June Wednesday 19th - Friday 21st 2013

Bresso (Milan) Italy

NPMED 2013
International Conference

Nanoparticles And Nanotechnologies In Medicine 2013

NPMED13 www.npmed13.eu

Abstract Book

Venue: "Zambon Open Circle", Via Meucci 12, 20091 - Bresso (MI)
Organised by University of Milano-Bicocca and BioTalentum Ltd.

Program at a glance

Time	Wednesday, 19 June 2013	Thursday, 20 June 2013	Friday, 21 June 2013
8.30		Registration	
9.00		Session 2 Nanoparticles for Therapy and diagnosis of Alzheimer Disease	Session 4 Industry and Nanotechnology
9.30			
10.00			
10.30			Coffee Break
11.00		Coffee Break	Session 4 Industry and Nanotechnology
11.30		Session 2 Nanoparticles for Therapy and diagnosis of Alzheimer Disease	
12.00			
12.30			End of the Congress
13.00			
13.30	Registration		
14.00	Welcome	Lunch Break	
14.30	Session 1 Desing of nanoparticles for Medicine	Session 3 Nanoparticles for Therapy and diagnosis of Human Diseases	
15.00			
15.30			
16.00	Coffee Break	Coffee Break	
16.30	Session 1 Desing of nanoparticles for Medicine	Session 3 Nanoparticles for Therapy and diagnosis of Human Diseases	
17.00			
17.30			
18.00	Poster Session with cocktail party		
18.30			
19.00			
19.30			

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Chairmen's Invitation

On behalf of the Scientific Organizing Committee of the conference, it is our pleasure to welcome you to the **International Conference Nanoparticles and Nanotechnologies in Medicine (NPMED) 2013** on 19-21 June 2013 at the Zambon Conference Center, Bresso (Milan) – Italy.

Dear Participants of the NPMED 2013 conference,

It is a real pleasure for us to welcome you to NPMED conference in Milan, Italy. This meeting is one of the largest annual international scientific conference devoted to medical application aspects of the nanoparticles, with particular attention to design, toxicology and use of nanoparticles in diagnosis and therapy.

A major goal of this congress is to bring partners and stakeholders from different disciplines together, ranging from the nanoparticle design for medical applications to new nanotechnologies for therapy and diagnosis of human diseases. This interdisciplinary approach shall generate new project ideas and business opportunities for participants from universities, hospitals, research centers as well as small and large enterprises.
The conference is organized as part of the final meeting of the NAD (Nanoparticles for therapy and diagnosis of Alzheimer's disease) Project which is aiming to develop nanoparticles for Alzheimer's diagnosis and therapy. The research of NAD is financed by the European Union's 7th Framework Program and includes 19 European partners. Grant agreement no: CP-IP 212043-2 NAD.
We trust that you will enjoy this NPMED 2013 meeting in Milan as well as the congress venue being located at a Zambon Conference Center close to the Milan city center.

Committee

Organizing Committee

Scientific Chair	**Massimo Masserini**, University of Milano-Bicocca
Organization Chair	**Adriana Monti**, University of Milano-Bicocca
	Ildiko Nagy, BioTalentum, Hungary
Local Arrangements	**Reina Cabiria**, Zambon Italia
Technical Support	**Silvia Sesana**, University of Milano-Bicocca
	Sarolta Peter, BioTalentum, Hungary
Organization Support	**Laura Bana**, University of Milano-Bicocca
	Stefania Minniti, University of Milano-Bicocca

Scientific Advisory Committee

(in alphabetical order)

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Moghimi S. Moein, *Full Professor of Nanomedicine, Department of Pharmacy, University of Copenhagen.*

Re Francesca, *Research Associate, Department of Health Science, University of Milano-Bicocca.*

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UNIMIB was founded ten years ago and today has 8 Faculties and 21 Departments. It has 30 thousand students attending the courses. It has two main campuses, one in Milan and one other in Monza (Medical School) acting in a strict cooperation with the San Gerardo Hospital.

UNIMIB participates to NAD with a wide expertise in the areas of biochemistry, neuroscience, physiology, chemistry and physics. Instrumentations include AFM, fluorescence microscopy, dynamic laser light scattering, DSC, MS (MALDI-TOF), IR and UV-vis spectrophotometers, NMR, BiaCore, Genechip, confocal microscopy, Circular Dicroism (CD), Cytofluorimetry. Equipments for centrifugation, ultrafiltration, lyophilisation, electrophoresis, chromatography, and facilities for cell culture, protein purification and molecular biology, radiochemistry are available.

BioTalentum Ltd.

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info@biotalentum.hu

BioTalentum Ltd. is a research start-up company established in 2005. Focus areas of its activities are animal biotechnologies, including stem cell research, transgenic cellular and animal models. The company has a mission of research and development of new animal models and cellular systems for biomedical research and drug testing, and to provide technical services for academic research teams and pharmaceutical industry based on it's state-of-the-art technological know-how.

The company is very active in European research, as BIO is full research partner in 4 FP6 projects and in 14 FP7 projects. In 9 of these BioTalentum is the coordinator of the project. Furthermore, BioTalentum has a branch specialized on project management for biomedical research, and provides such services for national and EU FP7 projects.

BioTalentum is a leading technology provider in Central & Eastern Europe in the field of discovery and development of new transgenic animal models, cellular models and stem cell systems for biomedical research and drug testing. The company offers these R&D tools to academic research groups and the related trainings to pharmaceutical / biotechnology industry worldwide.

http://www.biotalentum.hu

The Venue

Zambon
Open Circle

Via Lillo del Duca, 10
20091 Bresso (MI), Italy
Main Entrance :
Via Meucci 12

Established in Vicenza in 1906 and currently headquartered in Bresso, it operates on three continents - Europe, North and South America and Asia - with more than 2600 employees in 15 different countries and as at 31/12/2011, it had a consolidated revenue of € 562 Mio. Today, Zambon is a multicultural group which, in the patchy and constantly-changing scenario of the pharmaceutical industry, has managed to strengthen its competitive position on the market.

Zambon is one of the world's leading pharmaceutical and chemical multinationals, earning a strong reputation over the years for its high-quality products, flexibility and customer service.

Thanks to its new "Open Innovation" approach, Zambon pairs its internal research with the exploration of international scenarios, collaborating with Research Institutes, Technology Transfers, Start-ups and Biotec Companies.

General Information

Venue Location

"Zambon Open Zone" Via Meucci 12 - Bresso (Mi) Italy

Registration & Badge Pickup

The Conference registration Desk is located on the main level of the Zambon Conference Centre.
Desk Hours:
Wednesday, June 19 12.30pm – 6pm
Thursday, June 20 08.30am – 6pm

Lost & Found

Any lost items should be reported to or turned over to the Registration Desk. Items are turned into Zambon Security at the end of each day. To report or inquire about a lost item pleaserefer to the registration desk.

Wireless/Internet Access

Zambon Conference Centre is pleased to provide free WiFi access to all attendees. To access the wirelessnetwork please ask for it at the registration desk. They will provide username and password.

Taxi

Please contact the Registration Desk for assistance or phone the number: 848.814.781.

Local currency:

Official currency is the Euro (€).

Credit Cards / Debit Cards / ATMs

Major establishments accept credit cards whose logos are posted in their front windows, just as they do in the USA and Canada. You should check with your credit card company to see if they charge you an international transaction fee or foreign transaction fee. Visa and MasterCard charge a processing fee on international transactions, and most card-issuing banks add their own fees on top of that. They equal to generally a percentage of your overall purchase price, sometimes as high as 3% extra.
Beware that taxis can often be paid with cash (euro)
ATMs (Automatic Teller Machines) in Italy are known as Bancomat, and can be found anywhere in large cities as well as in small towns.
Withdrawing cash at Visa/PLUS ATMs using a secured PIN can save you money and makes it easy to take advantage of the favorable exchange rates offered by ATMs. Cash withdrawals are dispensed in local currency, and are debited from your account in your own currency - this eliminates additional currency conversion fees and commissions often assessed by traditional currency exchange bureaus.

