacro Cuore, Rome;[3] Department of Experimental Medicine and Pathology, Università la Sapienza, Rome

Email of presenting author: antonioamodeo@yahoo.it.

Despite of the traditional notion of a post-mitotic organ without the potential for self-renewal, the human heart is capable of regeneration and repair from endogenous and exogenous stem cells. When selecting a candidate stem cell type for clinical use, multiple factors need to be taken into consideration. Skeletal myoblasts engraft, differentiate and are arrhythmogenic; in contrast, bone marrow-derived cells do not engraft long-term with low arrhythmias incidence. Neither cell type, however, achieves true myocardial regeneration. Recognition of the existence of cardiac stem cells, and of the ability of mature myocytes to re-enter the cell cycle and proliferate, has motivated the development of new approaches in the cardiac regenerative medicine. It is now prooved that resident cardiac stem cells exist in the adult mammalian heart. Multiple extracardiac cell sources, including bone marrow mononuclear cells , bone marrow–derived mesenchymal stem cells, adipose tissue–derived mesenchymal stem cells, endothelial progenitor cells, and myoblasts, have been used clinically in attempts to regenerate the damaged heart. The implantation of these cells of extracardiac origin has been found to produce generally positive effects, mostly through paracrine mechanisms. Although resident cardiac stem cells can mediate direct cardiogenesis and angiogenesis, recent studies have demostrated that even these cells exert most of their benefits via indirect paracrine mechanisms. Therefore, no convincing evidence supports the superiority of heart-derived cells for myocardial repair. Two heart-derived cell products are in clinical trials namely CADUCEUS [1] and SCIPIO [2], without direct comparison.

The first, Cardiosphere-Derived Cells (CDCs), are a natural mixture of stromal, mesenchymal, and progenitor cells, the second cell product represents the c-kit subpopulation purified from mixed heart-derived cells. The magnitude of reduction in relative infarct size shown in the two trials is not dissimilar and seems to improve with increased follow-up. An increased LV-EF of 8.2% after 4 months (SCIPIO) not only persisted but was even greater in the eight patients studied after 12 months. A recent head-to-head comparison of 4 different stem cell types/subpopulations for functional myocardial repair by assessing multiple in vitro parameters, including secretion of relevant growth factors, and in vivo cell implantation into an acute myocardial infarction model in mice showed that the CDCs were superior in terms of paracrine factor secretion, angiogenesis, cardiomyogenic differentiation, ischemic tissue preservation, antiremodeling effects, and functional benefit [3]. They suggest that intracoronary infusion of autologous CSCs is effective in improving LV systolic function and reducing scar size in patients with heart failure after myocardial infarction, and warrant further, larger, phase 2 studies.

In conclusion several features should be included in the ideal cell type to treat heart disease: (i) be safe, i.e. not create tumors (ii) improve heart function; (iii) create healthy, functional cardiac muscle and vessels; (iv) be amenable to delivery by minimally-invasive clinical methods;(v) be 'off the shelf' available as a standardized reagent;(vi) be tolerated by the immune system; and (vii) circumvent societal ethical concerns. At present, it is not clear whether such a 'perfect' stem cell exists but some cell types are more promising than others.

Immune response elicited by saccharide-functionalized Gold nanoparticles

Luigi Lay,[a] Fabrizio Mancin,[b] Grazia Lombardi[c] and Paolo Scrimin[b]

[a] Department of Organic and Industrial Chemistry and CISI and ISTM-CNR, University of Milano, Via Venezian 21, 20133 Milano, Italy; [b] Department of Chemical Sciences and ITM-CNR, University of Padova, Via Marzolo 1, 35131 Padova, Italy; [c] DISCAFF, University of "Piemonte Orientale Amedeo Avogadro", Via Bovio 6, 28100 Novara, Italy

Email of presenting author: paolo.scrimin@unipd.it.

Clusters of gold atoms 2-10 nm in diameter may be easily covered by a monolayer made of organic molecules terminating with a thiol because of the relatively strong Au-SR bond formed. The resulting nanoparticles constitute examples of multivalent systems (i.e. systems presenting a collection of identical units on their periphery) providing the thiols are properly functionalized with different functional groups. Such functionalities may include: metal complexes, peptides, saccharides or oligonucleotides. The properties of these functionalized nanoparticles are defined by the nature of the units present on the monolayer. For instance a gold cluster covered with peptides is not much different morphologically from a protein. A saccharide-covered gold nanocluster is similar to a bacterium. But is it possible to extend this similarity to its functional properties?
We will provide evidence of the ability of gold nanoparticles covered with a monolayer constituted by saccharides modeled after those of the coat of Neisseria meningitidis bacteria to be recognized by mouse polyclonal antibodies specific for this bacterium.[1] Their affinity for the biological target is several order of magnitude larger than that of analogous monovalent saccharides and, even more important, they elicit specific immune response in nanoparticle-exposed cells.[2]

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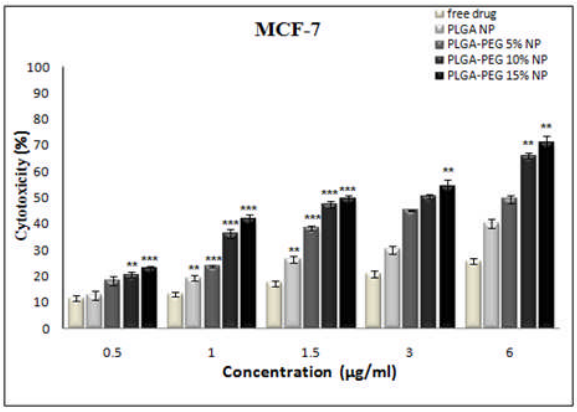
The surface modified nanocarrier: Anticancer efficacy, tissue distribution and blood pharmacokinetics of loaded anticancer drug

Katayoun Derakhshandeh

Department of Pharmaceutics & Nanosciences and Technology Research Center Faculty of Pharmacy, Kermanshah University of Medical Sciences, Kermanshah67149-67346, Iran.

Email of presenting author: kderakhshandeh@kums.ac.ir

Background and Aims: The major limiting factor for nanocarrier's success is uptake of nanoparticle (NP) by reticuloendothelial system (RES) and subsequent removal of the carrier from systemic circulation. An overview is presented of a potent approach for treating cancer that uses nanoparticles to deliver anticancer drugs.
Methods: To achieve this goal, 9-nitrocamptothecin (9-NC) loaded Poly lactic-glycolic acid (PLGA), PLGA-PEG with different ratio of PEG (0–15%) and folate coated PLGA nanoparticles were formulated by nanoprecipitation method and characterized that could efficiently encapsulate hydrophobic drug, and also have suitable release behavior.
In our study, we primarily assessed a rational approach for designing ideal long-circulating nanoparticles by optimizing the amount of polymers, emulsifier and internal and external phases by statistical artificial neuronal network.
Macrophage uptake efficiency and in vitro cytotoxicity of the formulated nanoparticles was also evaluated in different cancer cell lines (MCF7, AGS, Hela, PC-3, JC744A.1, and HT-29).
To comparatively investigate the pharmacokinetics of different formulations of 9-NC nanoparticles, a simple HPLC chromatography method was developed for the quantification of 9-NC (lactone and total forms) in plasma of rats after intravenous administration.
Results: PLGA-PEG nanoparticles showed dramatic prolongation in blood circulation, as well as reduced macrophage uptake, compared to free drug and PLGA nanoparticles. Superior anti-proliferative effect and cell cycle inhibition was observed in case of PLGA-folate nanoparticles over loaded PLGA, PLGA- PEG nanoparticles and native 9-NC.



Cytotoxicity of MCF-7 cells after 24 exposures with different concentrations of 9-NC, determined by LDH assay. Data are presented as the mean ± SEM of three separate experiments (n = 3). (*** p<00.01, **p<0.01, *p<0.05)

In pharmacokinetic studies, the area under the plasma concentration-time curve of PLGA-PEG and PLGA- folate was increased significantly ($P < 0.01$) in comparison with the values for the 9-NC-PLGA nanoparticles and solution. The MRT value of PLGA- PEG nanoparticles in plasma was greater than 3-5 times more than other formulations which means could be invisible for reticuloendothelial macrophage systems.
Conclusion: The present results suggest that, a combinational coating of PEG and folate may represent a significant step in the development of long-circulating and more cytotoxic target drug delivery carriers and could change the biofate of this valuable drugs.

Guanidinium and arginine clustering on calixarene macrocyclic scaffolds as a novel strategy for improved cell transfection

Francesco Sansone[a], Michele Bassi[a], Michela Lomazzi[a], Valentina Bagnacani[a], Valentina Franceschi[b], Gaetano Donofrio[b], Alessandro Casnati[a], Rocco Ungaro[a]

[a]*Dipartimento di Chimica Organica e Industriale, Università degli Studi di Parma, Parco Area delle Scienze 17/a, 43124 Parma, Italy;* [b]*Dipartimento di Salute Animale, Università degli Studi di Parma, Via del Taglio 6, 43126 Parma, Italy.*

Email of presenting author: francesco.sansone@unipr.it

Cell penetrating peptides (CPPs) are widely used as molecular transporters for the internalization inside cells of various cargos, including proteins and nucleic acids.[1] Among them a special role is played by arginine-rich peptides since the presence of guanidinium end groups strongly facilitates cellular uptake thanks to favorable electrostatic interactions with the negatively charged cell surface.[2] Due to these features, arginine-rich peptides can also help the delivery of nucleic acids across the cell membranes[3] opening interesting perspectives in gene delivery, a necessary prerequisite for gene therapy. In order to optimize the translocation properties of this type of carriers, we explored the strategy of clustering guanidinium and arginine units on macrocyclic scaffolds. We report here the application of this novel approach to DNA delivery and cell transfection. Instead of long oligoarginines or arginine-rich peptides, we covalently linked few guanidinium or arginine units either at the upper or at the lower rim of calix[n]arenes maintaining a 1/1 ratio between polar heads and lipophilic tails.[4] Some of the designed ligands (Figure) showed remarkable DNA condensation and transfection properties,[4] surpassing in efficiency commercially available lipofectamines. The structural features of the macrocyclic compounds resulted tightly correlated with their gene delivery ability.

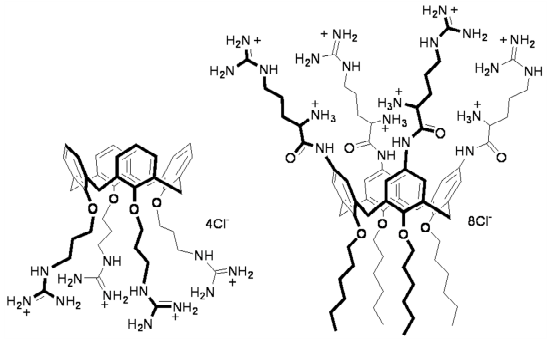


Figure 1. Examples of guanidino- and argininocalixarenes

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Cytotoxic effects on human breast adenocarcinoma MCF-7 cell line induced by multi-walled carbon nanotubes

Stefano Bellucci[1*], *Simona Dinicola*[2,3], *Pierpaolo Coluccia*[3], *Mariano Bizzarri*[4], *Angela Catizone*[5], *Federico Micciulla*[1], *Immacolata Sacco*[1], *Giulia Ricci*[6], *Alessandra Cucina*[3]

[1] *INFN-Laboratori Nazionali di Frascati, Via Enrico Fermi 40, 00044 Frascati, Rome, Italy*
[2] *Department of Clinical and Molecular Medicine, Sapienza University of Rome, Piazza Sassari 3, 00161 Rome, Italy*
[3] *Department of Surgery "Pietro Valdoni", Sapienza University of Rome, Via A. Scarpa 14, 00161 Rome, Italy*
[4] *Department of Experimental Medicine, Sapienza University of Rome, Viale Regina Elena 324, 00161 Rome, Italy*
[5] *Department of Anatomy, Histology, Forensic Medicine and Orthopaedics, School of Medicine, 'Sapienza' University of Rome, via Borelli 50, 00161 Rome, Italy*
[6] *Department of Experimental Medicine, Histology and Embryology Laboratory, School of Medicine, Second University of Naples, Via L. Armanni 5, 80138 Naples, Italy*

**Presenting and corresponding author. Tel/Fax: +39 06 94032883.*
Email of presenting author: s.bellucci@lnf.infn.it

Treatment of the human breast adenocarcinoma cell line, MCF-7, with 0.1 mg/ml of MWCNTs, MWCNTs-COOH, or MWCNTs-OH for 72 hours induced both a decrease in cell proliferation and a reduction of the percentage of cells in S-phase of cell cycle. Moreover, all types of MWCNTs induced an increase in apoptotic cells. Overall, these data indicated that the cytotoxic effects of all types of MWCNTs are mediated both from a decrease in the proliferation rate and from an increase of apoptotic cell death. The biological effects of all types of MWCNTs could be explained with their cellular internalization.

Bombesin labelled Liposomes as target selective delivery system for Doxorubicin: in vitro and in vivo studies.

Giancarlo Morelli[a], Antonella Accardo,[a] Carla Cicala,[b] Antonio Parisi,[b] Giuseppina Salsano,[c] Anna Morisco,[d] Michela Aurilio,[d] Diego Tesauro,[a] Luigi Aloj[d], Giuseppe De Rosa[c]

[a] *CIRPeB, Department of Biological Sciences & IBB CNR, University of Naples "Federico II", Via Mezzocannone, 16 80134 Napoli (Italy);* [b] *Department of Pharmacology, University of Naples "Federico II", Via Montesano, 49 80131 Napoli (Italy);* [c] *Department of Pharmaceutical Chemistry, University of Naples "Federico II", Via Montesano, 49 80131 Napoli (Italy);* [d]*Department of Nuclear Medicine and* [c] *Animal Experimentation Facility Istituto Nazionale per lo Studio e la Cura dei Tumori, Fondazione "G. Pascale", Via M. Semmola, 80131, Naples, (Italy).*

Email of presenting author: gmorelli@unina.it

This study addresses novel liposome composition approaches to specifically target tissues overexpressing bombesin (BN) receptors. A new bombesin analog peptide (BN-AA1) is used to decorate the external surface of doxorubicin loaded liposomes.
DOTA-Peg4-peptides containing the [7-14]bombesin wild type sequence and the new BN-AA1 sequences have been synthesized to compare the binding properties and in serum stability of the two peptide sequences. The amphiphilic peptide derivative (MonY-BN-AA1) containing the bombesin analog peptide BN-AA1, a hydrophobic moiety with two C18 alkyl chains, polyethylenglycole (PEG) spacers, and the chelating agent diethylenetriaminepentaacetate (DTPA), has been synthesized by solid-phase methods. Liposomes have been obtained by co-aggregation of MonY-BN-AA1 with 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC). The structural and in vitro and in vivo biological properties of these target selective drug delivery systems are studied.
Both ^{111}In labeled peptide derivatives, DOTA-Peg4-peptides, present high binding properties to PC-3 cells with Kd in the nanomolar range. On the contrary, the ^{177}Lu labeled peptide DOTA-Peg4-BN-AA1 is very stable with a half-life of 414.1 h, while the wild-type [7-14]bombesin peptide derivative, DOTA-Peg4-BN, shows a half-life of only 15.5 h. Liposomes with a DSPC/MonY-BN-AA1 (97/3 molar ratio) composition showed a mean diameter of 136.3 ± 42.4 nm and a polydispersity index of 0.20 ± 0.05. High doxorubicin (Dox) loading was obtained with the remote pH gradient method using citrate as the inner buffer. In vivo studies on the therapeutic efficacy of DSPC/MonY-BN-AA1/Dox targeted liposomes in comparison to already described DSPC/MonY-BN/Dox targeted liposomes, to DSPC/Dox liposomes and to saline were performed in PC-3 xenograft bearing mice. Treatment with DSPC/MonY-BN-AA1/Dox and DSPC/MonY-BN/Dox targeted liposome formulations showed similar inhibition of tumour growth inhibition compared to control animals treated with non-targeted DSPC/Dox liposomes or saline solution. For the new studied formulation, DSPC/MonY-BN-AA1/Dox, the maximum effect was observed 19 days after treatment (tumour growth inhibition was 43 % compared to DSPC/Dox liposomes, and 59 % compared to saline group).