From Milan airports to the Milan Central Station

Malpensa Airport
The international airport of the Malpensa is distant about fifty kilometres from the centre of Milan.

Taxi
The fare is about 90 euro

Malpensa Express by railway:
The MALPENSA EXPRESS travels straight into the airport, stopping at basement level in TERMINAL 1.
TERMINAL 1 [for international traffic] is connected to TERMINAL 2 [dedicated mainly to low-cost traffic] by a free 24-hour shuttle service.
Adult one way Fare: € 10
http://www.malpensaexpress.it/en/

Malpensa Shuttle by Bus:
Fare: € 10,00
http://www.malpensashuttle.it/e-index2.php

Linate Airport
The airport is to the extreme suburb of East Milan. Is about 10 km from centre town.

Taxi
The fare is about 70 euro

Air bus
Fare: € 5.00
http://www.atm-mi.it/en/AltriServizi/Trasporto/Pages/airbus.aspx

A.T.M. Bus 73
From Linate --> Milan Piazza S. Babila (MM1 subway station)
Starting from: 06:05 – ends at 00:55
Fare: € 1.95
http://www.milanolinate.eu/en/accessibility-and-parking/means-of-transport/public-buseness
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Orio al Serio Airport
Bergamo province is about 55 km from Milan.

Taxi
The fare is about 80 euro

Orio Shuttle by bus
One way ticket Fare: € 3.50
Ticket price includes: 1 luggage (max 20 kg) + 1 hand luggage (max 55x40x20 cm) + 1 personal bag/PC bag + 1 stroller.
Cost extra bagage: € 5.00
See timetable: http://www.orioshuttle.com/_eng/

Direction from Milan Central Station to the Venue of the conference NPMED13 (Via Meucci,12 Bresso Milan Italy)

By public transportation

From Milan Central station

ticket: 1,95 euro

Subway line 3 (S.DONATO - COMASINA)

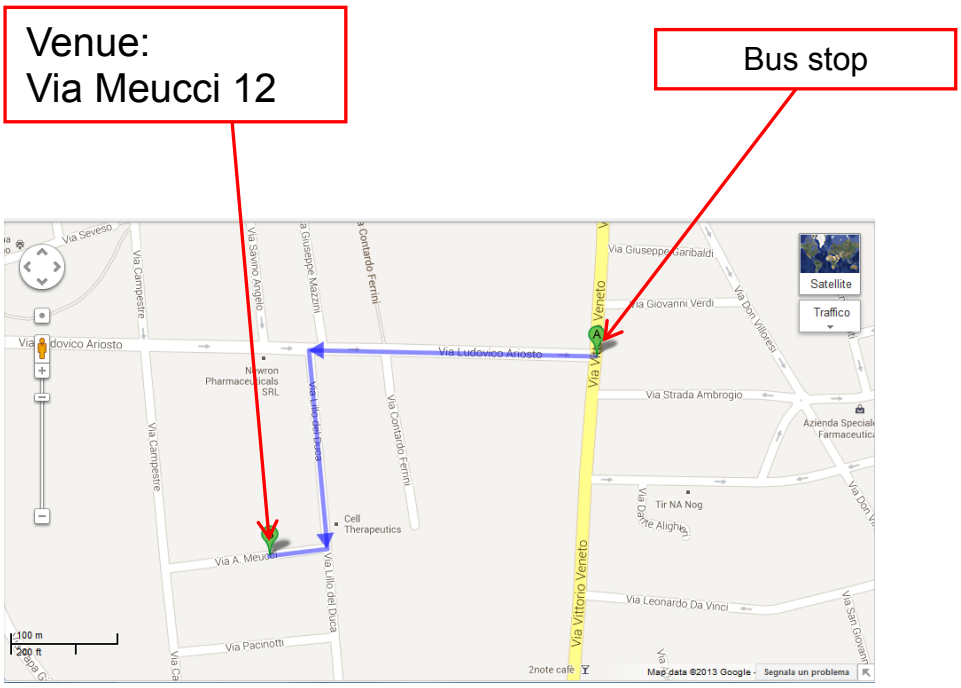
get off at COMASINA Station

Walk for about 150 mt. to bus stop

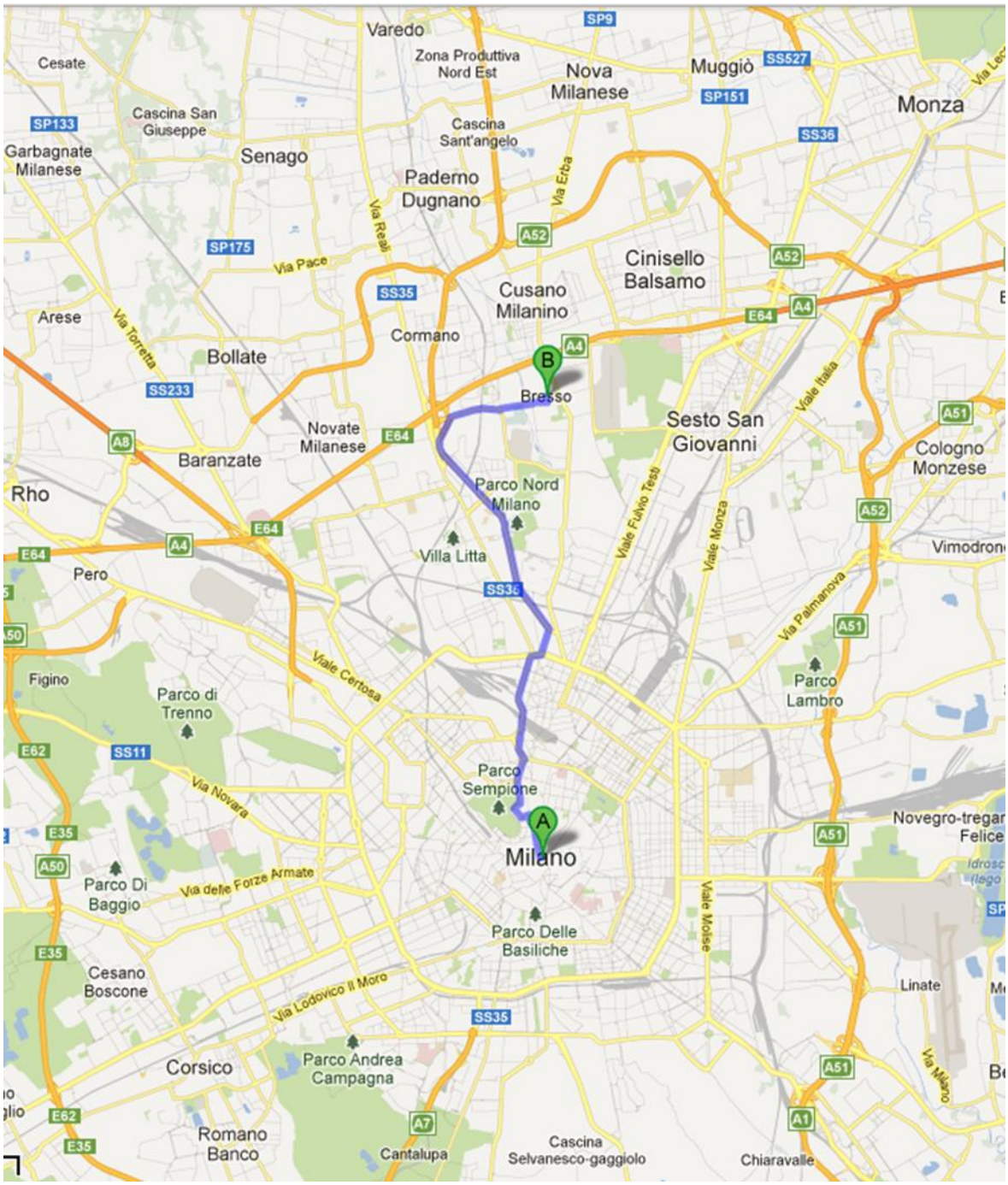
Take Bus n 83 (for 10 stops)

Get off at Via Vitt. Veneto Via Dante (Bresso)

Walk for about 500 mt. To Via Meucci 12 –Bresso



Bresso - Milano



About Milan

Milan is the second-largest city in Italy and the capital of Lombardy. The city proper has a population of about 1.35 million, while its urban area is the 5th largest in the EU and the largest in Italy with an estimated population of about 5.2 million. Milan metropolitan area is part of the so-called Blue Banana, the area of Europe with the highest population and industrial density.