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Biomimetic nanoparticles with sustained release: from conventional chemotherapy to combined strategies in treating cancer

Claudia Conte[a], Francesca Ungaro[a], Giuseppe Palma[b], Antonio Barbieri[b], Pasquale Tirino[c], Giovanni Maglio[c], Rosario Palumbo[c], Maria Teresa Sciortino[d], Antonino Mazzaglia[e], Claudio Arra[c], Fabiana Quaglia[a]

[a]*Department of Pharmaceutical and Toxicological Chemistry, University of Naples Federico II, Via Domenico Montesano 49, 80131, Naples, Italy;* [b]*Animal Facility, National Cancer Institute "G. Pascale", Via Mariano Semmola 100, 80131, Naples, Italy;* [c]*Department of Chemistry "Paolo Corradini", University of Naples Federico II, Via Cinthia 21, 80126, Naples, Italy,* [d]*Department of Life Sciences, Sezione di Microbiologia, Genetica e Biologia Molecolare, University of Messina, V.le F. Stagno d'Alcontres 31, 98166, Messina, Italy;* [e]*CNR-ISMN Istituto per lo Studio dei Materiali Nanostrutturati, V.le F. Stagno d'Alcontres 31, 98166, Messina, Italy.*

Email of presenting author:: claudia.conte@unina.it

Inspired by metronomic therapy of cancer, we are currently investigating the potential of sustained release nanocarriers pointing to their possible application as intravenous nanomedicines with better performance and lower toxicity as compared to free drugs. Up to know, we have designed and tested several core-shell micelles and nanoparticles (NPs) based on biodegradable amphiphilic block copolymers of poly(□-caprolactone) (PCL) and poly(ethyleneoxide) (PEO) with different architectures. In case of NPs, copolymers were assembled in core-shell structures by a simple *melting/sonication* technique previously developed in our labs [1]. Here, we report the results obtained on PCL/PEO NPs entrapping docetaxel (DTX) and describe their potential in the treatment of breast cancer. As a further step, we combined DTX with zinc-phtalocyanine (ZnPc), a second generation photosensitizer used in Photodynamic Therapy (PDT), with the aim to obtain a combined chemo/photodynamic nanomedicine.

Besides physical characterization in aqueous solution (size, zeta potential, drug loading, release, stability and spectroscopic properties), NPs were tested in biologically relevant media. All the NPs displayed a hydrodynamic diameter around 60 nm, a slightly negative zeta potential and a complete entrapment of both drugs. DTX-loaded NPs and ZnPc/DTX-loaded NPs showed a biphasic release profile of DTX, with an initial burst followed by a slower diffusion-erosion phase completed in around 60 days. On the contrary, ZnPc remained associated to NPs in monomeric form, highlighting a time-dependent singlet oxygen generation after irradiation at 610 nm. Stability of NPs in the presence of plasma suggested that PEO coating shielded NPs from hydrophobic interactions with plasma proteins and no toxicity toward red blood cells was found. Furthermore, a sustained DTX release from NPs in human plasma highlighted a strong stability of the system in simulated biological conditions.

NPs cytotoxicity in different cancer cell lines was evaluated. In breast cancer cells (MDA-MB231), DTX-loaded NPs showed a cell growth inhibition similar to that of free drug (80% after 72 h). ZnPc/DTX-loaded NPs were tested on Hep2 cells under irradiation with a halogen lamp. In this case, viability of cells treated with NPs containing both drugs in association strongly decreased as compared to NPs loaded only with DTX, showing a synergic early cytotoxicity of ZnPc followed by induction of mitotic catastrophe by DTX at longer times.

Finally, the potential of DTX-loaded NPs (10 mg/kg of DTX) was assessed in an **eterothopic** mice model of triple-negative breast cancer. Tumor Growth, Body Weight and survival (Kaplan-Mayer) of mice were monitored. Empty NPs did not show any obvious toxicity whereas DTX-loaded NPs showed strong tumor regression after a single administration without a significant difference as compared with the DTX commercial formulation Taxotere®.

We demonstrate that core-shell NPs prepared from PCL-PEO copolymers are versatile and attractive carriers for the passive targeting and sustained release of different lipophilic drugs used in the therapy of solid tumors, being able to maintain activity and strongly decrease treatment toxicity.

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Polysaccharide nanogels by Template Chemical Cross-Linking in Polyion Complex Micelle Nanoreactors

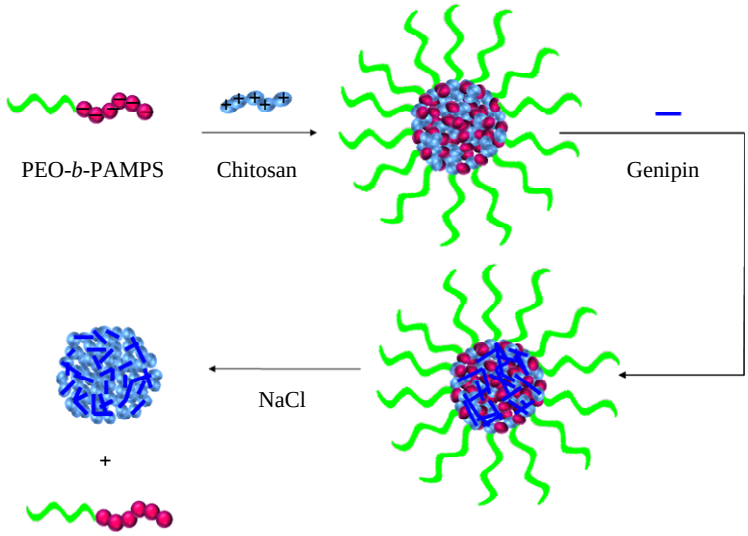
Giancarlo Masci

Department of Chemistry, Sapienza University of Rome, P.le A. Moro 5, 00185 Rome, Italy.

Email of presenting author: giancarlo.masci@uniroma1.it

Polysaccharide covalent nanogels were prepared by template chemical cross-linking in polyion complex micelle (PIC) nanoreactors. Chitosan, hyaluronic acid and alginate were used as polysaccharides. Block copolymers formed by a neutral hydrophilic block and a permanently negatively or positively charged block were used to form the PIC template with the polysaccharide by electrostatic interactions. PIC with small size (diameter about 50 nm) and low polydispersity were obtained up to 5 mg/mL. After covalent cross-linking of the polysaccharide the nanogels were obtained by dissociation of the nanoreactors by adding NaCl. Nanogels with size of about 50 nm in the swollen state and 20 nm in the dry state were obtained.

Figure 1. Schematic representation of PIC micelle formation, cross-linking and dissociation of the nanoreactor.



Endocannabinoids alone and in association with catalytically active bovine serum amine oxidase bound to magnetically drivable nanoparticles as a new anticancer therapy

Enzo Agostinelli

[1]*Istituto Pasteur Cenci Bolognetti and Department of Biochemical Sciences 'A. Rossi Fanelli', SAPIENZA University of Rome and CNR, Biology and Molecular Pathology Institutes, Piazzale Aldo Moro 5, 00185 Rome, Italy*

Email of presenting author: enzo.agostinelli@uniroma1.it

The in situ formation of cytotoxic polyamine metabolites by amino oxidase is a recent approach in cancer chemotherapy. It was demonstrated that bovine serum amine-oxidase (BSAO) and spermine (Spm) addition to cancer cells induces cytotoxicity and overcomes multidrug-resistance (MDR) phenotype. The cytotoxic effect was caused by polyamine metabolites, H_2O_2 and aldehyde(s) produced by the oxidative deamination reaction. Previous results have shown that resistant variants of human colon adenocarcinoma (LoVo) and melanoma (M14) cells were significantly more sensitive than their wild-type counterparts to the cytotoxic products.

Therefore, toxic polyamine metabolites, alone or in association with other drugs, are currently explored as probable candidates for a new approach in tumor therapy.

Pre-treatment with the endocannabinoid anandamide (AEA) (1), sensitized both cell lines to the subsequent exposure to spermine metabolites amplifying the ability of these products to induce cell death. Melanoma and LoVo cells were pre-treated with AEA, at concentrations ranging from 0 to 60 μM, for 24 or 48 hrs and then treated with BSAO in presence of various concentrations of spermine, between 6 and 18 μM, for 60 min. Strikingly, the sensitizing effect was higher on MDR cells than wild-type ones in both cell lines. These results are supported by Annexin V-FITC/ PI and cell cycle assays. Pre-treatment with AEA significantly increased the early apoptotic fraction induced by cytotoxic spermine metabolites on both WT and MDR cells. The involvement of apoptotic cell death was definitely confirmed by the presence of sub-G1 peak by flow cytometric analysis of cell cycle. BSAO/spermine enzymatic system is not only able to prevent tumour cell growth, but also prevents tumour mass growth, particularly well if the enzyme has been conjugated with a biocompatible hydrogel-polymer. In fact, the growth of a mouse melanoma (B16-F0) was reduced after injection of the immobilized enzyme, in comparison with the inhibition after injection of native enzyme. To deliver BSAO into cancer cells a new strategy using nanoparticles is under investigation.

Novel superparamagnetic maghemite nanoparticles (SAMNs, surface active maghemite nanoparticles), characterized by a diameter of 10 ± 2 nanometers, were modified with BSAO, using rhodamine-isothiocyanate adduct as fluorescent spacer arm (2). A fluorescent and magnetically drivable adduct comprising BSAO immobilized on the surface of specifically functionalized magnetic nanoparticles was developed. The multifunctional nanomaterial was characterized by several techniques and activity measurements. Results showed that bare magnetic nanoparticles form stable colloidal suspension in aqueous solutions. The maximum binding capacity of bovine serum amine oxidase was about 6.4 mg g^{-1} nanoparticles. The immobilization procedure reduced the catalytic activity to 30 ± 10 % with respect to the native enzyme. It could be used, in the presence of polyamines, as fluorescent magnetically drivable H_2O_2 and aldehydes producing system. These results might be of great interest in clinical application, suggesting a new and promising anticancer therapy.

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Multiscale type behaviours of drug loaded polymeric micro/nanoparticles in the in-vitro release process.

Elena Simona Bacaita[a,b], Cătălina Peptu[a], Gabriela Andrei[a], Marcel Popa[a], Maricel Agop[b]

[a] *Department of Natural and Synthetic Polymers, Faculty of Chemical Engineering and Environmental Protection, "Gheorghe Asachi" Technical University, Prof. dr. docent Dimitrie Mangeron Rd., No. 73, 700050, Iasi, Romania ;* [b] *Physics Department, Faculty of Machine Manufacturing and Industrial Management, "Gheorghe Asachi" Technical University, Prof. dr. docent Dimitrie Mangeron Rd., No. 59A, 700050, Iasi, Romania*

Email of presenting author: bsimona77@yahoo.com

Polymeric matrices can be produced in one of the forms: micro/nano-particles, micro/nano capsules, hydro gels, films, patches. Due to the multitude of biocompatible polymers that can be used in the experimental protocol, these are considered proper for delivery of drug via many routes of administration. Regardless their form, polymer matrices as drug carriers must have the following properties: biocompatibility, biodegrability, controlled release capacity. The last one refers to the fact that in order to have an efficient and non-toxic administration of drug, its concentration must be in the therapeutic window: a minimum concentration is required to produce a wanted effect but as levels are elevated, a toxic threshold is crossed. In this context, the identification of the function that describes the kinetics of drug release is very important.

A particular case of polymeric matrices are the micro/nanoparticles, following an original double crosslinking method of a CS-GEL mixture, both being biocompatible.

The experiments revealed different type behaviours at different time scales, the measured parameter being the released drug concentration.

Thus, if at small time scales, of hours order, their evolutions are described, with very good correlation coefficients by Peppas relation, of power type. This fact indicates the fact that self-structuring processes take place, these processes being described by power type laws, specific for fractal systems. We must mention that regardless the form of the polymer matrix, its behavior can be described by the same law, indicating thus the universal character of the behaviour at small time scale.

Following the experiments at large time scales, of days order, long enough so that all the phenomena to take place simultaneously (swelling, dissolution, diffusion, chemical and physical interactions between drug and polymer, drug and polymer degradation), large variations of released drug concentrations, specific for self-structuring processes were observed. To build a theoretical model, for this case, proved to be very difficult due to the complexity of the phenomena involved, but the empirical modelling lead us to the function that describes these evolutions at large time scales, namely the Weierstarss function, known as a „fractal" function (a countinous, but non-differentiable function).

The functions that describes small time and large time scales behaviours (power type law and Weierstrass function) give them fractal characteristic and offers the possibility of new approaches for drug release process from polymeric matrices.

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Industry Report

Multiplexed label-free bio-affinity analysis and MALDI-MS Characterisation of bound analyte

Massimo Placidi

Chiraz FRYDMAN, HORIBA Scientific, Chilly-Mazarin, France

Email of presenting author: Massimo.Placidi@horiba.com

Surface Plasmon Resonance (SPR) is an optical technique offering label-free analyses of molecular interactions in real time. It provides information on kinetic processes (association and dissociation), binding affinity and molecule detection.
SPR imaging (SPRi) uses SPR in a micro-array approach. It is the ideal solution for rapid and multiplexed investigations. It allows:

- the rapid quantification and monitoring of biomolecular interactions
- the study of up to several hundreds of label-free bindings simultaneously

The coupling of Surface Plasmon Resonance imaging (SPRi) and Matrix-Assisted Laser Desorption Ionization Mass Spectrometry (MALDI-MS) is an innovative approach for biomarker discovery in biological fluids. Multiplexed SPRi analysis allows the direct visualization and thermodynamic analysis of molecular avidity, and is advantageously used for ligand fishing of captured biomolecules on multiple immobilized receptors on a SPRi-Biochip surface. MALDI-MS is a powerful tool for the identification and characterization of molecules by their molecular weight and peptide sequence. Therefore, the combination of SPRi and MS into one concerted procedure, using a unique dedicated surface, is of great interest for functional and structural analysis of bound molecules. Results will be shown using the Lymphocyte Activation Gene 3 (LAG3) protein, a potential biomarker of breast cancer and tuberculosis. LAG3 was captured in human plasma by SPRi down to several femtomoles/mm². Then, after MS pre-processing, LAG3 was successfully identified by MALDI-MS directly on the SPRi biochip. The coupling of SPRi to MS analysis is possible and is a valuable tool for biomarker identification.