Piazza del Duomo ("Cathedral Square") is the main piazza (city square) of Milan, Italy. It is named after, and dominated by, the Milan Cathedral (the Duomo). The piazza marks the center of the city, both in a geographic sense and because of its importance from an artistic, cultural, and social point of view.

The Galleria Vittorio Emanuele II is the world's oldest shopping mall. Housed within a four-storey double arcade in central Milan, the Galleria is named after Vittorio Emanuele II, the first king of the Kingdom of Italy. It was designed in 1861 and built by Giuseppe Mengoni between 1865 and 1877.

Navigli is one of the most romantic neighborhoods in Milan. Situated southwest of the city's historic center, the neighborhood is named for the navigli, canals that were once ubiquitous in this former port area.

Subway (metro) maps and stations of Milano

Bus transfer

Bus transfer organized for the participants:

19 June 2013

•12.00 from Milan Central station (Piazza IV Novembre, Hotel Gallia side) To Zambon Conference Center

•12.30 from Milan Central station (Piazza IV Novembre, Hotel Gallia side) To Zambon Conference Center

•13.00 from Milan Central station (Piazza IV Novembre, Hotel Gallia side) To Zambon Conference Center

•19.30 from Zambon Conference Center to Milan Central station ((Piazza IV Novembre, Hotel Gallia side)

20 June 2013

•8.00 from Milan Central station ((Piazza IV Novembre, Hotel Gallia side)) To Zambon Conference Center

•8.10 from Milan Central station (Piazza IV Novembre, Hotel Gallia side) To Zambon Conference Center

•8.20 from Milan Central station (Piazza IV Novembre, Hotel Gallia side) To Zambon Conference Center

•18.30 from Zambon Conference Center to Milan Central station (Piazza IV Novembre, Hotel Gallia side)

•22.00 from Zambon Conference Center to Central station (Piazza IV Novembre, Hotel Gallia side)

21 June 2013

•8.10 from Milan Central station (Piazza IV Novembre, Hotel Gallia side) To Zambon Conference Center

•8.20 from Milan Central station (Piazza IV Novembre, Hotel Gallia side) To Zambon Conference Center

•12.30 from Zambon Conference Center to Central station (Piazza IV Novembre, Hotel Gallia side)

Bus transfer

Parking Lot:

For who is coming by car we have parking lot available.

Please inform the organizing chair that you need it, if you haven't done it yet (email: Sarolta.peter@biotalentum.hu / ildiko.nagy@biotalentum.hu).

International conference for
NANOPARTICLES AND NANOTECHNOLOGIES IN MEDICINE
-
NPMED 2013
19-21 June 2013 Milan – Italy

NPMED 2013 provides a forum for chemists, clinical physicians, nanoscientists, industry expert as well as educational institution and small and large enterprises for discussing current, emerging and future trends of the converging fields of Nanotechnology, Biotechnology and Medicine.

Exciting lectures and invited talks given by leading international scientists as well as poster presentations offer delegates a good opportunity to discuss pioneering developments and also initiate cooperation projects.

NPMED 2013 will focus on the following topics:

- **Session 1- Design of Nanoparticles for Medicine**

  Engineering nanoparticles for medical application, nanosafety, novel drug delivery system, nanotoxicology.

- **Session 2- Nanoparticles for therapy and diagnosis of Alzheimer Disease**

  NAD (Nanoparticles for therapy and diagnosis of Alzheimer's disease) Project presentation, updating on Alzheimer disease, strategy to overcome the blood-brain barrier, disease targets, nanotherapy of Alzheimer disease, nanoparticles biodistribution and pharmacokinetics.

- **Session 3- Nanotechnology for therapy and diagnosis of human diseases**

  Theranostic nanotechnologies, nanomedicine in cancer therapy, regenerative medicine, biological sensors systems, magnetic nanodevices.

- **Session 4- Industry and Nanotechnology**

  The point of view of Companies on Nanotechnology, regulatory aspects in Nanomedicine development.

Wednesday - 19 June, 2013

12:30 –14.00 Registration

14.00-14.30 **M. Fontanesi** (UNIMIB Rector)
E. Zambon (Zambon S.p.a. President)
M. Masserini (NAD project Coordinator)
M. Cacace (NAD Project PTA)

Session 1: Design of Nanoparticles for medicine

Chairmen: M. Masserini and K. Andrieux

14.30-15.00 **M. Moghimi** (UCPH- Denmark)
"Nanoparticle Engineering for Medical Applications: Current Status, Future Medicine and iMedicine"

15.00-15.30 **WH De Jong** (RIVM - The Netherlands)
"Safety evaluation of nanomaterials and pitfalls in toxicity testing"

15.30-15.45 **F. Cellesi** (CEN – Italy)
"Functionalization of hybrid nanoparticles. From stealth to immune-active polymer coatings"

15.45-16.30 **Coffee break**

Chairmen: W. Scheper and G. Sancini

16.30-17.00 **F. Stellacci** (EPFL - Switzerland)
"Cell membrane penetrating nanoparticles: basic science and nanomedicine applications"

17.00-17.15 **F. Baldelli Bombelli** (UEA-UK/CEN-Italy)
"Engineered Nanoparticles: the Bionano Interface in a Biological Environment"

17.15-17.30 **S. Argentiere** (Filarete Foundation - Italy)
"Engineered silver nanoparticles: protein corona formation and toxic effects on brain cells"

17.30-18.00 **R. Consonni** (Zambon Company CEO)
"Open Zone Scientific Campus"

18.00-19.30 **Poster session and Cocktail party**

Thursday– 20 June 2013

Session 2: Nanoparticles for therapy and diagnosis of Alzheimer Disease

Chairmen: M. Moghimi and F. Wandosell

9.00- 9:10 **M. Cacace** (NAD project PTA)
"Welcome and opening"

9.10-9:30 **C. Ferrarese** (UNIMIB - Italy)
"State of the art of AD"

9.30-9.50 **M. Masserini** (NAD project coordinator)
"NAD Project Overwiew"

9.50-10.10 **S. Antimisiaris** (UPAT - Greece)
"Functionalization of NPs with Antibodies for targeting the BBB and/or Amyloid plaques"

10.10-10.30 **K. Andrieux** (UPS - France)
"Functionalization of NPs with Curcumine derivative"

10.30-10.50 **C. Duyckaerts** (UPMC - France)
"Studies with NPs designed for therapy of AD on post-mortem human brains"

10.50-11.30 **Coffee break**

Chairmen : M. Salmona and C. Duyckaerts

11.30-11.50 **F. Re** (UNIMIB- Italy)
"Liposomes functionalized with acidic phospholipids"

11.50-12.20 **G. Forloni** (IRF - Italy)
"Efficacy of NP tailored for AD"

12.20-12.35 **J. Rokka** (UTURKU – Finland)
"Synthesis of [^{18}F]Liposomes for PET imaging within NAD"

12.35-12.50 **S. Krol** (IRCCS - IFOM-IEO-campus - Italy)
"How protein-binding influences biodistribution in nanoparticulated drug delivery! Is the blood brain barrier really impermeable to nanoparticles?"

13.00 **Lunch**

Session 3: Nanotechnology for Therapy and Diagnosis of human diseases

Chairmen: S. Antimisiaris and P. Gasco

14.30- 15.00 **P. Couvreur** (UPS -France)
"Nanomedicine for the therapy of tumors"

15.00-15.30 **F. Nicotra** (UNIMIB- Italy)
"Nanostructured biomaterials for regenerative medicine"

15.30-15.45 L. Sitia (IRF – Italy)
"Optimization of an integrated system for the quantitative measurements of nanoparticles cellular uptake and cellular localization"

15.45-16.00 T. Pellegrino (IIT – Italy)
"Highlighting some MAGNIFYCO project results: iron oxide nanocubes as heat mediators for combining hyperthermia treatment with drug delivery"

16.00-16.30 Coffee break

Chairmen: P. Couvreur and O. Flores

16.30-17.00 S. Logotethidis (AUTH - Greece)
" Nanotechnology Approaches for Cardiovascular Stents"

17.00-17.15 Y. Li (UMA – Portugal)
"pH Sensitive Laponite/Doxorubicin/Alginate Nanohybrids with Improved Anticancer Efficacy"

17.15-17.30 M. Morpurgo (UNIPD – Italy)
"In vivo fate of Avidin-Nucleic-Acid-Nanoassemblies as a novel theranostic tool"

17.30-17.45 R. M. Ion (ICECHIM – Romania)
"Nano-porphyrin drugs for clinical applications in dermatological photodynamic therapy"

17.45-18.30 POSTER SESSION

Friday – 21 June 2013

Session 4: Industry and Nanotechnology

Chairmen: M. Haaparanta-Solin and M Cacace

9.00- 9:30 D. Bazile (Sanofi-France)
"Nanotechnologies in drug delivery – An industrial perspective"

9.30-10.00 P. Gasco (Nanovector - Italy)
"Industry and Nanotechnology: The point of view of SME Companies"

10.00-10.30 B. Sarkadi (EMEA - Hungary)
"European legislation on nanotechnology"

10.30-11.00 Coffee break

Chairmen: M. Masserini and J. J. Pei

11.00-11.30 S. Farhangrazi (Biotrends Foundation and University of Denver -USA)
"Realistic solutions for the future of Nanomedicine: To boldly go where no one has gone before"

11.30-12.00 F. Gramatica (ETP - Italy)
"Open innovation in Nanomedicine: challenges and achievements towards a real personalized medicine"

ORAL PRESENTATIONS

Nanoparticle Engineering for Medical Applications: Current Status, Future Medicine and iMedicine

S. Moein Moghimi
Nanomedicine Research Group, Centre for Pharmaceutical Nanotechnology and Nanotoxicology, University of Copenhagen, Universitetsparken 2, DK-2100 Copenhagen O, Denmark
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Nanoparticulate drug carriers and multifunctional nanoparticles provide a range of unique therapeutic and diagnostic opportunities. In terms of site-specific targeting, pharmacokinetics and biodistribution of nanoparticles are controlled by a complex array of interrelated core, dynamic interfacial physicochemical and biological factors. Pertinent to realizing clinical goals, definitive maps that establish the interdependency of nanoparticle size, shape, and surface characteristics in relation to interfacial forces, biodistribution, controlled drug release, excretion, and adverse effects must be outlined. For instance, the protein adsorption phenomenon has received much attention, but often over interpreted and not analysed within the broader context of dynamic pathophysiological processes and the immune system function. Today, the complex nature of many engineered nanoparticles/nano carriers makes such studies cumbersome and difficult, but this may be overcome by improving nanoparticle characterization techniques, introducing methodologies that generate precisely defined nanoparticles and offering the ability to independently alter one variable at a time. Nevertheless, promising findings have resulted from trials at the clinical level and, indeed, there are a number of nanopharmaceuticals/nanomedicines currently in the market. Economic is also a key driving force. The sheer complexity and the know-how of particulate drug delivery design, development, and production issues potentially offer market exclusivity to the pharmaceutical industry and reduce the threat of generic competition. Such technological innovations may reduce and/or prevent the rapid fall-off of revenue for proprietary nano-based medicines even after patent expiration. Finally, it is envisaged that the boundaries toward development of nanopharmaceuticals/nanomedicines can be pushed further. With concomitant advances in extensive computational knowledge of the genomics and epigenomics of interindividual variations in drug and nanomaterial responses, development of personalized nanomedicines could materialize. Functional nanoparticles and semiconductive nanowires are also finding their way into mobile technologies complimenting App developments for clinicians and patients to track, monitor and record health status more efficiently. These innovative platforms will be the pillars of eHealth in the digital age and are expected to transform clinical research, medical practice, healthcare delivery and health spending.

References:

1. **Moghimi, S. M., Hunter, A. C. and Andresen, T. L**. (2012) Factors controlling nanoparticle pharmacokinetics: an integrated analysis and perspective. *Anun. Rev. Pharmacol. Toxicol.* **52:** 481–503.
2. **Moghimi, S. M., Peer, D. and Langer R.** (2011) Re-shaping the future of nanopharmaceuticals: ad iduicium. *ACS Nano* **5:** 8454–8458.
3. **Moghimi, S. M., Wibroe, P. P., Helvig, S., Farhangrazi, Z. S. and Hunter, A. C.** (2012) Genomic perspectives in inter-individual adverse responses following nanomedicine administration: the way forward. *Adv. Drug Deliv. Rev.* **64:** 1385–1393.
4. **Moghimi, S. M. and Farhangrazi, Z. S.** (2013) Nanomedicine and the complement paradigm. *Nanomedicine: Nanotechnol. Biol. Med.* http://dx.doi.org/10.1016/j.nano.2013.02.011 (in press).
5. **Special Report: Healthcare and Technology. Medicine Goes Digital.** *Economist* 16th April 2009. http://www.economist.com/node/13437990.

Financial support by the Danish Agency for Science, Technology and Innovation (Det Frie Forskningsråd for Teknologi og Produktion, references 274-08-0534 and 12-126894; Det Strategiske Forskningsråd, reference 09-065746/DSF), Lundbeckfonden (Denmark) and the European Community's Seventh Framework Program under grant agreement number 212043 is gratefully acknowledged.

Safety evaluation of nanomaterials and pitfalls in toxicity testing

Wim H. De Jong

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Similar to other medical products medicines developed and manufactured using nanotechnology need to be evaluated for their efficacy and safety (i.e. possible adverse effects). Nanoparticles have a relative high surface area that is beneficial for loading molecules making them good candidates for drug delivery. In addition, pharmaceutical formulas themselves may be prepared as nanoparticles aiming for increased biological availability. However, the high surface area may be accompanied by an increase in reactivity that may be useful in catalytic reactions but could be harmful in terms of toxicity. In addition, the safety evaluation of nanomaterials and thus also nanomedicines may require adaptation of the assays used for the evaluation as the particulate nature of a nanomedicine is quite different from medicines that show a good dissolution.
As for every safety evaluation the identification of the substance is essential. For nanomaterials this may pose a problem in view of a multitude of manufacturers that can produce nanomaterials with the same chemical identity but differences in physicochemical properties like size and size distribution and the presence or absence of a coating on the nanoparticle surface. Risk can be determined using both in vitro and in vivo assays. In vitro assays have, with the exception of certain assays for hazard identification (e.g. genotoxicity), a limited contribution in the safety evaluation and risk assessment. In vivo assays have a limitation that they act as a kind of "black box" and may have extrapolation problems (intra- and interspecies variation), but they can provide information on possible organ specific toxicity and no effect levels in dose response studies. The safety evaluation of nanomaterials is hampered by a number of issues: diversity of the nanomaterials, (lack of) solubility, stability (agglomeration/aggregation), dispersion problems, reproducibility of production, size distribution, matrix interactions (there are no "naked"nanoparticles), and choice and preparation of test medium. Also the dose metric to be used is an issue of discussion. As the nanoparticle is the entity that interacts with biological systems the amount of molecules present as indicated by the mass, may not be a good descriptor of the dose. It might be that the total surface area or number of particles administered may be a better description of the dose. As for every pharmaceutical the toxicokinetics and tissue distribution is important. A problem with drug targetting using nanoparticles is the fact that most particles end up in the liver and spleen the major organs of the so called reticulo-endothelia system (RER). So, when testing nanomedicines it is essential to keep in mind the particulate nature of the nanomedicine in view of its implications on the testing methodology.