Posters

FOLATE TARGETED MRI IMAGING PROBES

Diego Alberti[a], Simonetta Geninatti Crich[a], Maria Rosaria Ruggiero[a], Silvio Aime[a].

[a] *Department of Chemistry IFM, University of Turin, Via Nizza 52, 10126, Turin, Italy.*

Email of presenting author: albertidiego@hotmail.com.

In respect to other imaging modalities such as PET or SPECT, the low sensitivity is the main limitation of the Magnetic Resonance-Molecular Imaging approach. The use of nanoparticles as carriers for MRI contrast agents (CA) has both the advantage to transport a high number of CA units at the site of interest and an improved efficiency of their contrast enhancement properties. Nanoparticles have to be functionalized with characteristic ligands that bind specifically to receptors that are expressed primarily on malignant cells. Folic acid is a vitamin essential for the proliferation and maintenance of cells. Significant levels of proton-coupled folate transporter (PCFT) are expressed in the majority of human solid tumor cell lines and folate receptor alpha (FR-α) is overexpressed in particular on ovarian and colon human cancer. In this study, liposomes containing lipophilic Gd-complexes, functionalized with folic acid have been considered to visualize both in vitro and in vivo ovarian (OVCAR-3, IGROV-1) and breast cancer cells (MDA-MB-231).
The uptake by ovarian and breast cancer cells of folate targeted liposomes was significantly higher with respect the non-targeted liposomes. This indicated that the non-specific binding of pegylated non-targeted liposomes was almost nil and demonstrated that the uptake of folate targeted liposomes involves folate receptors and transporters. In IGROV-1 tumor bearing mice, targeted and non targeted liposomes showed enhanced uptake in the tumor, however, no difference was observed in tumor accumulation between the two different liposomes. Likely, passive targeting due to EPR effect dominated the biodistribution of both formulations, partly loosing the targeting effect of the folate vector. On the contrary, in MDA-MB-231 tumor bearing mice, targeted liposomes showed a higher tumor uptake with respect to non-targeted liposomes. This could be the consequence higher folate transporter (PCTF) efficiency and expression by these breast cancer cells. Furthermore, since PCFT is a symporter that functions optimally at pH 5.5, its transport function may be enhanced in solid tumors characterized by an acid microenvironment.
Conclusions. Folate targeted liposomes display enough sensitivity to allow ovarian and breast cancer in vivo MRI visualization. Furthermore, targeted liposomes can also be loaded with antitumor drugs in order to perform imaging guided therapy.

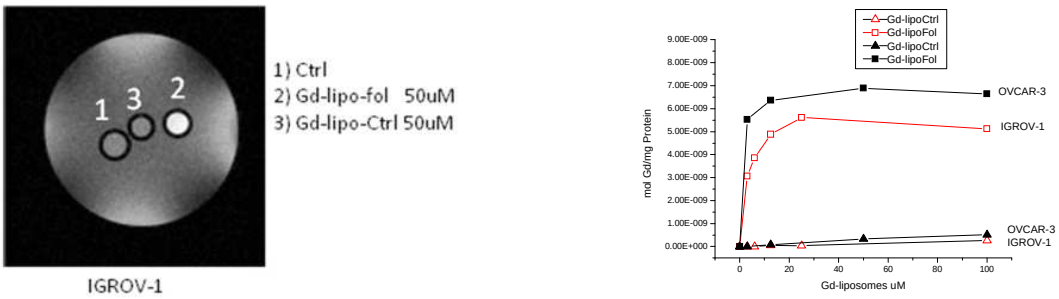


Figure 1. Left) T_1-weighted spin echo MRI images (measured at 7 T) of an agar phantom containing unlabeled cells (1) or cells incubated with 50 μM Gd of Gd-lipo-fol (targeted liposome) (2), Gd-lipo-ctrl (non-targeted liposome) (3) for 6 h at 37°C. Right) Uptake of targeted and non targeted Gd containing liposomes in OVCAR-3 and IGROV-1 cells on incubation for 6 hours at 37°C in the presence of increasing concentrations of Gd containing liposomes (3 – 100 μM).

Lamellar phases including a calixarene-based glucose functionalized bolaamphiphile for multivalent targeted drug delivery

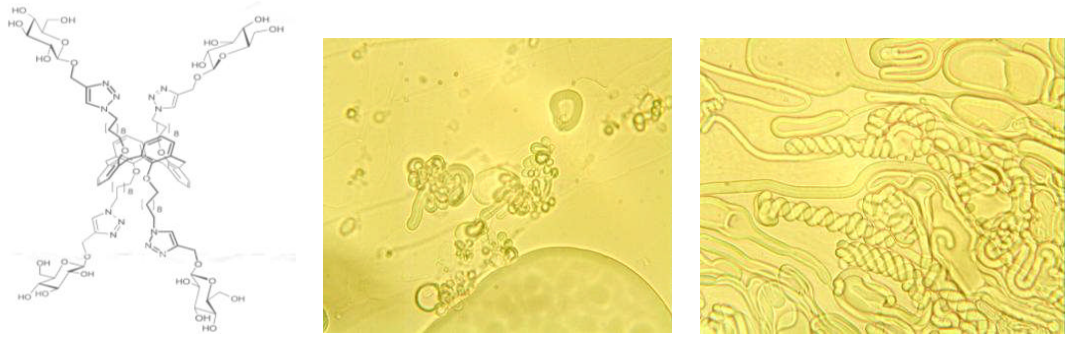
Aleandri S.[a], Casnati A.[b], Mancini G.[c], Rispoli G.[b], Sansone F.[b]

[a] Dipartimento di Chimica Università degli Studi di Roma "Sapienza" P. le A. Moro 5, 00185 Roma, Italy; [b] Dipartimento di Chimica dell'Università degli Studi di Parma, Parco Area delle Scienze 17- A, 43124 Parma, Italy; [c] CNR-IMC and Dipartimento di Chimica Università degli Studi di Roma "Sapienza" P. le A. Moro 5, 00185 Roma, Italy.

Email of presenting author: simone.aleandri@uniroma1.it

Bolaamphiphiles are formed by two polar regions connected by one or more hydrophobic chains. The study of bolaamphiphiles based liposomes showed that they feature increased rigidity and lower permeability with respect to conventional liposomes thus being of great interest as drug delivery systems.[1]

We herein describe the synthesis, the characterization and the inclusion in liposomes and lamellar phases of the bolaamphiphile **1** built on a calixarene scaffold. The macrocycle was shaped in the 1,3-alternate structure and functionalized with four hydrophobic tails terminating with glucose head-groups. The glycosyl units render glycocalixarene **1**, and its liposome formulations, potentially useful as multivalent ligands[2] for carbohydrate binding proteins (lectins) to be used in site-specific drug delivery systems. Because of the rigid, calixarene scaffold bola **1** can be included only in liposome formed by unsaturated lipids. Optical microscopy was exploited to investigate the growth, shape and size of myelin structures composed of dioleoyl-sn-glycero-phosphatidylcoline (DOPC) and bola **1**. The affinity of the bolaamphiphile **1** containing liposomes for the lectin was also investigated by fluorescence experiments in solution, towards fluorescence labeled Concanavalin A.



1 **a)** **b)**

Figure 1. Calixarene-based bolaamphiphile **1**, myelin figures formed by a 9:1 DOPC/**1** mixture (a), and by pure DOPC (b).

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Synthesis of Cross-linked Block Ionomer Complexes of Superoxide Dismutase and PLL-PEG for Biomedical Applications.

Aleksashkin A.D.[a,c], Nukolova N.V.[a,b], Klyachko N.L.[a], Kirzhanova E.A.[a], Pechyonkin M.A.[a], Balabushevich N.G.[a], Kabanov A.V.[a,d], Manickam D.S.[d]

[a]Faculty of Chemistry, Lomonosov Moscow State University, Moscow, Russia; [b]The Serbsky State Scientific Center for Social and Forensic Psychiatry, Moscow, Russia; [c] Faculty of Materials Science, Lomonosov Moscow State University, Moscow, Russia; [d]Center for Drug Delivery & Nanomedicine, Nebraska Medical Center, Omaha, NE, USA.

E-mail of presenting author: AntonD.Aleksashkin@gmail.com

Numerous central nervous system (CNS) disordes accompanied with activation of microglial cells, which is linked to overproduction of the reactive oxygen species and subsequent neuronal damage. The use of the antioxidant enzymes (such as superoxide dismutase (SOD) and catalase) is considered for therapy of pathological processes in CNS. For the treatment antioxidant enzymes should be resistant to proteolysis and able to overcome the blood-brain barrier. A possible solution is to modify the catalase and SOD. The aim of this work was to obtain the immobilizing active SOD in cross-linked nanoparticles.

Formulations of SOD were prepared by electrostatic coupling of these enzymes with cationic block copolymer (poly(L-lysine)-poly(ethylene glycol), followed by covalent cross-linking with glutaraldehyde, DTSSP and other cross-linkers to stabilize nanoparticles. Also different methods to improve properties have been tried.

All samples were characterized by several parameters: protein yeild, specific activity, total activity, particle size. We have studied the effect of the linker, the amount of copolymer, the way of synthesis on the result.

Obtained SOD nanoparticles were 4-5 times bigger than native enzyme. The loss of specific activity is about 70%.

Thus, active SOD nanoparticles were obtained, giving perspectives to overcome the blood-brain barrier and deliver the enzyme in the CNS.

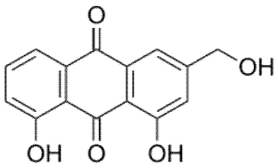
Remote loading of Aloe-Emodin in liposomes for cancer therapy.

Barbara Altieri [a], Cecilia Bombelli [b], Giorgio Cerichelli [a], Luisa Giansanti [a], Chiara Giuliani[c], Giovanna Mancini [b], Annarita Stringaro [d], Marisa Colone [d].

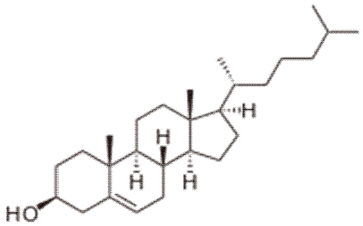
[a] Dipartimento di Scienze Fisiche e Chimiche, Università degli Studi dell'Aquila, Via Giovanni Falcone 25, 67100 L'Aquila, Italy; [b] CNR-IMC and Dipartimento di Chimica Università degli Studi di Roma "La Sapienza", P. le A. Moro 5, 00185 Roma, Italy ; [c] Dipartimento di Chimica Università degli Studi di Roma "La Sapienza", P. le A. Moro 5, 00185 Roma, Italy; [d] Dipartimento Tecnologie e Salute, Istituto Superiore di Sanità, Viale Regina Elena 299, 00161 Roma, Italy

Email of presenting author: barbaraltieri@gmail.com; kia.giuliani@virgilio.it

Aloe extracts have long been used for medicine, dietary supplements and cosmetic purposes. Aloe-Emodin (AE), a hydroxyanthraquinone compound, has identified as one of the main active components in Aloe. This bioactive compound shows an antiinflammatory, antibacterical effects and possesses an antioxidant [1], radical scavenging and antiproliferative activity in some types of cancer cells. [2,3] Therefore the inclusion of AE into liposomes for therapeutical applications is an interesting goal. In fact liposomes increase the specificity, the pharmacological activity and reduce the toxicity of various drugs.
A large number of loading strategies are currently available. The selection of the optimal procedure for the encapsulation and the localization of the drug into liposomes depends on its hydrophobicity/hydrophilicity. Hydrophilic compounds localized in the internal aqueous bulk, meanwhile lipophilic molecules are associated with the liposome bilayer. The hydrophobicity/hydrophilicity of same molecules can be modulated by pH conditions.
The remote loading of AE into the aqueous compartment of vesicles, can be drived by generating a pH gradient across the lipid membrane[4].
Herein the investigation of the remote loading of AE into DPPC/chol liposomes is reported.



Aloe Emodin



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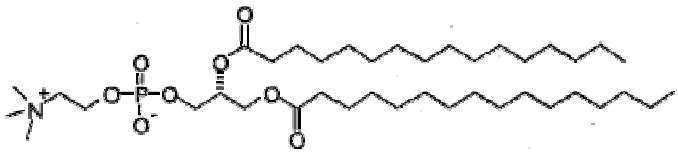

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Stereochemistry of the gemini surfactant influences the internalization pathways of cationic liposomes in human tumor cells

Giuseppina Bozzuto[a], Cecilia Bombelli[b], Luisa Giansanti[b], Annarita Stringaro[a], Marisa Colone[a], Laura Toccacieli[a], Giuseppe Formisano[a], Agnese Molinari[a], Giovanna Mancini[b]

1. Department of Technology and Health, Italian National Institute of Health, Viale Regina Elena 299, 00161 Rome, Italy. 2. Institute of Chemical Methodologies, CNR and Department of Chemistry University of Rome "Sapienza", Piazzale A. Moro 5, 00185 Rome, Italy.

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

In a previous investigation we reported that the presence of cationic gemini surfactant (S,S)-2,3-dimethoxy-1,4-bis(N-hexadecyl- N,N-dimethylammonio)butane bromide **1a** in the liposomes formulated with dimyristoyl-*sn*-glycero-phosphatidylcholine, DMPC, increases both cell uptake of the photosensitizer *m*-tetrahydroxyphenylchlorin, *m*-THPC[1, 2], and its cytotoxic effect after laser irradiation with respect to the pharmaceutical formulation (Foscan®) in human colon adenocarcinoma cells[1] and murine and human glioblastoma cells [2]. In order to explore the influence of the stereochemistry of the gemini surfactants on the delivery efficiency of *m*-THPC to malignant glioma cell lines, liposomes were formulated with DMPC, and either cationic gemini surfactant **1a**, or (S,R)-2,3-dimethoxy-1,4-bis(N-hexadecyl-N,N-dimethylammonio) butane bromide (**1b**). The stereochemistry of the gemini was found to strongly influence the delivery efficiency of *m*-THPC to cells and its intracellular distribution.

Endocytosis includes a variety of pathways: clathrin-mediated and caveolae-mediated endocytosis; phagocytosis, and clathrin- and caveolae-independent endocytosis. Because the pathway chosen depends on the size and nature of the extracellular cargo, we investigated the influence of the stereochemistry of the surfactant on the interaction with the cell membrane and the internalization pathway of liposomes. In a first approach, a combination of different inhibitors was used to selectively block different pathways. The inhibition effects were evaluated both by flow cytometry and by LSCM on human (LN229) and murine (C6) glioblastoma cells. The analysis performed revealed that both DMPC/**1a** and DMPC/**1b** liposomes followed the endocytic pathway but that the internalization of the two formulations were influenced by different endocytic inhibitors. The results strongly suggest that DMPC/**1a** liposomes enter into the cells preferentially through the interaction with rafts and caveolae as demostrated by the filipin-induced inhibition. On the other hand, DMPC/**1b** liposomes seem to enter into the cells preferentially upon interaction with clathrin, as showed by the inhibition exerted by chlorpromazine.

In the second approach, several antibodies specific for organelles involved in endocytic routes were employed. The observations performed on cells treated with DMPC/**1a** or DMPC/**1b** confirmed data obtained by flow cytometry. In addition, it was observed that DMPC/**1a** liposomes colocalized preferentially with early endosomes (Rab5+) whereas DMPC/**1b** liposomes were found in early and late endosomes (Rab7+), and in lysomomes (Lamp1+).

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Voacamine as chemosensitizer included in liposome formulations

Maria Condello[a], Giuseppe Arancia[a], Giovanna Multari[b], Francesca Gallo[b], Simone Aleandri,[c] Luisa Giansanti,[d] Giovanna Mancini,[e] Stefania Meschini[a]

[a]Department of Technology and Health,[b]Department of Drug Research and Evaluation, Istituto Superiore di Sanità, Viale Regina Elena, 299, 00161 Roma, [c]Dipartimento di Chimica, Università degli Studi di Roma "La Sapienza", P.le A, Moro 5, 00185 Roma, [d] Dipartimento di Scienze Fisiche e Chimiche, Università degli Studi de L'Aquila, Via Vetoio Coppito 67100 L'Aquila, [e]CNR, Istituto di Metodologie Chimiche Sezione Meccanismi di Reazione c/o Dipartimento di Chimica, Università degli Studi di Roma "La Sapienza", P.le A, Moro 5, 00185 Roma.

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

Tumors of different histological origin may be little or not sensitive to drug treatments. It is therefore necessary to identify new strategies and combined therapies that may improve the efficacy of treatments and expectancy of patients life. In the scientific community there has been recently a renewed interest in the use of liposomes as drug delivery systems. The therapeutic treatment with liposomal formulations does not cause an inflammatory response and does not alter the immune system. Moreover, liposomal carriers can greatly affect the pharmacokinetics and tissue distribution of chemotherapeutic drugs. The use of liposomes incorporated with anticancer drugs can lead to greater efficacy of the drug itself and a decrease of its side effects.

We reported previously that the bisindolic alkaloid voacamine (VOA), extracted from the stem bark of a Brazilian plant, showed a powerful chemosensitizing effect against Doxorubicin (DOX) in drug-resistant cell lines derived from osteoarticular and gastrointestinal districts and no effect on normal human lines (1, 2).

Herein we report on the treatment of osteosarcoma resistant cells (U2 OS-R) with VOA included in liposomes composed of 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC). The clonogenic survival test was used to determine the sensitivity of U2 OS-R to DOX, DOPC, VOA alone, VOA plus DOX and DOPC/VOA plus DOX. Flow cytometric analyses were used to evaluate the presence of cell surface P-glycoprotein (P-gp) in resistant cells and DOX accumulation after combined treatments. Finally, the DOX distribution in the presence and in the absence of VOA (either used free or included in DOPC liposomes) was evaluated by confocal microscopy. Cell survival was not affected by VOA and DOPC. DOX treated resistant cells were scarcely damaged by the drug given alone, whereas a strong cytotoxic effect was observed following treatment with both combination, VOA plus DOX and DOPC/VOA plus DOX. Cytofluorimetric analysis showed a remarkable increase in DOX accumulation in DOPC/VOA treatment (very similar to treatment with free VOA) compared to DOX alone. These results indicate that the chemiosensitizing effect is maintained even upon treatment with VOA included in liposomes. In addition the observation performed by confocal microscopy indicated that the DOX fluorescent drug molecules were located mainly inside the nuclei only when treatment with DOX was carried out in the presence of VOA (either free or in DOPC).

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Synthesis of new amphphilic copolymers of sucrose

M. Teresa Barros,[a] Krasimira T. Petrova[a] and Paula Correia-da-Silva [a,b]

[a] *REQUIMTE, CQFB, Departamento de Química, Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa, 2829-516 Caparica, Portugal . Fax: +351212948550; Tel:+351212948300;*
[b] *Instituto Superior de Ciências da Saúde Egas Moniz, 2829 - 511 Caparica, Portugal.*

Email of presenting author: pml.silva@campus.fct.unl.pt

Carbohydrates are a sustainable source of feedstocks due to the fact that they are natural substances, abundant and often cheap. In nature these compounds are consumed and this makes their derivatives potentially biodegradable and biocompatible.
The chemoselectivity of the hydroxyl groups of sucrose permits the formation of mono- and di-substituted sucrose derivatives in the 6 and 6' positions. Moreover, besides the conventional synthesis conditions, microwave irradiation has also been used, for the purpose of reducing the amount of solvents used and also to operate under less aggressive conditions.
Cationic living polymerization has been used to obtain new copolymers of ethylvinyl ether and sucrose, with potential biological applications. The amphiphilic linear block copolymers achieved by this method are expected to have the ability to self-assemble, which will confer on them interesting physical, mechanical and thermal properties. The results obtained using this polymerisation technique will be compared to those obtained previously.

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Synthesis of polymeric nanoparticles consisting of steroid, carbohydrate, and polymer conjugates

Carina Crucho[a], Maria T. Barros[a]

[a] *REQUIMTE, CQFB, Departamento de Química, Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa, 2829-516 Caparica, Portugal*

Email of presenting author: cic17468@campus.fct.unl.pt

Nature continues to be the ultimate in nanotechnology, where polymeric nanoarchitectures play a central role in biological systems. Nature's efficiency in assembling her versatile building blocks on the submicron scale has inspired synthetic chemists in their quest to make nanostructures and to incorporate these into macrostructures as nature does. (1) In this context, chemistry, and in particular, supramolecular chemistry is consolidating itself as a central science, as it finds ways to increase the dimensionality and complexity of these new materials and allows strict control and tunability of their properties and functionalities. Taking this into consideration, in the present work we have studied the formation of polymeric nanoparticles based on amphiphilic polymeric conjugates composed of a cholic acid or cholesterol derivative and/or carbohydrate moiety, and different size polymer chains with either a vinyl or polyethylene glycol backbone. Such conjugates are expected to be capable of self organizing under the proper conditions to form nanoparticles having diverse functionality, with their main application in target drug-delivery systems. These synthetic tasks were carried out using methods based upon our experience in carbohydrates chemistry. (2, 3)

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Preliminary approaches to prepare biocompatible and antibacterial cotton fabrics

Gabriella Di Carlo[a], Daniela Ferro[a], Gabriel Maria Ingo[a], Sergey M. Barinov[b], Giuseppina Padeletti[a]

[a] *Istituto per lo Studio dei Materiali Nanostrutturati (ISMN) – CNR, Via Salaria Km 29300, 00015, Monterotondo Stazione (Rome), Italy;* [b] *A.A. Baikov Institute of Metallurgy and Materials Science, Russian Academy of Sciences, Leninsky prospect 49, 119991, Moscow, Russia.*

Email of presenting author: gabriella.dicarlo@ismn.cnr.it.

The effectiveness of nano-hydroxyapatite (nHA) has been attested in various biomedical areas. During the last years our activity started to consider some strategies for the synthesis of hydroxyapatite as a fully biocompatible material and in a physical state suitable for biomedical applications [1,2]. The actual research is mainly focused on the functionalization of cotton fabrics with hydroxyapatite and silver in order to obtain biocompatible and bactericide textiles, which could be used for the treatment of burns and wounds. In particular, the antibacterial properties have a crucial role for the prevention of cross-infection is in medical care and welfare institutions.
With this aim, the present study is designed to functionalize cotton fabrics with hydroxyapatite and silver nanoparticles in order to realize biocompatible and antibacterial textiles. The cotton fabrics have been modified with hydroxyapatite using two different types of binders: chitosan and silica sol. In addition to the deposition of hydroxyapatite, the cotton fabrics were also functionalized with silver nanoparticles by using a novel green and facile synthetic route. The formation of silver nanoparticles was confirmed by UV-vis spectroscopy, due to the presence of the typical band plasmon resonance.

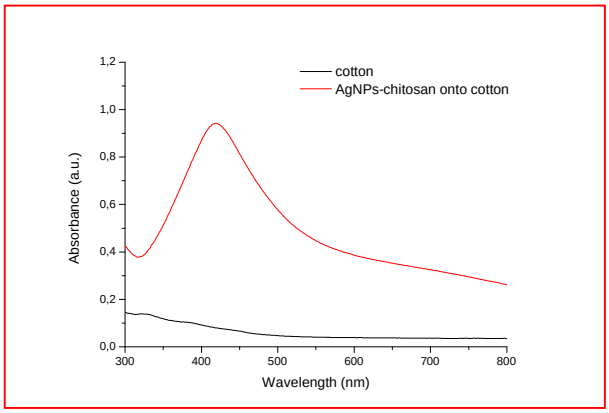

Figure 1. UV-vis of cotton fabrics functionalized with chitosan-stabilized Ag nanoparticles and of bare cotton.

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Study of the interactions of core-shell magnetite nanoparticles with lipid bilayers in the relevant biological environment

Desire' Di Silvio[a], Paul McNaughter[b], Andrew Mayes[b], Roger Parker[c] and Francesca Baldelli Bombelli[a]

[a]School of Pharmacy, University of East Anglia, Norwich Reasearch Park, NR4 7TJ, Norwich, UK; [b] School of Chemistry, University of East Anglia, Norwich Reasearch Park, NR4 7TJ, Norwich, UK; [c] Institute of Food Research, Reasearch Park, NR4 7UA, Norwich, UK.

Email of presenting author: D.Di-Silvio@uea.ac.uk.

Background: The use of nanoparticles (NPs) in medicine is ever increasing. The development of drug delivery systems that can help to overcome most of the limits of the conventional drugs (solubility, toxicity, dosage, targeting, *in vivo* stability and circulation half-life) is a challenge and the versatile properties of NPs allow their application in therapeutics, diagnostics and imaging[1]. One of the major advantages of the NPs is their targeting ability: either passive or active. NPs accumulate spontaneously in diseased tissues thanks to the enhanced permeability and retention effect (EPR) due to a leaky vasculature respect to that of normal tissues. Active targeting is achieved functionalizing NPs and exploiting specific interaction such as antibody-antigen, ligand –receptor and aptamers. *In vivo* the specific interaction between the functionalized NPs and the biological counterpart is affected by the surrounding environment. Proteins interact with NPs forming a corona around the surface that changes completely its chemical-physical properties[2]. The present work aims to characterize polymer coated 20-30 nm magnetite NPs (Fe_3O_4-NPs) with different surface moieties and study the interactions with models of cell membrane through Quartz Crystal Microbalance (QCM-D) and Atomic Force Microscopy (AFM) in the relevant biological environment.

Methods: Fe_3O_4-NPs were synthesized in organic solvent by thermal decomposition (*Sun et al. 2003*) and transferred to water solution by amphiphilic polymer coating (*Parak et al. 2008*). NPs with carboxylic and PEG groups were purified (dialysis and size exclusion chromatography) and characterized by DLS, Z potential, TEM, ICP-AES, agarose gel. Protein corona were characterized by SDS-PAGE. QCM-D and AFM experiments were performed incubating NPs in 55% of serum and forming a lipid bilayer from monodispersed vesicles solution.

Results: Different surface-functionalized Fe_3O_4-NPs are characterized by diverse size and Z-potential. These properties influence the interaction with the proteins in the biological medium affecting the interactions with the lipid bilayers.

Discussion or Conclusion These preliminary experiments showed the role of the environment proteins in mediating the interactions between the NPs and the biological matter. The different functional groups on the surface of the NPs determine the formation of a diverse protein corona. The optimized methodology developed here will be useful for extending this study to investigate the role of the environmental protein in the specific binding between engineered targeted NPs and the desired biological substrate.

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Preliminary approach to realize cotton fabrics containing calcium phosphate particles and biopolymers.

S.M Barinov[a], A.Yu.Fedotov[a], I.V.Fadeeva[a], N.V.Petrakova[a], V.S.Komlev[a]
D.Ferro[b] ,G.Padeletti[b], G.Di Carlo[b] , G.Ingo[b]

[a] *Baikov Institute for metallurgy and materials Science RAS (IMET)- Moscow, Russia;*
[b] *CNR Istituto per lo Studio dei Materiali Nanostrutturati (ISMN)- Roma, Italia*

Email of presenting author : daniela.ferro@cnr.it

In the frame of the Program "Innovative ceramics, cements and composites for diseased bone tissue repair: research, development and innovations" in the bilateral accordance between Consiglio Nazionale delle Ricerche and the Russian Academy of Science , CNR-ISMN and RAS-IMET, several studies have been carried out about the use of calcium phosphates in medicine [1-3]. In the present study a new approach has been undertaken to support calcium phosphate nanoparticles on textile supports. Treatments of burns and wounds requires the application of dressing to absorb drainage and to isolate the wound from the environment. As dressing are generally fabricated using various cotton materials, more often as cotton gauze, and contain antimicrobial agents to prevent bacterial contamination, this natural fibers has been considered as support of the nano-calcium phosphate (HA) and its functionalized forms such as silver ions to enhance the antimicrobial properties [4]

The chemical process to obtain HA cotton coverage with different characteristics, from the biocompatibility to the particles structure and dispersion without makings substantial modifications of the tissue mechanical properties requires the selection of adequate procedures. The two Institutes involved in the study, experimented various techniques of to fabricate cotton tissues containing calcium phosphates by starting from the infiltration with biopolymers solution containing HA particles.

Diagnostic investigations to control the phase composition, particles size, fibres morphology have been carried out with the instrumentations of both the two Institutes.

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Gold hydrophilic nanoparticles interacting with dexamethasone: loading and release study

Laura Fontana[1], Francesca Fabber[1], Ilaria Fratoddi[1], Iole Venditti[1], Maria Vittoria Russo[1], Cesare Cametti[2].

[1]Dipartimento di Chimica Università " La Sapienza" Piazzale Aldo Moro 5, Roma, Italia
[2]Dipartimento di Fisica Università " La Sapienza" Piazzale Aldo Moro 5, Roma, Italia

Email of presenting author: laura3fontana@yahoo.it

The target of this work has been the systematic study of the behavior of different thiols used for the stabilization of gold nanoparticles (AuNPs). The thiols used for the functionalization of NPs, showed in figure 1, have been chosen for their peculiar features: sodium_3-mercapto-1-propansolfunate **(3MPS)**, because of the presence of terminal charged groups, that induce a good solubility in water and in polar solvents; 3-mercaptopropyl-trimethoxysilane **(3MPT)**, that has been used together with 3MPS, considering that its terminal groups can be able to give a crosslinking reaction or anchorage on surfaces. The AuNPs have been prepared by using Shiffrin-Brust's synthesis, in which a strong reducing agent, as sodium borohydride, reduce the metallic ions ($AuCl_4^-$) in the presence of the chosen thiol [1].

Figure 1: Thiols used for the AuNPs functionalization: **3MPS** and interaction scheme of **AuNPs** with **DXM**

The obtained nanomaterials have been investigated by means of FTIR, NMR and UV-vis spectroscopies to obtain information about theirs chemical structure. DLS, Z-scan, TEM and FE-SEM measurements have been carried out to study their size, shape and surface charge. Furthermore, through a careful method of separation, it has been possible to obtain a good modulation of the size of the nanoparticles, that are the range between 5 nm and 180 nm for AuNPs. The Surface Plasmon Resonance (SPR) was observed at the value of ~ 520 nm for AuNPs. This material can be available in nanomedicine and in particular in nanodrugs delivery by means of surface interactions with bioactive molecules, which can be released in appropriate conditions [2,3]. AuNPs functionalizated by 3MPS, have been herein used for the bioconjugation with dexamethasone (**DXM**), that is a potent synthetic glucocorticoid with an anti-inflammatory action. From our preliminary studies, it has been observed that the DXM is able to be loaded to the functionalized AuNPs, by a simple and straightforward approach, in about four hours. The observed loading was very high, *i.e.* approximately 80%. The studies revealed a very slow release of the drug in PBS (phosfate buffer, pH = 7.4 and T = 37°C).