Functionalization of hybrid nanoparticles. From stealth to immune-active polymer coatings

Francesco Cellesi

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CEN - European Centre for Nanomedicine
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Hybrid multifunctional nanoparticles have recently drawn intensive research in the field of drug delivery and in vivo diagnostics, taking advantage of the combination of physicochemical properties of inorganic/organic nanomaterials and the biocompatibility/bioactivity of functional biopolymers[1]. Nanomaterials based on titania and silica were successfully developed in our lab to obtain inorganic oxide nanoparticles for in vivo administration[2, 3], exploiting their unique characteristics ofbeing photoactive, in vivo traceable, and able to nano-encapsulate bioactive payloads. The fate and the bioactivity of nanoparticles in vivo, however, mainly depend on their surface properties. Free circulation in body fluids can be achieved by covering the nanoparticle with a layer of a water soluble, protein-repellent polymers(such as poly(ethylene glycol)) , which prevent recognition and clearance by phagocytic cells. This “stealth” character was obtained with a bio-inspired approach, utilizing a library of hydrophilic polymers terminated with chelating enediol ligands, which irreversibly adsorb onto TiO2 nanoparticles[4]. Alternatively, a surface initiated polymerization technique was developed for the functionalization of SiO2 nanoparticles. Cationic macroinitiators were adsorbed onto the anionic surface ofcolloidal silica to initiate the formation of a hydrophilic polymer corona by controlled living radical polymerisations, thus providing stealth character and chemical functionality. Immunoactive nanomaterials were also achieved by synthesizing multifunctional poly(glycerol methacrylate)s which contain dangling immunogenicsugar moieties. Controlled living radical polymerizations, combined with thiol-ene click chemistry, were used to vary polymer composition and architecture, and to develop a new class of bio nanomaterials for specific cell targeting, antigen delivery and controlled immunostimulation[5].

References:

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Acknowledgement / funding:
CEN startup package
EPSRC funding (grant EP/H027092/1)

Cell membrane penetrating nanoparticles: basic science and nanomedicine applications

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A bird eye view of any folded protein shows a complex surface composed of hydrophobic and hydrophilic patches closely packed. To date little is known on the fundamental properties that such packing determines. In this talk I will present my group's endeavor into the synthesis, characterization, and understanding of a family of nanomaterials (mixed monolayer protected nanoparticles) that posses a surface coexistence of patches of opposite hydrophilicity resembling that present on folded protein. I will show that these materials are ideal model compound to uncover the basic properties that such coexistence determines at the solid liquid interface, and will conclude with example of application of these nanoparticles when used as mimic of biological entities (e.g. as cell penetrating peptides, as nano-enzymes, etc.).

Engineered Nanoparticles: the Bionano Interface in a Biological Environment

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Nanomedicine is a new branch of medicine where nano-scaled materials are used for the detection and treatment of human diseases. It is a growing multidisciplinary area of research aiming to develop novel nanomaterials that can combine diagnostic and therapeutic components in one unique particle. Huge efforts have been done to better understand how the physical-chemical properties of nanoparticles (NPs) affect their interaction with the cell. It is now accepted that NP surfaces in a biological environment are immediately modified by the adsorption of proteins leading to a protein "corona" defining the biological identity of the particle[1,2]. For NPs of the same material differences in size and surface charge alter the composition of the corona significantly. This implies that extreme care must be taken in the development of nanomedicine and nanotherapeutics in terms of controlling the manufacturing process of nanoparticles and control of the surface properties of the final product.

Here, different approaches to functionalize the surface of nanoparticles designed for biomedical applications are presented. A methodology to address the different aspects governing the formation and the function of the protein corona in the biological environment is outlined by using different techniques such as dynamic light scattering, ultracentrifugation, quartz microbalance and SDS-PAGE[3]. Moreover, different biological fluids have been investigated to determine how small variations in the fluid composition can alter the properties at the bionanointerface. These datahighlight the importance of this methodology as an analysis to be used in advance of the application of engineered NPs in biological environments.

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Engineered silver nanoparticles: protein corona formation and toxic effects on brain cells

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Because of their enhanced antiseptic activity, silver nanoparticles (Ag-NPs) are currently used in many consumer products ranging from food packaging to odour resistant textiles.[1] Even though the potential for adverse health effects is very high,[2] little is known about the health impact of the prolonged exposure to Ag-NPs. In particular, since Ag-NPs can penetrate the blood-brain barrier (BBB) and gain access to the brain, there is the need for a quantitative risk assessment in brain cells.[3]Proteins in biological fluids associate with nanoparticles and lead to a protein corona, which defines the biological identity of the particle.[4] Therefore, this work was aimed at studying the protein corona formation and the potential toxicity of Ag-NPs on astrocytes as a function of nanoparticle properties (coating, size, concentration).In this study, commercial Ag-NPs of 10, 40 and 100 nm in size were employed, both nude and coated with polyvinylpyrrolidone (PVP). To study the protein corona formation, Ag-NPs were incubated in a cell-free medium at different concentrations and their diameter was measured by Dynamic Light Scattering (DLS) at fixed time points. Samples were also fixed in a glutaraldehyde solution and analysed by transmission electron microscopy (TEM).In vitro cell viability of primary astrocytes in the presence of increasing amount of Ag-NPs was evaluated in real time by xCELLigence apparatus (Roche), whereas intracellular uptake was determined by confocal microscopy.
The Ag-NPs/proteins complex was observed by TEM after incubation with the cell medium (Fig. 1). The DLS data suggested that Ag-NPs concentration did notaffect significantly the protein corona, which in turn was highly dependent on both coating and size of Ag-NPs. Indeed, the lower the size, the higher was the increase of Ag-NPs diameter. The in vitro experiments showed that 100 nm sized Ag-NPs showed the highest level of toxicity. The PVP coating seemed to reduce the toxicity of Ag-NPs. According to confocal microscopy analysis, Ag-NPs were internalized into astrocytes after an overnight exposition. After 6 days of incubation, they formed aggregates inside the cells. Overall, it was found that the greater the Ag-NPs diameter, the smaller the protein corona, the higher the toxicity on astrocytes. Therefore, these results suggested that the protein corona could represent an effective tool to reduce the Ag-NPs toxicity.

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Acknowledgement / funding:
This research was supported by Cariplo Foundation on "Toxicology of chronic exposure to engineered silver nanoparticles" project.