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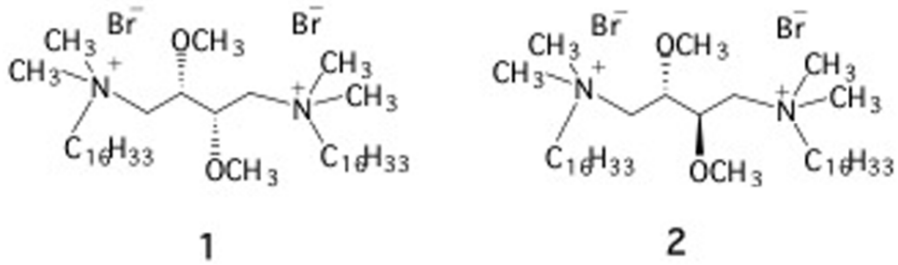
Interaction of cationic liposomes with cell membrane models: correlation with their biological activity

Cecilia Bombelli[a], Maria Grazia Bonicelli[b], Federico Bordi[c,d], Luisa Giansanti[a], Giovanna Mancini[a], Simona Sennato[c,d]

[a]CNR-IMC and Dipartimento di Chimica Università degli Studi di Roma "Sapienza" P.le A. Moro 5, 00185 Roma, Italy: [b]Dipartimento di Ingegneria Chimica, dei Materiali e dell'Ambiente (ICMA), Università degli Studi di Roma "Sapienza", via del Castro Laurenziano 7, 00161 Roma, Italy; [c]Dipartimento di Fisica, Università degli Studi di Roma "Sapienza", P.le A. Moro 5, 00185 Roma Italy; [d]CNR-IPCF, Università degli Studi di Roma "Sapienza", P.le A. Moro 5, 00185 Roma, Italy

Email of presenting author: luisa.giansanti@uniroma1.it

Detailed investigations on liposomes formulated with 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC) and the cationic gemini surfactants (*S*,*S*)-2,3-dimethoxy-1,4-bis(*N*-hexadecyl-*N*,*N*-dimethylammonium)butane bromide **1** or its stereoisomer (*S*,*R*)-2,3-dimethoxy-1,4-bis(*N*-hexadecyl-*N*,*N*-dimethylammonium)butane bromide, **2**, showed that the stereochemistry of the gemini components significantly affects important features of the mixed liposomes as carriers: the efficiency of the delivery, the intracellular distribution of the drug and the DNA condensation and transfection in gene delivery.[1-6] Moreover, the delivery efficacy was shown to depend on cell lines.[4,5] Herein an investigation on the interaction of neutral and anionic phospholipid liposomes, used as cell models, with cationic liposomes by differential scanning calorimetry, fluorescence experiments and electrophoretic mobility is reported. This study was aimed at rationalizing the different biological features shown by liposomes based on different gemini stereoisomers.



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Strategies for development of high-sensitive immunodiagnostic tools for the detection of bacterial pathogens

Arina V.Kozyr, Alexander V. Kolesnikov, Anna E. Khlyntseva, Nina E. Luneva, Alyona K. Kutsenko, Olga N. Krasavtseva, Igor G. Shemyakin

State Research Center for Applied Microbiology and Biotechnology,
142279, Obolensk, Russia;

E.mail of presenting author: AVKozyr@gmail.com

Creation on novel high-sensitive tools for the detection of bacterial infection is a priority task for organization of efficient epidemiological control and health protection. Most of widespread conventional immunodiagnostic kits, majority of which is based of IFA techniques, do not supply desirable sensitivity necessary for the detection of a certain important life-threatening bacterial pathogens, which may be present in nanoscale amounts at early stages of disease.

Immuno-PCR, an actively developing approach, based on combination of antibody affinity molecule with DNA as a detection target, offers an opportunity to achieve up to 10^6-fold increase in sensitivity of traditional immunoassay. Diagnostic efficiency of immuno-PCR technique was vastly investigated employing both monoclonal antibody couples and polyclonal antibodies for the detection of nosocomial infections caused by **A.baumannii,** NDM-1-carrying bacterial strains, and hazardous gastrointestinal disease, caused by enteropathogenic **E. coli** O157:H7 strain, as well as for detection of dangerous infections such as anthrax and botulism. Methods for obtaining of antibody-DNA conjugates comprised direct chemical coupling of DNA to antibody as well as formation of complexes between biotinylated antibody and biotin-labeled DNA via streptavidin bridges. Experimental data has shown that sensitivity as high as 1 pg/ml can be routinely achieved by this assay in the detection of proteinaceous targets, such as bacterial toxins. Sensitivity of detection for whole bacterial cells by immuno-PCR mounted up to 1 bacteria/ml. Application of real-time PCR detection for immuno-PCR assays permitted witnessing the dynamics of pathogen accumulation in the infection course. Comparison of strategies for antibody immobilization has outlined preferential employment of paramagnetic particles as a solid phase for antibody binding over utilization of immunoplates.

Potential of immuno-PCR technology was assayed in multiplex format for the selective detection of pathogenic bacteria. For this purpose pathogen-immobilizing monoclonal antibodies to three different pathogenic bacterial strains (*E.coli* O157:H7, *P.aeruginosa* and *A.baumannii*) were linked to paramagnetic particles, and detecting antibodies to these pathogens were coupled with unique DNA fragments and bound to golden particles. Amplification of specific DNA fragments linked with monoclonal antibody to certain bacterial pathogen permitted selective detection of bacteria species in liquid samples.

Our data shows, that immuno-PCR, as well as other diagnostic formats employing DNA-antibody complexes as detection modules, such as antibody-DNA microarrays, appears an efficient strategy for nanomedicine in acquisition of selectivity and improvement of sensitivity in routine bacterial immunoassays.

Arginino-based calix[4]arenes for gene delivery

Michela Lomazzi,[a] Valentina Bagnacani,[a] Michele Bassi,[a] Francesco Sansone,[a] Gaetano Donofrio,[b] Alessandro Casnati,[a] RoccoUngaro [a]

[a]*Dipartimento di Chimica Organica e Industriale, Università degli Studi di Parma, Parco Area delle Scienze 17/a, 43124 Parma;* [b]*Dipartimento di Salute Animale, Università degli Studi di Parma, Via del Taglio 8, 43126 Parma, Italy.*

Email of presenting author: michela.lomazzi@gmail.com

The future success of Gene Therapy is intimately linked to development of carrier systems able to efficiently cross cell membrane and transport nucleic acids in the target cells.[1] A number of nonviral gene delivery carriers, in particular cationic lipids and polymers, have been developed as alternatives to the viral ones. Due to their properties, Cell Penetrating Peptides (CPPs) have also received much attention in the field of gene delivery as molecular transporters. [2] In particular a special role is played by arginine-rich peptides, where the structure and basicity of this specific aminoacid facilitate cellular uptake and the direct intecation with the DNA phosphodiester skeleton.
On the basis of these data, in the last years, we prepared new potential nonviral vectors based on calixarenes functionalized with guanidinium groups.[3] Some of them showed interesting transfection properties.[4,5]
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Carbosilane dendrimers are potential nanoparticles for autoimmune therapy.

Effects of carbosilane dendrimers on PBMCs and CD4+T cells under Th17 differentiating conditions

María I. García[a], Rafael Correa-Rocha[b], Inmaculada de la Torre[c], Rafael Gómez[d], F. Javier de la Mata[d], Luis Carreño[c], María A. Muñóz-Fernández[b] and Luis A. López-Fernández[a]

[a] *Laboratory of Pharmacogenetics and Pharmacogenomics,* [b] *Laboratory of Immunobiology, and* [c] *Servicio de Reumatología, Hospital General Universitario Gregorio Marañón, Doctor Esquerdo 46, 28007, Madrid, Spain;* [d] *Departmento de Química Inorgánica, Universidad de Alcalá de Henares, Campus Universitario, 28871, Alcalá de Henares, Madrid, Spain.*

Email of presenting author: llopezf.hgugm@salud.madrid.org.

Introduction: T helper type 17 (Th17) cells are a subset of effector CD4+ T cells involved in response to fungal and extracellular bacteria and several autoimmune diseases. Carbosilane dendrimers have shown potential as drug delivery vehicles and our group recently demonstrated successful regulation of Th17 differentiation by use of the second-generation dendrimer 2G-NN16 [1, 2, 3].
Objective: The aim of this work was to measure the capacity of 2G-NN16 and three families of new carbosilane dendrimers (JS, BR and EF) to regulate Th17 differentiation in healthy and psoriatic arthritis patients.
Results: Two families of dendrimers (JS and BR) demonstrated a repressive effect on IL17 production in stimulated PBMCs that was similar to 2G-NN16. This IL17 repression was inversely correlated with dendrimer generation. Dendrimers BDJS07 and 2G-NN16 decreased memory-activated CD4+T cells cultured under Th17 differentiating conditions. Dendrimers 2G-NN16 or BDBR02 were associated with down-regulation of transcription of Th17 markers (IL17A, IL17F, IL22, IL23R, CCR6, and RORC) in in-vitro cultures of CD4+T cells from psoriatic arthritis patients.
Conclusion: 2G-NN16 and BDBR02 are potentially valuable nanocompounds for treatment of autoimmune diseases.

Polysaccharide nanohydrogels as drug carriers

G. D'Arrigo[a], C. Di Meo[a], E. Montanari[a], S. Chichiarelli[b], E. Gaucci[b], P. Matricardi[a]

[a]*Department of Drug Chemistry and Technologies, "Sapienza" University of Rome, Roma, 00185 Italy.* [b]*Department of Biochemical Sciences "A. Rossi Fanelli", "Sapienza" University of Rome, Roma, 00185 Italy.*

Email of presenting author: pietro.matricardi@uniroma1.it

The nanohydrogel systems are gaining an increasing interest in the field of drug delivery and for biomedical applications, as they can combine the favourable properties of nanotechnologies and the features of the hydrogels. In this contest, the present work is focused on the preparation of a new nanoparticulate carrier showing nanohydrogel features, using the biocompatible polysaccharide gellan as starting material. The nanoparticles were obtained by self-assembly of gellan chains previously derivatized with prednisolone moieties (fig.1). Similarly to other nanoparticulate systems described in literature [1,2], the one that is presented here can be considered as a nanohydrogel, as the hydrophilic polysaccharide chains can retain a great amount of water within the particulate form. The rationale of present system is the use of a well known gel-forming polysaccharide and a drug as the hydrophobic moiety responsible for the polymer chain assembly.

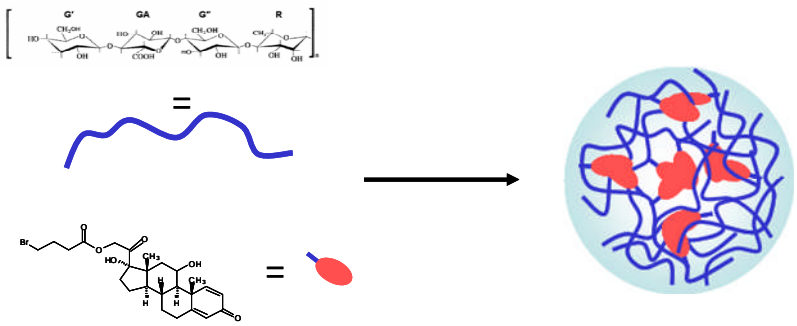

Figure 1. Scheme of nanohydrogels formation from gellan chains derivatized with prednisolone moieties.

In this study, in order to obtain a more suitable polymeric system, before the conjugation reaction, the polysaccharide chains were previously depolymerized by means of ultrasound treatment, and the prednisolone moiety was derivatized by means of a four carbon atom chain in order to reduce the steric hindrance thus improving the reaction course.
Nanohydrogels were characterized in terms of dimensions, polydispersity, ζ-potential and stability. They were also tested for their citocompatibility.

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Biochemical perturbations induced by miR34a in multiple myeloma: molecular rationales for its delivery through nanotechnological devices

Gabriella Misso[a], Angela Lombardi[a], Chiara Bertolino[a], Mayra Zarone[a], Michele Caraglia[a], Giuseppe De Rosa[b], Virginia Campani[b], Maria Teresa Di Martino[c], Pierosandro Tagliaferri[c], Pierfrancesco Tassone[c].

[a]*Department of Biochemistry and Biophysics, Second University of Naples, Via Costantinopoli, 16 80138, Naples, Italy;* [b]*Department of Pharmaceutical Chemistry and Toxicology, ''Federico II'' University of Naples, Via Montesano, 49 80131, Naples, Italy;* [c]*Tommaso Campanella Foundation, Magna Graecia University of Catanzaro, Viale Europa, Loc. Germaneto, 88100, Catanzaro, Italy.*

Email of presenting author: gabriella.misso@unina2.it.

Introduction. Multiple myeloma (MM) is a clonal B-cell malignancy characterized by the aberrant expansion of plasma cells (PCs) within the bone marrow, as well as into extramedullary sites. The actual understanding of cellular events underlying MM development is consistent with the involvement of increased cell proliferation through the activation of NFκB, PI3K/AKT and STAT-3. Stated the emerging role of micro- RNA (miRNA) in the pathogenesis of many types of human cancer including MM, we have investigated the function of the p53 transcriptionally regulated tumor suppressor miR-34a, whose expression resulted deregulated in MM. For this purpose, we have transiently transfected SKMM1 MM cells with pre-miR-34a mimics in order to evaluate the resulting modulation of survival and proliferation main mediators Akt and Erk and of the stress involved p38MAPK. We have also analyzed the modulation of the apoptosis executioners Caspase-3 and -6 and of the tumor suppressor p53, mutated for this cell line. Moreover a new nanotechnological Stable Nucleic-Acid-Lipid Particles (SNALPs) formulation encapstulating miR-34a was evaluated *in vivo*.

Methods. SKMM1 MM cell lines, which display low constitutive miR-34a levels, were electroporated, for transient expression, with both pre-miR-34a mimics and scrambled sequence. Cells were harvested 12h, 24h, 48h and 72h after transfection and processed for cell extract preparation. Equal amounts of cell proteins were separated by SDS-PAGE. The proteins on the gels were electro-transferred to nitrocellulose and reacted with the different MAbs for Western blot determination of the proteins. *In vivo* experiments were performed injecting intravenously pre-miR34a or SNALP carrying miR-34a on SCID mice bearing MM SKMM1 cells in the flank. The mature miR-34a$_s$ were encapsulated as single strands in SNALPs using a controlled step-wise dilution method.

Results. We have evaluated by Western blot experiments both the expression and activity of Akt and Erk, showing already 12h after transfection with miR-34a a reduction in activity of over 50% compared to non-transfected or scrambled sequence-transfected SKMM1 cells. We also found an about 50% decrease of pro-caspase 3 expression at 48h and of pro-caspase 6 expression at 24h and 48h. The decrease of both the pro-caspases suggested that they were cleaved and, therefore, activated to trigger the apoptotic program. Moreover a significant reduction of p53 expression was observed, an interesting outcome since SKMM1 cells expressed mutated p53. We have also found that SNALP formulation encapsulating miR34a was more active than intravenously injected pre-miR34a in SCID mice bearing MM SKMM1 in the flank.

Conclusion. In conclusion our preliminary results showed the advantage of miR34a re-expression in SKMM1 cells. Moreover we have found a new molecular weapon for the treatment of MM.

Neutral liposomes able to complex DNA: a combined experimental and computational study

Roberta Galeazzi[a], Milvia Marini[b], Luca Massaccesi[a], Giovanna Mobbili[a], Michela Pisani[b]

[a] *Di.S.V.A. Università Politecnica delle Marche, Ancona, Italy;* [b] *SIMAU, Università Politecnica delle Marche, Ancona, Italy;*

Email of presenting author: g.mobbili@univpm.it

With the aim to design novel vectors able to transfect genetic material into target cells, our group started few years ago studying neutral liposomes able either to form stable complexes with plasmid DNA in the presence of bivalent metal cations (Ca, Mg, Mn), either to transfect this material to cells (1). In order to improve the ability of neutral liposomes to complex DNA, our strategy has been to develop liposomal gene delivery systems containing new synthetic lipids lacking in positive charge but acting as effective cationic lipids (2).

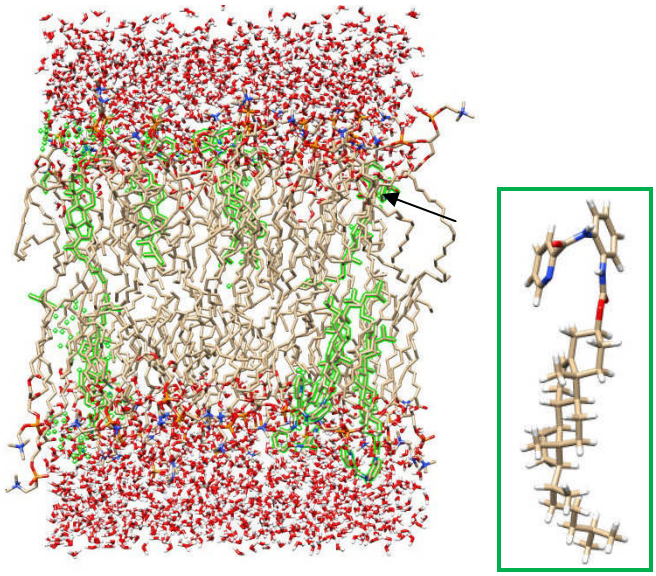

Figure 1. Structure of mixed DOPC based bilayer at 25% of cholesteryl-2-(picolinamido) phenylcarbamate.

To this purpose, trying to optimize the structure of the vectors, lipids with different chelating agents in the polar heads have been synthesized. Here we report the synthesis of a cholesteryl-2-(picolinamido)phenylcarbamate together with the results of the computational study. The neutral synthetic lipid has been mixed with commercial zwitterionic lipids (DOPC and DOPE) in different percentage and employed in the preparation of multilamellar liposomes. *In silico,* we studied first the single molecule at high level DFT, then the mixed DOPC based bilayer with different percentage composition of the chelating agent using PME molecular dynamics simulations at 310 K. Finally, the ability of these systems to form stable complexes with plasmid DNA in the presence of bivalent metal cations (Ca, Mg, Mn) has been investigated by means of synchrotron X-ray diffraction.

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Visualization and quantification of magnetic nanoparticles into vesicular systems by atomic force microscopy

Daniele Passeri[a], Chunhua Dong[a,b], Stella Corsetti[a], Livia Angeloni[a], Marco Rossi[a,f], Federica Rinaldi[c], Carlotta Marianecci[c], Maria Carafa[c], Fabrizio Pantanella[d,f], Angela Sorbo[e]

[a] *Department of Basic and Applied Sciences for Engineering, University of Rome Sapienza, via A. Scarpa 14, 00161, Rome, Italy;* [b] *Department of Physics, University of Rome Sapienza, piazzale A. Moro 2, 00185, Roma, Italy;* [c] *Department of Drug Chemistry and Technologies, University of Rome Sapienza, piazzale A. Moro 5, 00185, Rome, Italy;* [d] *Department of Public Health and Infectious Diseases, University of Rome Sapienza, piazzale A. Moro 5, 00185, Rome, Italy;* [e] *Department of Food Safety and Veterinary Public Health, Istituto Superiore di Sanità, viale Regina Elena 299, 00161, Rome, Italy;* [f] *Research Center for nanotechnology for Engineering (CNIS), University of Rome Sapienza, piazzale A. Moro 5, 00185, Rome, Italy.*

Email of presenting author: Dong.Chuanhua@roma1.infn.it.

Vesicular systems incorporating magnetic nanoparticles (MNPs) have been attracting much interest for their possible application in active drug delivery. To this aim, suitable procedures have to be developed to enhance the efficiency of inclusion of MNPs into vesicular systems. Therefore, it is crucial to dispose of reliable control tools to quantify the presence of MNPs in such systems. To verify the homogeneity of the nanosystems, such techniques are required to give information not only about the overall amount of MNPs in the sample, but also about the presence/absence into the single vesicle.
To this aim, we developed a methodology based on magnetic force microscopy (MFM), which is a particular atomic force microscopy (AFM) based technique where a tip coated with a magnetic ultra-thin film is used to probe the sample magnetic properties. Firstly, the sample surface is scanned in semi-contact mode to reconstruct its morphology. Then, the same area is scanned again imposing a constant tip-sample distance during the image, the cantilever is oscillating and the cantilever oscillation phase shift is recorded. This is related to the gradient along the vertical direction of the tip-sample interaction force, which is dominated by the magnetic interaction.
Vesicular systems incorporating MNPs have been produced by the thin film technique, hydrating with the MNPs solution. The presence of MNPs in the samples have been previously verified and quantified by inductively coupled plasma mass spectrometry (ICP-MS), which nevertheless does not allow the identification of their position and distribution into the vesicles. Drops of vesicles/MNPs sample have been poured on glass substrates and imaged by AFM either in water (using an *ad hoc* designed and realized cell) or in air (after dehydration). MFM has been used to investigate the sample. The MFM phase images revealed the presence of MNPs into the vesicles. By analyzing such phase images using a phenomenological calibration specifically developed for the purpose, we quantified the amount of MNPs incorporated in the vesicles.

Synthesis of PLGA-Galactose Polymeric Conjugates and Preparation of Polymeric Nanoparticles for Target Drug Delivery

Krasimira Petrova[a], Ines Peca[a], M. Margarida Cardoso[a], M. Teresa Barros[a]

[a] *REQUIMTE, CQFB, Departamento de Química, Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa, Quinta da Torre, 2829-516 Caparica, Portugal.*

Email of presenting author: k.petrova@fct.unl.pt

The work to be presented aimed at the synthesis of novel type of targetable polymeric conjugates of PLGA, a known biopolymer, with a sugar moiety (galactose) with potential application for liver-specific drug delivery. Simple and effective synthesis of poly (DL-lactide-co-glycolide)-galactose conjugate and poly (DL-lactide-co-glycolide)-co-poly (ethylene glycol)-10%-Triblock-galactose conjugate by esterification of the end carboxyl groups will be reported. Spherical hydrophilic nanoparticles with elevated galactose content on the surface were prepared from these polymeric conjugates, and their physical properties were determined by DLS, SEM, XPS and DSC.

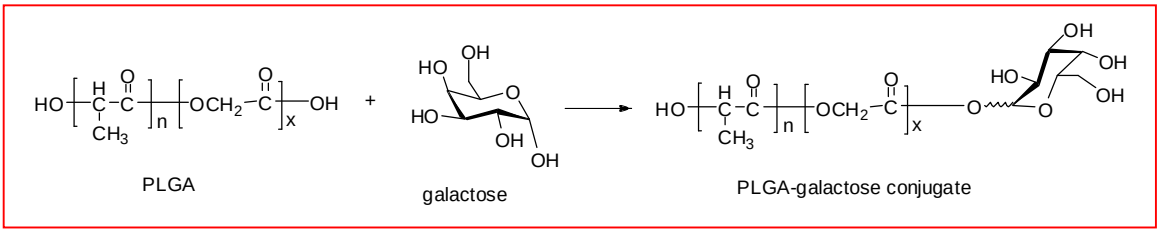

Figure 1. Synthesis of PLGA-galactose conjugate.

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Aknowledgments: This work has been supported by Fundação para a Ciência e a Tecnologia through grant no. PEst-C/EQB/LA0006/2011 and contract PTDC/EQU-EPR/119631/2010. INP is grateful to Fundação para a Ciência e a Tecnologia for the Ph.D. grant (SFRH/BD/48773/2008).

Fe_3O_4/MWCNT Nanocomposites as Potential Tools for Tissue Engineering Applications

Anna Piperno,[a] Carla Cunha,[b] Silvia Panseri,[b] Daniela Iannazzo,[c] Alessandro Pistone,[c] Marianna Fazio,[c] Alessandro Russo,[d] Maurilio Marcacci,[d] Signorino Galvagno[c]

[a] Department of Chemical Sciences, University of Messina, Street F. Stagno D'Alcontres, 98168, Messina, Italy; [b] Laboratory of Bioceramics and Bio-hybrid Composites, Institute of Science and Technology for Ceramics, National Research Council, Faenza 48018, Italy; [c] Department of Electronic Engineering, Industrial Chemistry and Engineering, University of Messina, C.da Di Dio, 98166, Messina, Italy; [d]Laboratory of Biomechanics and Technology Innovation, Rizzoli Orthopaedic Institute, Bologna 40136, Italy

Email of presenting author: apiperno@unime.it

Carbon nanotubes decorated with magnetic nanoparticles are very interesting as new materials for applications in biomedicine; these nanotubes can be used in a functionalized state and as nanocontainers for different human medical treatments including magnetically guided hyperthermia, as drug delivery/carrier/release in the treatment of tumors and in tissue engineering.[1-2] In this study, we report the development of a high-strength magnetic material for biomedical applications by combining the remarkable mechanical properties of multiwalled carbon nanotubes (MWCNT) and the superparamagnetism of Fe_3O_4 nanoparticles. Fe_3O_4/MWCNT hybrid composites were analysed *in vitro* by incubation with mesenchymal stem cells either in the presence or absence of a static magnetic field. Results demonstrate that the introduction of magnetite into the MWCNT structure increases biocompatibility of oxidized MWCNT. In addition, the presence of a static magnetic field further increases Fe_3O_4/MWCNT influence on cell behaviour. Such results confirm that this novel Fe_3O_4/MWCNT hybrid composite is having good potential for tissue engineering applications.

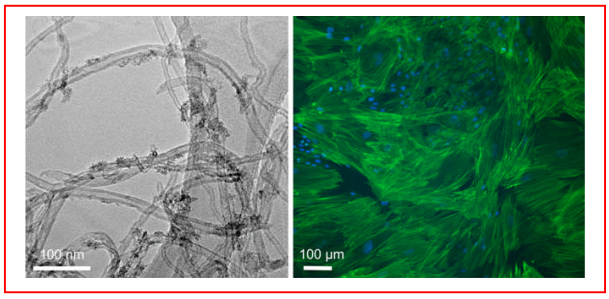

Figure 1. Morphology of Fe_3O_4/MWCNT composites and mesenchymal stem cells after Fe_3O_4/MWCNTs incubation.

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Study of protein binding onto cat-anionic vesicles for nanomedicine applications

Carlotta Pucci[a], Anita Scipioni[a], Camillo La Mesa[a]

[a] *Department of Chemistry, University of Rome "La Sapienza", P.le Aldo Moro 5, I-00185, Rome, Italy.*

Email of presenting author: Carlotta.pucci@uniroma1.it.

Cat-anionic vesicles are aggregates formed by mixing two oppositely charged surfactants, in non-stoichiometric ratios [1,2]. Their structure is similar to cellular membranes and, for this reason, they could be employed as vectors for bio-active molecules in drug delivery technologies and transfection, particularly in non-viral gene therapy. For this reason, it is fundamental to study their interaction with biomacromolecules.

Vesicular entities were obtained by mixing didodecyldimethylammonium bromide and sodium dodecylsulfate in non-stoichiometric [DDAB]/[SDS] ratios (3.8) and the total surfactant concentration is 4 mmol kg^{-1}. Vesicles bear a positive surface charge, due to the cationic species in excess. In our study, the binding of bovine serum albumin (BSA) was studied. At its spontaneous pH, BSA has a negative effective charge. In these conditions, vesicles adsorb significant amount of protein by electrostatic interactions, presumably. Moreover, we modulated the bovine serum albumin net charge by pH and dealt with its binding onto the above vesicles.

Binding is controlled by the net charge of vesicles and albumin: it is substantial when albumin has negative charges in excess and is negligible, if any, below its isoelectric point. For pH > 6.0, the binding efficiency increases in proportion to protein charge. Surface coverage changes in proportion to pH, when the number of charges neutralized upon binding remains the same. The size of protein-vesicle lipo-plexes was inferred by Dynamic Light Scattering and their charge by ζ-potential. The structure of albumin was evaluated by Circular Dichroism spectroscopy and estimates of α-helix, β-strand and random coil content were achieved. Increasing of β-strand and random coil content subsequent to binding suggests a strong interaction between vesicles and albumin. Attempts to determine the binding efficiency were made by elaborating surface charge density values by ζ-potential ones. The results were interpreted in terms of a Gibbs adsorption isotherm. Accordingly, it is possible calculating the binding energy in different pH conditions.

The results obtained so far indicate the existence of strong electrostatic interactions between protein and vesicles with fast formation of stable complexes, and highlight the pontentialites of these cat-anionic vesicles in nanomedicine.

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PMA_{sh} microcapsules and breast cancer cells

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

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

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

Various drug delivery systems such as nanoparticles, liposomes, microparticles and implants have been demonstrated to significantly enhance the preventive/therapeutic efficacy of many drugs by increasing their bioavailability and targetability.

Herein, we report the preparation of capsules from hydrogen-bonded polymer multilayers that are cross-linked through disulfide (S-S) bonds. These capsules are both stable at physiological pH and amenable to deconstruction under reducing conditions or via thiol-disulfide exchange, such as those occurring within cells. We employed a pair of polymers that form stable multilayers when alternately deposited at moderately acidic conditions, pH 4, poly(methacrylic acid) containing thiol moieties, PMA_{SH}. The resulting single-component PMA hydrogel capsules are colloidally stable in a range of conditions, including the presence of blood serum proteins. Furthermore, and of particular importance for biomedical applications, the capsules degrade in the presence of intracellular concentrations of a natural reducing agent, glutathione (1, 2).

In this study we provide PMA_{sh} biocompatibility by MTT test on a cell line of human breast adenocarcinoma (SKBR3). These studies have shown that PMA_{sh} are not toxic. To evaluate the potential of PMA_{SH} capsules as a carrier to deliver anticancer drugs, we incorporated doxorubicin (DOX) into the capsules. DOX has an intrinsic fluorescence spectrum (excitation at 480 nm, emission at 550-650 nm) that can be exploited to monitor localization of the drug. The amount of DOX loaded into the PMA_{SH} capsules ($2\ 10^{12}$ molecules/caps) was determined by UV vis spectrophotometry. The successful encapsulation of DOX was confirmed by the confocal laser scanning microscopy (CLSM) images of DOX-loaded PMA_{SH} capsules (PMA_{SH}-DOX), showing red DOX fluorescence within PMA_{SH} capsules.