State of the art of Alzheimer Disease

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Alzheimer's disease (AD) is a progressive neurodegenerative disease linked to aberrant metabolism of beta-amyloid (Aβ), a toxic peptide playing a key role in the neuronal death occurring in this disorder. Several studies have nowadays established the mechanisms of Aβ production (through beta and gamma-secretases) and clearance (along two major pathways: LRP-mediated transport through the blood-brain barrier, and proteolytic degradation). Moreover, the effects of Aβ on the intracellular redox balance, mitochondrial function, glutamate and calcium homeostasis, apoptosis, proteasome activity and TAU phophorylation have been demonstrated as fundamental mechanisms of neurodegenerative pathways in AD. The same mechanisms have been also involved in regulating either Aβ production or degradation.
Biological fluids and peripheral tissues obtained from patients represent valid models for ex-vivo studies of these processes and to validate new biomarkers for early diagnosis and/or more specific therapeutic interventions for AD patients.
Based on these biological pre-clinical and clinical evidences, several therapeutic strategies, aiming to counteract beta amyloid accumulation and its toxic effects, have been proposed and are under investigation in animal models and in AD patients.
Acetylcholinesterase inhibitors and NMDA antagonist Memantine are so far the only drugs approved for treatment of AD, but contrasting results have been obtained by anti-amyloid strategies, antioxidant vitamins, statins, metal ion-chelators and anti-inflammatory drugs. It reasonable to conceive, however, that early or preclinical diagnosis (such as at the stage of Mild Cognitive Impairment), based also on biological markers, may lead to more effective therapeutic or preventive strategies. Indeed, demonstration of amyloid accumulation in brain by Positron Emission Tomography (PET) and analysis of beta-amyloid and TAU levels in cerebrospinal fluid may predict conversion from the stage of MCI to AD. For this reason, new therapeutic trials will be focused on pre-clinical stages of the disease, with positive biomarkers, to prevent the onset of dementia.
New ways to deliver drugs (including anti beta-amyloid antibodies), with the help of nanoparticles, may be extremely important to overcome problems related to blood-brain barrier and to toxic side effects of these drugs.

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Akcnowledgement / funding
The research leading to these results has received funding from the European Community's Seventh Framework Program (FP7/2007-2013) under agreement No. 212043 NAD Project.

NAD PROJECT OVERVIEW

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Over three million people in the EU have Alzheimer Disease (AD), with one in 20 people over 65, and one in five over 85, with associated immense societal and economic problems. The use of new diagnostic and therapeutic methods based on nanotechnology is one of the potential future answers to the problem. The objective of NAD project is to use nanoparticles (NPs) specifically engineered for the diagnosis and therapy of AD, even combined (theranostics), by targeting Aβ peptide.
For this purpose, different NPs (liposomes, SLN, polymeric) have been multiple-functionalized with: i) molecules interacting with the different pools and forms of Aβ, ii) molecules stimulating BBB crossing to reach the brain, ii) PET or MRI contrast agents.
Ligands with high affinity in vitro for Aβ peptide have been selected among naturally occurring small molecules (phosphatidic acid and its derivatives; curcumin) or synthesized within the Consortium (antibodies). Selected ligands have been utilized for decoration of NPs, taking advantage of their amphiphilic nature or after chemical modification, e.g. through a "click" reaction with an azido group in the case of curcumin derivative, or via a cysteine-maleimide linkage in the case of antibodies. Different molecules, potentially able to cross the Blood Brain Barrier (BBB) have been identified (tat peptide, anti-TfR antibody, modified ApoE peptides) and linked to NPs, alone or in combination with Aβ ligands. Experiments utilizing in vitro BBB cellular models have shown the ability of such NPs to cross the barrier.
Artificial and cellular models have been used to improve and fine-tune NPs binding to Aβ, biocompatibility, BBB crossing and physical stability.
Finally, in vivo experiments have shown that NPs functionalized to bind Aβ and to cross the BBB, administered to transgenic mouse models of AD are able to decrease brain Aβ , the amount of plaques in the brain and to improve cognitive functions .
The results of the project, carried out on a large set of cellular and animal models, provide new chances for the treatment and the diagnosis of AD in humans..

References:
4 patents and 35 papers have been published on the main journals of Biotechnology and Nanomedicine

Acknowledgement / funding
The research leading to these results has received funding from the European Community's Seventh Framework Program (FP7/2007-2013) under agreement No. 212043 NAD Project.

Functionalization of NPs with Antibodies for targeting the BBB and/or Amyloid plaques

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BBB-targeting: In order to construct NP formulations for brain targeting, anti-transferrin receptor MAb (TfRMAb)-decorated liposomes (LIP) were formulated [1] and evaluated for important physicochemical properties. The effect of using different ligation methodologies and increasing surface densities of ligands on their physicochemical properties was evaluated. After establishing which types of immunoliposomes fullfil requirements for in vivo applications they were evaluated for BBB targeting potential in vitro (using hCMEC/D3 cells and monolayers), in vivo (live-animal imaging) in wild-type mice, and ex-vivo (imaging of explanted mice organs). In vitro, in vivo and ex vivo results prove that immunoliposomes target the brain at substantially higher amounts compared to control liposomes; however, in vitro and in vivo results are not well correlated, especially for dually targeted LIP on which a peptide to target the BBB LDLr [2] was additionally immobilized. Interestingly, when in vivo studies were modified and performed in presence of increasing amount of serum proteins their results were better correlated with the in vivo and ex vivo results, in line with recently published data for transferrin targeting liposomes [3].
Aβ targeting: Anti-Aβ MAb (AβMAb)-decorated LIP, TfRMAb-decorated and dually-decorated ones (ddLIP) with TfRMAb and AβMAb were constructed [4]. Uptake and transcytosis of all LIP types and control vesicles by human brain endothelial hCMEC/D3 cells was measured and AβMAb-LIP uptake was higher than control PEGylated liposomes, while uptake of ddLIP was similar to that of TfRMAb-LIP. In the cases of AβMAb-LIP and ddLIP the uptake increased significantly when cells were pre-incubated with Aβ 1-42 peptides; while transcytosis of Aβ MAb-LIP through monolayers was also increased (by 2.5 times) when the monolayers were pre-incubated with Aβ 1-42 peptides. The peptides did not modulate the barrier tightness and integrity, as determined by trensendothelial resistance and Lucifer Yellow permeability evaluations. Additionally, hCMEC/D3 cell viability was not affected by Aβ peptides or by Aβ MAb –LIP. After blocking RAGE (receptor for advanced glycation end-products, known to regulate transcytosis of Aβ peptides across the BBB [5]) by a specific MAb, it was proved that the Aβ peptide-induced increase in binding (and transport) of Aβ MAb-decorated LIP-types is regulated by the membrane receptors for Aβ1-42 peptides (RAGE). This finding may have serious implications for nanosystems constructed to target Aβ species in the brain or in the blood.

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Acknowledgement / funding

The peptide to target LDLr was developed by the group of Dr. Mario Salmona, Mario Negri Institute, Italy; The AβMAb was developed by STAB VIDA, Portugal, Group of Dr. Orfeu Flores.
The research leading to these results has received funding from the European Community's Seventh Framework Programme (FP7/2007-2013) under grant agreements n° 212043 (to SGA) and 260524 (to GTS).

Functionalization of NPs with Curcumine derivative

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A versatile and efficient functionalization strategy of PEGylated, biodegradable poly(alkyl cyanoacrylate) (PACA) nanoparticles has been reported and successfully applied in vitro and in vivo to active targeting in the field of Alzheimer's disease (AD). Based on copper-catalyzed azide-alkyne cycloaddition (CuAAC) and concomitant self-assembly in aqueous solution of amphiphilic copolymers, the resulting functionalized polymeric nanoparticles exhibited requisite characteristics for drug delivery purposes: (i) a biodegradable poly(alkyl cyanoacrylate) core, (ii) a hydrophilic poly(ethylene glycol) (PEG) outer shell leading to stealth features, (iii) fluorescent properties provided by the covalent linkage of a rhodamine B-based dye to the polymer backbone and (iv) biologically active ligands displayed at their surface to achieve active targeting. The construction method is very versatile and this was here illustrated by the design of targeted, fluorescent nanoassemblies decorated by curcumine derivatives, resulting in high affinity towards: (i) the β-amyloid peptide 1-42 (Aβ1-42), believed to be the most representative and toxic species in AD, and (ii) Aβ1-42 fibrils, usually located in AD brains. In comparison with only PEGylated NPs, the curcumin decorated NPs exhibited higher affinity toward Aβ1-42 species and led to significant aggregation inhibition and toxicity rescue of Aβ1-42 at low molar ratios. These NPs have been evaluated in vivo in a model of transgenic mice evidencing an improvement of the memory of treated animals.

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Akcnowledgement / funding

The research leading to these results has received funding from the European Community's Seventh Framework Programme (FP7/2007-2013) under agreement n°212043. The CNRS and the French Ministry of Research are also warmly acknowledged for financial support.

Studies with NPs designed for therapy of AD on post-mortem human brains

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Alzheimer disease (AD) is characterized by the intracellular accumulation of tau protein and the extracellular deposition of Aβ (amyloid) peptide[1]. Mice bearing human mutated genes of the Aβ precursor (APP) and of the enzymes involved in its production have been developed (APPxPS1 mice)[2]. Aβ is both a diagnostic and a therapeutic target. The major problems met in the development of Aβ molecular probes are the hydrophobicity of several of them and the difficulty in having them cross the blood–brain barrier. Nanoparticles may solubilize and transport hydrophobic molecules; it may also incorporate proteins that facilitate the crossing of the blood brain barrier (BBB). In the NAD EU project nanoparticles targeting Aβ have been developed. We have tested them on post mortem brain tissues.
As a proof of concept, we have shown that fluorescent nanoparticles coupled with anti-Aβ antibodies label a large number of Aβ deposits in sections of AD cortex[3].
Curcumin is a naturally fluorescent molecule that has a strong affinity for Aβ. We showed that the nanoparticles were stable, non-toxic, and decorated the amyloid deposits with a high sensitivity and specificity. After intracerebral injection in APPxPS1 mice, the nanoparticles were shown to migrate in the brain and to label Aβ deposits[4]. Curcumin nanoparticles with additional proteins to facilitate the crossing of the BBB are currently developed.

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Akcnowledgement / funding

This investigation has been entirely funded by the NAD project (Nanoparticles for therapy and diagnosis of Alzheimer disease) - 7th EU Framework program. Adina Lazar is currently paid by the Assistance Publique des Hôpitaux de Paris (APHP), IMAD Project (PHRC: Clinical Hospital Research Project).

Liposomes functionalized with acidic phospholipids

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The neurotoxic beta-amyloid peptide (Abeta), formed in anomalous amounts in Alzheimer's disease (AD), is released as monomer and then undergoes aggregation forming oligomers, fibrils and plaques in diseased brains. Abeta aggregates are considered as possible targets for therapy and/or diagnosis of AD. Since nanoparticles (NPs) are promising vehicles for imaging probes and therapeutic agents, we realized and characterized lipid-based NPs (liposomes, 100 nm diameter) functionalized to target Abeta with high affinity. Preliminary immunostaining studies identified anionic phospholipids [phosphatidic acid (PA) and cardiolipin (CL)] as suitable Abeta ligands. PA/CL-functionalized, but not plain, NPs interacted with Abeta aggregates as indicated by ultracentrifugation experiments, in which binding reaction occurred in solution, and by Surface Plasmon Resonance (SPR) experiments, in which NPs flowed onto immobilized Abeta. SPR studies indicated that, when exposed on NPs surface, PA/CL display very high affinity for Abeta fibrils (22-60 nM), likely because of the occurrence of multivalent interactions which markedly decrease the dissociation of PA/CL NPs from Abeta [1], as also demonstrated by Molecular Dynamic simulation (Maestro Schrodinger program). Moreover, these NPs are able to prevent complement activation in vitro [2], to rescue Abeta toxicity, to decrease the tau phosphorylation levels in neuroblastoma cells [3] and to decrease the levels of Abeta in plasma of transgenic mice of AD. These characteristics make our NPs a very promising vector for the targeted delivery of potential new diagnostic and therapeutic molecules to be tested in appropriate animal models.

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Acknowledgement / funding
The research leading to these results has received funding from the European Community's Seventh Framework Program (FP7/2007-2013) under agreement No. 212043 NAD Project.

Efficacy of NP tailored for AD

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Alzheimer's disease (AD), the major form of dementia in elderly, it is neuropathologically characterized by the βamyloid (Aβ) accumulation in the brain parenkyma and the formation of neurofibrillary tangles intracellularly. Although numerous aspects of the neurobiology of the disease have been delucidated, the elaboration an efficacious therapy remains one of the main biomedical challenge of this century. The central role of Aβ deposits in AD pathogenesis is supported by the genetic studies showing that the familial form of the disease was associated with the mutations of the gene encoding for precursor of amyloid protein (APP) and presenilin 1 and 2 (PS1/2), proteins involved in APP metabolism. The common experimental models of AD are transgenic mice overexpressing the mutated form of human APP alone or in combination with mutated form of PS1 or 2 and in some case with the addition of the transgene encoding for the mutated form of tau (1). In these mice the accumulation of Aβ at the cortical and hippocampal level occurred with different onset according to the severity of their phenotypes. The neuronal dysfunction demonstrated in these transgenic mice is due to the small soluble aggregates of Aβ named oligomers rather than Aβ fibrils Within the frame of the European consortium NAD, these models have been used to test the efficacy of nanoparticles functionalized to pass the blood brain barrier and to exert anti-amyloidogenic activity. In vitro studies have identified these characteristics in nanoliposomes decored with a peptide sequence derived from the LDL-receptor binding domain of human ApoE and phosphatidic acid. Single (APP with Swedish mutation) and double (APP/PS1) transgenic mice 9 and 12 month old were treated for three weeks with the nanoparticles. The effect on cognitive decline was determined by object recognition test, a long term memory test based on spontaneous animal behavior without the need of stressor elements. The consequence of the treatment on Aβ levels was evaluated by ELISA determinations in the brain and in the blood, while immunocytochemical staining has been used to determine Aβ plaques and the astroglial and microglial reactivity. In the initial results a reduction of Aβ plaques as well as an improvement of cognitive condition has been found after the treatment with the nanoliposomes indicating the possibility to develop new therapy based on this approach.

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Acknowledgement / funding

The research leading to these results has received funding from the European Community's Seventh Framework Program (FP7/2007-2013) under agreement No. 212043 NAD Project.

Synthesis of [^{18}F]Liposomes for PET imaging within NAD

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Liposomes are spontaneously formed phospholipid bilayer vesicles used in nanomedicine for drug or imaging agent delivery. In Alzheimer's disease appropriately functionalized liposomes can be use for specific drug administration or to induce the 'sink effect' where liposomes bind to amyloid oligomers. [1-4] The purpose of this study was to synthesize functionalized ^{18}F-labeled nanoliposomes which bind to amyloid plaques of Alzheimer's disease. With these [^{18}F]liposomes and positron emission tomography (PET) imaging *in vivo,* the behavior of functionalized liposomes can be studied in experimental animal models. We describe synthesis of functionalized [^{18}F]liposomes using nucleophilic ^{18}F-fluorination, thin-film hydration and extrusion.

Fluorine-18 was produced with the ^{18}O(p,n)^{18}F nuclear reaction using ^{18}O-enriched water. With suitable treatment, the ^{18}F-fluorine was converted to a more reactive form. For radiofluorination the precursor, a mesyl derivative of diacylglycerol was reacted with the ^{18}F-fluoride anion yielding the product, [^{18}F]diacylglycerol ([^{18}F]DAG). [^{18}F]DAG was then separated using semi-preparative HPLC.

[^{18}F]liposomes were synthesized using a thin film hydration method. [^{18}F]DAG was mixed with cholesterol, sphingomyelin, phosphatidic acid and mal-PEG-PE dissolved in chloroform-methanol. The solvent was then evaporated. Functionalized [^{18}F]liposomes were made by adding a phosphate buffer solution to the vessel and by sonication. The particle size of the functionalized [^{18}F]liposomes were adjust with extrusion at 55 °C using a 100 nm pore size filter. Finally these [^{18}F]liposomes were purified using gel chromatography.