Further insight into the internalization and intracellular distribution of PMA_{SH}-DOX capsules was gained by CLSM. The resulting observations show the effective internalization of PMA_{SH}-DOX capsules inside the cells. Although some DOX still appears to colocalize with PMA_{SH} capsules, strong DOX fluorescence is observed in both the cytoplasm and the nucleus, indicating release of DOX from the capsules after internalization. These results were confirmed both by flow cytometric analysis of time course experiments of PMA_{sh}-DOX uptake and scanning electron microscopy observations.

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Carbon based nanostructures for tissue engineering.

M.L. Terranova [a] , I.Cianchetta [a] , V. Guglielmotti [a] , E. Tamburri [a] , T. Lavecchia [a] , M.Rossi [b]

[a] Dipartimento di scienze e Tecnologie Chimiche , Universita' di Roma Tor Vergata, Via della Ricerca Scientifica 1, 00133, Rome, Italy;
[b]*Dipartimento di Scienze di Base e Applicate all'Ingegneria e CNIS, Università di Roma Sapienza*,

Email of presenting author: terranova@roma2.infn.it MINIMA LAB http://Minima.stc.uniroma2.

With the fast growing of tissue engineering, the research of new materials and multimodal architectures able to substitute or implement human tissues or whole organs, has become of vital importance. In this context fabrication of micro- and nano-scale scaffolds represents a key task. However, for biomedical applications, in order to design culture platforms able to improve tissue reconstruction, composition and structure of the scaffolds must meet strict requirements in terms of biocompatibility and long-time reliability.
Sp^2 and sp^3 hybridized carbon materials have the primacy among the materials used in the nanomedicine field. Diamond has already been proven as a good candidate for bio-implants thanks to its excellent properties (non-cytotoxic, chemical inertness) [1,2]. Moreover, nanosized diamond (grains < 10 nm) revealed exceptional abilities in cell proliferation and differentiation [3].
As regards sp2 carbon, fullerenes, graphite nanoplatelets, carbon nanofibers and nanotubes have been tested as drug delivery platforms as well as sensing elements and reinforcing agents for bio-devices [4].
In our lab several different carbon based nanostructures are synthesized by means of Chemical Vapour Deposition techniques in order to obtain differently shaped substrates (porous, dendritic, functionalized) to be tested as multivalent architectures for biomedicals applications. In particular, graphitic dendrimers with various size, shape, branching and surface functionality and nanodiamond systems are proposed as attractive scaffolds for tissue growth and also for selective attachment of targeting groups.

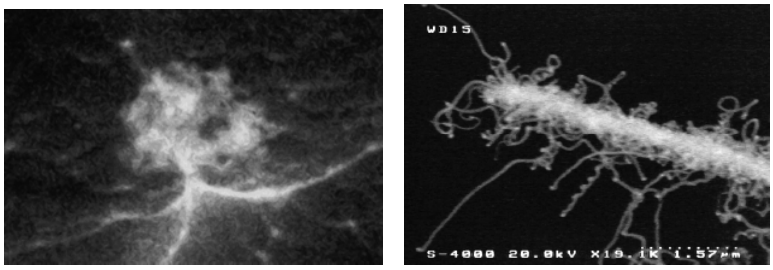

Fig. 1 Some examples of dendritic carbon structures

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Determination of loading and release efficiencies of doxorubicin by using chitosan coated of magnetic nanoparticles designed for targeted drug delivery

Gozde Unsoy[a], Serap Yalcin[b], Rouhollah Khodadust[a], Pelin Mutlu[c], Ufuk Gunduz[a]

[a]*Department of Biotechnology, Middle East Technical University, Ankara 06800, Turkey*

[b]*Kaman Vocational High School, Ahi Evran University, Kırşehir 40100, Turkey*

[c]*Central Labratory Molecular Biology and Biotechnology R&D, Middle East Technical University, Ankara 06800, Turkey*

Email of presenting author: gozdeunsoy@hotmail.com

Biodegradable polymeric nanoparticles, particularly those coated with polymer, have been used as potential drug delivery devices because of their ability to circulate for a prolonged period time target a particular organ, as carriers of DNA in gene therapy, and their ability to deliver drug, antibody, and genes. Chitosan is a natural, biodegradable, biocompatible, linear polymer and has many reactive functional groups that can serve as anchors for conjugation of therapeutics, targetting ligands, and imaging agents. The aim of this study is to create the most effective delivery system for anticancer agents like Doxorubicin, by using chitosan coated magnetic nanoparticles (Cs MNPs). In this study, CS MNPs were synthesized at four different ammonium ion concentrations. For each synthesis homogen size distributions were obtained at four different size ranges (6-8 nm (CS MNP-S_1), 5-7 nm (CS MNP-S_2), 3-5 nm (CS MNP-S_3) and 1-3 nm (CS MNP-S_4). Doxorubicin loading and release efficiencies were studied by using four different types of Cs MNPs. The highest Doxorubicine loading efficiency was obtained in CS MNP-S_1 (around %98) at room temperature in pH 7. 4 buffer. The CS MNP-S_1 has the biggest particle size and largest chitosan amount. Because the Doxorubicine was loaded on to the chitosan polymer, the highest loading efficiency was obtained in CS MNP-S_1. The drug release studies were done with acidic pH that mimics endosomal conditions. According to results, approximately 75 % of the loaded drug was released at 6 hours. The remaining 25 % of the loaded Doxorubicine is continued to be released from the nanoparticles through two days. The Doxorubicin loading and release were observed most efficiently in CS MNP-S_1. The results of this study will provide new insights to the development of new drug delivery systems for cancer therapy.

Activation of superoxide dismutase 1 immobilized on the surface of iron oxide magnetic nanoparticles by application of AC magnetic field.

Vlasova K.Y.[1], Sokolsky M.[2], Vishwasrao Hemant M.[2], Gulin D.A.[1,2]. Golovin Yu.I.[1,3,] Klychko N.L.[1,2], Kabanov A.V.[1,2],

1 *Department of Chemistry, Lomonosov Moscow State University,117234, Moscow, Russia*
Email of presenting author: vlasova_k.y@mail.ru
[2] *University of Nebraska Medical Center, Omaha, USA*
[3] *G.R.Derzhavin Tambov State University, Tambov, Russia*

Email of presenting author: vlasova_k.y@mail.ru

Nanomedicine formulations aim to improve the biodistribution and the target site accumulation of systemically applied therapeutics. Theranostic nanomedicine formulations offer both the visualization of drug distribution and drug release at the target site, while the optimized strategy is both triggered drug release and the prediction and monitoring of therapeutic responses. Example of such systems is metal nanoparticles, which can be used for both MRI imagining and drug loading.
The goal of this study is preparation of theranostic nanodrug- immobilized superoxide dismutase 1 (SOD1) on magnetic particles surface- and evaluation of changes in enzymatic activity following exposure to AC magnetic field.

Methods.
First, iron oxide magnetic nanoparticles (MNPs) were coated with $mPEG_{5K}$-b-$PLKC_{100}$ block-copolymer. Second, SOD1 was immobilized on polymer-coated MNPs. MNPs solution (in DI water) was added dropwise to SOD1 solution (in HEPES, pH 7.4). The unbound enzyme was removed by size-exclusion chromatography, the MNPs concentrate was washed with HEPES buffer and reconstituted with 10 mM HEPES buffer, pH 7.4 or 10 mM HEPES-buffered saline, pH=7.4. The solution was filtered through a 0.2 μm Teflon™ filter. The protein concentration in MNPs was determined by ICP-MS (by the ratio of Cu to enzyme). The size and surface charge of the nanoparticles was determined using DLS and NTA. Immobilized enzyme activity before and after magnetic field application was measured using pyrogallol autoxidation.

Results and discussion.
Salt addition into HEPES buffer confirmed the electrostastic nature of binding between negatively charged SOD1 (at pH=7.4) and positively charged polymer-coated MNPs.
Size of new formulations was 86,1±0,2 nm and ς-potential (–12,2±0,3) mV. Concentration of SOD1 on the surface of MNPs was 3,36 μg/mL.
Immobilization of SOD1 resulted in decrease in activity. But it's interesting to note that after field application activity of enzyme increased. It might be associated with deformation of enzyme and increasing access to active site of SOD1.

In **future studies** we would like to optimize the filed strength and/or exposure time to further increase the enzymatic activity. We will also evaluate the stability of MNPs-SOD1 complex in vivo.

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Loading, release, stability of anti cancer drug from in situ formation PHB coated magnetic nanoparticles

Serap Yalcin[a], Gozde Unsoy[b], Pelin Mutlu[c], Rouhollah Khodadust[b], Ufuk Gunduz[b]

[a]*Kaman Vocational High School, Ahi Evran University, Kırşehir 40100, Turkey*

[b]*Department of Biotechnology, Middle East Technical University, Ankara 06800, Turkey*

[c]*Central Labratory Molecular Biology and Biotechnology R&D, Middle East Technical University, Ankara 06800, Turkey*

Email of presenting author: syalcin@ahievran.edu.tr

Biodegradable polymeric magnetic nanomaterials gained importance in biomedical and bioengineering research such as targeting drug delivery, tissue engineering, cancer diagnosis and therapy. For targeted drug delivery, the magnetic nanocarriers should have a certain size distribution to avoid rapid uptake by RES, and be coated with biocompatible, biodegradable polymers to form a stable dispersion. Poly hydroxy butyrate (PHB) is a nontoxic, biodegradable, biocompatible polymer and hence is suitable for medical applications. In this study, PHB coated magnetic nanoparticles were prepared by co-precipitation of iron salts (Fe^{2+} and Fe^{3+}) by ammonium hydroxide. Doxorubicin loading and release efficiencies were studied. The drug loading was obtained around 92% at room temperature in pH 7.2 buffer. The drug loaded magnetic nanoparticles were highly stable (97%) up to 2 months in neutral pH which is the same pH of blood. The drug release studies were done with pH 5.2 and 4.2 that mimics endosomal conditions. The most of the drug was released within first 15 hours (around 85%). The Doxorubicin loading, stability and release were observed more efficiently in PHB coated magnetic nanoparticles. The results of this study will provide new insights to the development of new drug delivery systems for cancer therapy.

Preparation of antibody-FITC binding dextran coated magnetic nonoparticles for imaging and cancer therapy

Serap Yalcin[a], Gozde Unsoy[b], Rouhollah Khodadust[b], Pelin Mutlu[c], Ufuk Gunduz[b]

[a]*Kaman Vocational High School, Ahi Evran University, Kırşehir 40100, Turkey*

[b]*Department of Biotechnology, Middle East Technical University, Ankara 06800, Turkey*

[c]*Central Labratory Molecular Biology and Biotechnology R&D, Middle East Technical University, Ankara 06800, Turkey*

Email of presenting author: syalcin@ahievran.edu.tr

In recent years, magnetic nanoparticles have high importance for cancer therapy. Because, they are biocompatible, targetable, and small dose requirement. Magnetic nanoparticles in order to be applied in medical application need to be coated with various polymers. These polymers can be dextran, chitosan, dendrimer, PLGA, PEG etc. Dextran is a polysaccharide polymer composed exclusively of a-D-glucopyranosyl units ($C_6H_{10}O_5$) with varying degrees of length (n) and branching points (usually 1,3 glucosidic linkages). In this study, IgG antibody modified dextran coated magnetic nanoparticles (DexMNPs) were synthesized for imaging and cancer therapy. In situ coating method was chosen for the synthesis of DexMNPs. Then, the fluorescent IgG antibody was linked to these DexMNPs by a reaction catalyzed by EDC/NHS. Thus, IgG conjugated fluorescent magnetic polymeric nanoparticles were formed and the resultant IgG-FITC-conjugated dextran coated magnetic nanoparticles were applied onto cell cultures. Visualization by confocal microscopy indicated that these antibody conjugated polymeric magnetic nanoparticles may have application potential in imaging and cancer therapy.

The identification of cytotoxicity of various polymers coated magnetic iron oxide nanoparticles in MCF-7 cells

Serap Yalcin[a], Rouhollah Khodadust[b], Gozde Unsoy[b], Pelin Mutlu[c], Ufuk Gunduz[b]

[a]*Kaman Vocational High School, Ahi Evran University, Kırşehir 40100, Turkey*
[b]*Department of Biotechnology, Middle East Technical University, Ankara 06800, Turkey*
[c]*Central Labratory Molecular Biology and Biotechnology R&D, Middle East Technical University, Ankara 06800, Turkey*

Email of presenting author: syalcin@ahievran.edu.tr

Magnetic iron oxide nanoparticles (MNPs), being biodegradable and suitable for surface modifications, are the most prominent class of MNPs for biomedical applications such as magnetic resonance imaging, magnetic hyperthermia for cancer treatment and tissue-specific delivery of therapeutic agents. The aim of this study is to investigate the cytotoxicity of different polymers coated magnetic nanoparticles. For this purpose, dendrimer, chitosan and polyhydroxybutyrate (PHB) coated MNPs were synthesized via aminosilane modified and coprecipitation methods, respectively. The breast cancer cell line was exposed to polymers coated MNPs to determine the toxicity of magnetic nanoparticles during bioapplication. The antiproliferative effects of polymers coated nanoparticles on MCF-7 cells were evaluated by means of the Cell Proliferation Kit (Biological Industries) according to the manufacturer's instructions. Assay was a colorimetric test based on the reduction tetrazolium salt, XTT to colored formazan products by mitochondria of live cells. As a result, survival rates indicated that there is no significant cytotoxic effect of the nanoparticles on MCF-7 cells.

Zoledronic acid encapsulated in self-assembly pegylated nanoparticles as a new delivery system against brain tumors.

Silvia Zappavigna[1], Amalia Luce[1], Alida Panico[1], Manuela Porru[2], Giuseppina Salzano[3], Sara Lusa[3], Giuseppe De Rosa[3], Carlo Leonetti[2] and Michele Caraglia[1]

[1]Department of Biochemistry and Biophysics, Second University of Naples; [2]Experimental Chemotherapy Laboratory, Regina Elena Cancer Institute, Rome; [3]Department of Chemistry and Pharmaceutical Chemistry Toxicology, University "Federico II" of Naples.

Email of presenting author: silvia.zappa@libero.it

Glioblastomas are highly aggressive brain tumors of adults with poor clinical outcome. Despite a broad range of new and more specific treatment strategies, therapy of glioblastomas remains challenging and tumors relapse in all cases. The blood–brain barrier (BBB) is the most important limiting factor for the development of new drugs and drug delivery for the central nervous system (CNS). Different methods have been used to facilitate the transporting of drugs into the brain (1, 2). Recently, small peptide-vectors have been used to enhance the uptake of several therapeutic drugs into the brain, and most of them can facilitate transportation safely and efficiently. Zoledronic acic (ZOL) is a drug used for the treatment of bone metastases and recent data report that beneficial effect of ZOL may result from a direct anti-tumour activity. One of the most important limits of ZOL is its limited delivery in tumour tissues and excessive accumulation in the bone. On these bases, there is a need to develop new ZOL formulations with a lower affinity for bone and a longer half-life in the circulation that would result in increased probability to affect peripheral tumours. Moreover, the functionalization of these nanoparticles with transferrin (TRF) could allow their crossing through BBB.
The delivery system consists in self-assembly PEGylated nanoparticles (NPs), based on calcium/phosphate NPs and cationic liposomes. The preparation conditions were optimized in order to achieve NPs easily prepared before use, with colloidal dimensions and high ZOL loading. Moreover, to improve the targeting of ZOL in the brain we designed ZOL-containing NPs (NPs-ZOL) functionalized with transferrin (TRF) able to bind specific receptors on endothelial cells of BBB. We have evaluated the effects of NPs-ZOL functionalized or not with transferrin on growth inhibition of Ln229, U373MG and U87MG glioblastoma cells by MTT assay. The encapsulation in NPs functionalized with TRF resulted in higher *in vitro* cytotoxic activity than free ZOL on all three glioblastoma cell lines. However, the potentiation of anti-proliferative activity of TRF-conjugated NPs-ZOL was equal (Ln229) or less (U373MG and U87MG) than that one induced by NPs-ZOL not functionalized with TRF and correlated with TRF receptor expression on tumour cells. On the other hand, TRF-NPs-ZOL showed a higher antitumor efficacy if compared with that one caused by naked NPs-ZOL in mice intramuscularly bearing glioblastoma tumors, inducing a significant tumor weight inhibition (TWI) of 41%, a tumor growth delay (T-C) of 10 days and an increase of ILS (increase of mice survival) of 23%. Moreover, we have performed interaction experiments with temozolomide (TMZ), a gold standard for the treatment of glioblastomas, nanoZOL functionalized with TRF or not, and free ZOL alone and in combination on growth inhibition using, as evaluation method of the synergism, the dedicated software Calcusyn. The sequences TMZ at the day 1 followed by TRF-NPs-ZOL or NPs-ZOL or free ZOL at the day 2 were in all cases strongly synergistic in inducing growth inhibition on glioblastoma cells while the reverse sequence TRF-NPs-ZOL followed by TMZ was synergistic only in LN229, which expressed TRF receptor at higher levels than U373MG and U87MG. All other combinations were additive or antagonistic.
Conclusion. These preliminary results showed that the delivery of ZOL by NPs increases the antitumor efficacy of this drug in glioblastoma cells also in combination with standard cytotoxic agents. These data strongly warrant further investigations in intracranially injected glioblastoma cells preclinical models.

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Molecular Dynamics Analysis of Bone Morphogenetic Protein-2 Conformations and Mechanical Properties

Iuliana Aprodu[a], Iuliana Banu[a], Adrian Istrate[b]

[a] *Faculty of Food Science and Engineering, "Dunarea de Jos" University of Galati, Domneasca 111, Galati, Romania;* [b]*Computer Science and Engineering Department, "Dunarea de Jos" University of Galati, , Domneasca 111, Galati, Romania.*

Email of presenting author: iuliana.aprodu@ugal.ro.

Bone tissues have good regenerative abilities in the human body. One of the most important growth factors in bone formation and healing are bone morphogenetic proteins (BMPs) (Bessa et al., 2008). The aim of the study was to evaluate the thermal stability of BMP-2 at single molecule level, using Molecular Dynamics (MD) approach.

In order to investigate the conformational particularities of BMP-2, the atomic coordinates of 3BMP.pdb (Scheufler et al., 1999) were obtained from the Brookhaven Protein Data Bank. The BMP-2 model was minimized, solvated and equilibrated at temperatures resembling the room temperature (25°C) and human body conditions (37°C). The equilibrated models were further characterized in terms of conformational properties. The root mean square error obtained by least squares fitting the BMP-2 models equilibrated at 25°C and 37°C was 5.34 Å indicating important conformational rearrangements at human body temperature with respect to the room temperature. Analyzing the results presented in Table 1 one can see that the conformational changes induced by the temperature are related both to the strands and helices content.

Table 1. Secondary structure details of BMP-2 model equilibrated at 25°C and 37°C

	25°C	37°C
Beta sheets	3 antiparallel beta sheets: A with 2 strands (Lys^{15}-Val^{21}; Tyr^{38}-His^{44}), B with 3 strands (Ile^{32}-Ala^{34}; Leu^{84}-Tyr^{91}; Val^{99}-Val^{108}) and C with 2 strands (Cys^{78}-Pro^{81}; Cys^{111}-Arg^{114})	3 antiparallel beta sheets: A with 2 strands (Lys^{15}-His^{17}; Tyr^{42}-His^{44}), B with 2 strands (Val^{80}-Leu^{84}; Val^{108}-Gly^{112}) and C with 2 strands (Ile^{87}-Leu^{92}; Val^{98}-Tyr^{103})
Strand (%)	40.6	26.4
α-helix (%)	9.4	13.2
3-10 helix (%)	2.8	0.0

The temperature increase from 25°C to 37°C causes the reduction of the strands and the increase of helices content within protein structure. Regardless of temperature, the secondary structure is dominated by the strand motif. The temperature increase from 25 to 37°C causes a slight decrease of the hydrophobic surface available to the solvent which represents 58-59% of the total solvent accessible surface of the protein.

The steered MD simulation carried out to stretch the protein up to 10% elongation showed that BMP-2 exhibits a more rigid behaviour at room temperature. According to our results, BMP-2 behavior is influenced by small temperature fluctuations.

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List of Authors and Partecipants

Agostinelli Enzo	Università degli Studi di Roma "La Sapienza", Italia	**SL22**
Alberti Diego	Università di Torino, Italia	**P1**
Albrecht Volker	Biolitec Research GmbH, Jena, Germany	
Aleandri Simone	Università degli Studi di Roma "La Sapienza", Italia	**P2**
Aleksashkin Anton D.	Lomonosov Moscow State University, Moscow, Russia	**P3**
Altieri Barbara	Università degli Studi dell'Aquila, Italia	**P4**
Amodeo Antonio	Università Cattolica del Sacro Cuore, Roma, Italia	**SL14**
Angelini Giancarlo	CNR – IMC – Roma, Italia	
Arancia Giuseppe	Istituto Superiore di Sanità, Roma, Italia	
Aubert Dimitri	IZON, The Oxford Science Park, United Kingdom	
Bacaita Elena Simona	"Gheorghe Asachi" Technical University, Iasi, Romania	**SL23**
Baldelli Bombelli Francesca	University of East Anglia, United Kingdom	
Bellucci Stefano	INFN-Laboratori Nazionali di Frascati, Roma, Italia	**SL18**
Bézière Nicolas	Technische Universität Müchen and Helmholtz Zentrum Müchen, Germany	**IL3**
Bombelli Cecilia	CNR – IMC - Sezione di Roma, Italia	
Bozzuto Giuseppina	Istituto Superiore di Sanità, Roma, Italia	**P5**
Brioschi Chiara	Bracco Imaging spa, Milano, Italia	**SL2**
Calcabrini Annarica	Istituto Superiore di Sanità, Roma, Italia	
Carafa Maria	Università degli Studi di Roma "La Sapienza", Italia	
Caraglia Michele	Seconda Università degli studi di Napoli, Italia	**IL12**
Caschera Stefano	Università degli Studi di Roma "La Sapienza", Italia	
Cedola Alessia	CNR- IFN, Roma, Italia	**SL9**
Chunhua Dong	Università degli Studi di Roma "La Sapienza", Italia	**P20**
Cianchetta Ilaria	Università di Roma "Tor Vergata", Italia	
Ciofani Gianni	Istituto Italiano di Tecnologia, Pontedera, Italia	**SL8**
Colombo Miriam	Università di Milano "Bicocca", Italia	**SL5**
Colone Marisa	Istituto Superiore di Sanità, Roma, Italia	**SL5**
Condello Maria	Istituto Superiore di Sanità, Roma, Italia	**P6**
Conte Claudia	Università di Napoli "Federico II", Italia	**SL20**
Correia da Silva Paula	Universidade Nova de Lisboa, Portugal	**P7**
Corvaglia Valentina	Università di Trieste, Italia	**SL11**
Costantini Francesca	Università degli Studi di Roma "La Sapienza", Italia	**SL3**
Crucho Carina	Universidade Nova de Lisboa, Portugal	**P8**
Cullis Pieter	The University of Brithish Columbia, Canada	**IL10**
Derakhshandeh Katayoun	Kermanshah University of Medical Sciences, Iran.	**SL16**
Di Carlo Gabriella	CNR – ISMN - Roma, Italia	**P9**
Di Meo Chiara	Università degli Studi di Roma "La Sapienza", Italia	
Di Silvio Desirè	University of East Anglia, Norwich, United Kingdom	**P10**
Diaspro Alberto	Università degli Studi di Genova, Italia	**IL2**
Duncan Ruth	Cardiff University, United Kingdom	**IL6**
Fahr Alfred	Friedrich Schiller University of Jena, Germany	

Ferro Daniela	CNR – ISMN - Roma, Italia	**P11**
Fontana Laura	Università degli Studi di Roma "La Sapienza", Italia	**P12**
Formisano Giuseppe	Istituto Superiore di Sanità, Roma, Italia	
Gabizon Alberto Abraham	University-School of Medicine, Jerusalem, Israel.	**KL**
Galeazzi Roberta	Università Politecnico delle Marche, Italia	
Gallo Annalisa	Istituto Superiore di Sanità, Roma, Italia	
Garaci Enrico	Istituto Superiore di Sanità, Roma, Italia	
Genchi Giada Graziana	Scuola Superiore Sant'Anna, Pontedera, Pisa, Italia	**SL12**
Gerosa Gino	Università di Padova, Italia	**IL8**
Giansanti Luisa	Università degli Studi dell'Aquila, Italia	**P13**
Giuliani Chiara	Università degli Studi di Roma "La Sapienza", Italia	**P4**
Grigioni Mauro	Istituto Superiore di Sanità, Roma, Italia	**SL13**
Gun'ko Yourii K.	Trinity College Dublin, Ireland	**IL1**
Guzman Luis	Talca University, Chile	
Khodadust Rouhollah	Middle East Technical University, Ankara, Turkey	**SL4**
Kozyr Arina V.	State Research Center for Applied Microbiology and Biotechnology, Obolensk, Russia	**P14**
Aprodu Iuliana	University of Galati, Romania	**P32**
Leroux Jean-Christophe	ETH Zürich, Switzerland.	**IL9**
Lomazzi Michela	Università degli Studi di Parma, Italia	**P15**
Lòpez-Fernàndez Luis	Istituto de Investigatción Sanitaria Gregorio Marañón, Madrid, Spain	**P16**
Luciani Paola	ETH Zürich, Switzerland.	
Macellari Velio	Istituto Superiore di Sanità, Roma, Italia	
Maiocchi Alessandro	Bracco Imaging spa, Milano, Italia	
Mancini Giovanna	CNR – IMC - Sezione di Roma, Italia	
Mandolini Luigi	Università degli Studi di Roma "La Sapienza", Italia	
Marianecci Carlotta	Università degli Studi di Roma "La Sapienza", Italia	**SL6**
Marini Bettolo Chiara	Imperial College, London, United Kingdom	
Masci Giancarlo	Università degli Studi di Roma "La Sapienza", Italia	**SL21**
Matricardi Pietro	Università degli Studi di Roma "La Sapienza", Italia	**P17**
Mauceri Alessandro	CNR – IMC - Sezione di Roma, Italia	
Meschini Stefania	Istituto Superiore di Sanità, Roma, Italia	
Mikaia Nona	Sokhumi State University Tblisi, Georgia	
Misso Gabriella	Seconda Università degli Studi di Napoli, Italia	**P18**
Mobbili Giovanna	Università Politecnica delle Marche, Italia	**P19**
Molinari Agnese	Istituto Superiore di Sanità, Roma, Italia	
Montanari Elita	Università degli Studi di Roma "La Sapienza", Italia	
Morelli Giancarlo	Università di Napoli "Federico II", Italia	**SL19**
Nelofer Syed	Imperial College, London, United Kingdom	
Pagano Livio	Università Cattolica del Sacro Cuore, Roma, Italia	**IL11**
Pasquato Lucia	Universitaà di Trieste, Italia	**SL7**
Passeri Daniele	Università degli Studi di Roma "La Sapienza", Italia	

Petaccia Manuela	Università degli Studi di Roma "La Sapienza", Italia	
Petrova Krasimira	Universidade Nova de Lisboa, Portugal	**P21**
Piperno Anna	Università di Messina, Italia	**P22**
Pittori Martina	Università degli Studi di Roma "La Sapienza", Italia	
Placidi Massimo	HORIBA Scientific, Chilly-Mazarin, France	
Prato Maurizio	Università di Trieste, Italia	**IL7**
Prosperi Davide	Università di Milano "Bicocca", Italia	**IL4**
Pucci Carlotta	Università degli Studi di Roma "La Sapienza", Italia	**P23**
Quaglia Fabiana	Università di Napoli "Federico II", Italia	
Qureshi Muhammad	Brunel University, London, United Kingdom	
Raffa Vittoria	Scuola Superiore Sant'Anna, Pisa, Italia	**SL10**
Rinaldi Federica	Università degli Studi di Roma "La Sapienza", Italia	
Romanelli Valentina	Università degli Studi di Roma "La Sapienza", Italia	
Sansone Francesco	Università di Parma, Italia	**SL17**
Scrimin Paolo	Università di Padova, Italia	**SL15**
Scipioni Anita	Università degli Studi di Roma "La Sapienza", Italia	
Shin Irina	Weizmann Istitute of Science	
Sperduto Claudio	Università degli Studi dell'Aquila, Italia	
Stringaro Annarita	Istituto Superiore di Sanità, Roma, Italia	**P24**
Terranova Maria Letizia	Università di Roma "Tor Vergata", Italia	**P25**
Tiggelaar Roald	University of Twente, The Netherlands	
Toccacieli Laura	Istituto Superiore di Sanità, Roma, Italia	
Toppino Antonio	Università di Torino, Italia	**SL1**
Unsoy Gozde	Middle East Technical University, Ankara, Turkey	**P26**
Vergara Claudia	Talca University, Chile	
Vlasova Kseniya	Lomonosov Moscow State University, Moscow, Russia	**P27**
Walpoth Beat H.	Geneva University Hospital, Switzerland	**IL5**
Yalcin Serap	Ahi Evran University, Kırşehir , Turkey	**P28** **P29** **P30**
Zappavigna Silvia	Seconda Università degli Studi di Napoli, Italia	**P31**

Congress Coordinators

Giovanna Mancini
Institute of Chemical Methodologies – CNR
Tel. 06 4991 3078
E-mail: giovanna.mancini@uniroma1.it

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

Scientific Advisory Board

Giancarlo Angelini
Director Institute of Chemical Methodologies, CNR, Rome

Enrico Garaci
President Istituto Superiore di Sanità, Rome

Velio Macellari
Director Dept. of Technology and Health, Istituto Superiore di Sanità, Rome

Scientific Committee

Giuseppe Arancia, **Stefania Meschini**, **Agnese Molinari**, **Annarita Stringaro**
Department of Technology and Health - ISS

Cecilia Bombelli, **Giovanna Mancini**
Institute of Chemical Methodologies - CNR

Paola Luciani
Institut f. Pharmazeut. Wissenschaften ETH Zürich

Organizing Committee

Giuseppina Bozzuto, **Marisa Colone**, **Maria Condello**, **Giuseppe Formisano**, , **Laura Toccacieli**
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Institute of Chemical Methodologies - CNR

Monica Brocco, Alessia Medici
Department of Technology and Health - ISS