The total synthesis time for the functionalized [^{18}F]liposomes was 90 min. The radioactivity concentration of these [^{18}F]liposomes was 131 ± 51 MBq/ml and the radiochemical purity > 97 % (n=6). The particle size of functionalized [^{18}F]liposomes was 126 ± 13 nm.

Functionalized [^{18}F]nanoliposomes were successfully synthesized. Studies of these [^{18}F]liposomes in APP-23 mice are in progress.

Acknowledgements: This study received funding from the European Community's Seventh Framework Programme (FP7/2007-2013, acronym NAD) under grant agreement no. 212043.

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How protein-binding influences biodistribution in nanoparticulated drug delivery! -Is the blood brain barrier really impermeable to nanoparticles?

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Nanomedicine is a fast evolving field involving nanoparticles or nanostructures for medical applications. Especially in the underdeveloped field of drug delivery to the brain, there are high expectations for the ability of multifunctional nanoparticles (NPs) to cross the blood-brain barrier (BBB). In the present overview the challenges nanoparticles face after injection into the body will be summarized. There is a broad range of biological, chemical and physical hurdles for NPs to reach the brain1.Perhaps the most challenging task will be to design and develop nanoparticles that specifically target that right subset of diseased neurons without affecting other healthy neurons. This is of immense importance especially in the case of targeting toxic drugs to highly invasive brain tumors.
Already, without the additional obstacle in the form of the BBB, targeting nanoparticles against a small subset of cells in the body is a big challenge. While the permeability of the blood vessels in other tissues is comparably higher the brain microvasculature is highly restrictive. The reason for this is that uncontrolled invasion of nano-objects or molecules may lead to a pathological change in neurons responsible for memory, personality, senses and movement. With nanomedicine we have for the first time the possibility to design systems to meet requirements such as reduced side-effects, controlled release, targeted delivery as well as higher drug bioavailability at the target site. If the brain delivery of drugs for neurodegenerative disease or cerebral cancer is to be successful, a far better understanding of the complex processes taking place on the nanoparticles surface, as well as in cell-NP contact with the different transit organs and tissues, will be required. Recent research2–6 indicate that nanoparticles depending on their surface properties are immediately covered by a corona of blood derived proteins. This corona has a significant influence on their biodistribution7,8. We performed some experiments binding on purpose two proteins on the surface of nanoparticles developed as drug for neurodegenerative prion disease and investigated their influence on biodistribution and BBB penetration. Those proteins are frequently found on the surface of nanoparticles. We observed that the proteins significantly influenced the pattern of nanoparticle accumulation in different tissues as well as the amount of nanoparticles detected in the brain at different time points.

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Acknowledgement / funding:
F.S. was financially supported by Besta5xmille and by an AIRC research grant. S.K. was supported by Besta5xmille

Nanomedicine for the therapy of tumors

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Even if new molecules are discovered to treat severe diseases, the clinical use and efficacy of conventional chemotherapeutics is hampered by the following limitations: (i) drug resistance at the tissue level due to physiological barriers (non cellular based mechanisms), (ii) drug resistance at the cellular level (cellular mechanisms), and (iii) non specific distribution, biotransformation and rapid clearance of the drugs in the body. It is therefore of importance to develop nanodevices able to overcome drug resistance in various pathologies resistant to chemotherapies, incl. cancer and infectious diseases. This is illustrated by the camouflage of doxorubicin into biodegradable polyalkylcyanoacrylate nanoparticles (PACA), allowing to overflow the PgP detoxification capacity, thus inducing reversion of the multidrug resistance (MDR). The higher cytotoxicity of doxorubicin when loaded onto poly(isohexylcyanoacrylate) nanoparticles has been shown on the X/myc transgenic mouse model of hepatocellular carcinoma which mimics several steps of human hepatocarcinogenesis (1). Based on these data, a phase III multicentric clinical trial is currently performed on patients with resistant hepatocarcinoma or liver metastasis. Recently, advanced multifunctional PACA nanoparticles have been constructed which combine: (i) a biodegradable nanoparticle core for drug entrapment, (ii) equipped with single (2) or multiple (3) fluorescent tag for imaging, (iii) coated with a polyethyleneglycol shield and (iv) functionalized with specific ligands for drug targeting (4). Another illustration of this approach is the “squalenoylation” (5, 16), a technology that takes advantage of squalene's dynamically folded conformation to link this natural and biocompatible lipid to anticancer and antiviral nucleoside analogues in order to achieve the spontaneous formation of nanoassemblies (100–300 nm) in water without the aid of surfactants (6). When applied to the anti-cancer compound gemcitabine (7), this original concept was demonstrated to be able to overcome different mechanisms of resistance to gemcitabine (8), ie. deamination of gemcitabine by the blood deaminases, down regulation of nucleoside transportersn and/or insufficient phosphorylation by the deoxycytidinekinases (dCK). Indeed, the squalenoylated gemcitabine nanoparticles were found (i) to be resistant to deaminases, (ii) to diffuse intracellularly independently of the presence of nucleoside transporters (9) and (iii) to improve the phosphorylation of gemcitabin by dCK. This breakthrough concept has been further enlarged to other anticancer drugs, including paclitaxel (10), cisplatin and small interfering RNA for the inhibition of the ret/PTC fusion oncogene in the papillary thyroid carcinoma (11), as well as to the treatment of resistant intracellular infections (12). The entrapment of ultrasmall iron oxide nanoparticles in those squalene-based nanoassemblies has further allowed to design multifunctional nanoparticles combining therapeutic and imaging properties (ie. the so-called “nanotheragnostics”) (13). This new concept open the way to the personalized medicine. Finally, the use of nanohybrids (14) constructed with metal organic frameworks (nanoMOFs) will be reviewed for their ability to encapsulate unprecedent high quantities of the anticancer compound busulfan also allowing imaging of tissues and organs in vivo (15).

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Acknowledgement / funding:
Part of this presentation benefits from the financial support of the European Council under the ERC Advanced Grant n°249835 "TERNANOMED"

Nanostructured biomaterials for regenerative medicine

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Humankind's use of materials to repair the body dates to antiquity, when natural materials such as wood were used in an attempt to structurally replace injured tissues by diseases or trauma. In the beginning of the twentieth century, synthetic polymers, ceramics and metal alloys, were introduced in place of natural materials, offering better performance, increased functionality and more reproducibility than their naturally derived counterparts. Research on nanostructured biomaterials surface functionalisation has become one of the hottest topics in biomaterials for regenerative medicine.
Since cell contact with the biomaterial surface is a key point, in recent years, biomaterial design has focused on the exposition and incorporation of signalling molecules into scaffold materials. Carbohydrates are well-known to have a wide variety of biological functions and participate in a number of recognising processes. Synthetic carbohydrate based polymers are increasingly being explored as biodegradable, biocompatible and bio-renewable materials for use as water absorbent, chromatographic supports and medical devices. In addition, membrane proteins, i.e. lectins, bind specific carbohydrates. This binding is extremely specific, and may present an attractive target for rational design of smart biomaterials. Thus, it is clear that carbohydrates may be used in the bioactivation of material surfaces toward tissue engineering applications.
Innovative and recent examples of material functionalisation for tissue engineering applications ("biodecoration") with signalling and relevant glycidic scaffolds will be outlined. Particular attention will be drawn to the chemistry used for covalent attachment of relevant carbohydrates to materials of different chemical nature [1,2].

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Akcnowledgement / funding
The work has been supported by Fondazione Cariplo, grant n° 2008-3175, 2010-0378 and 2011-0270, and PRIN 2011 2010L9SH3K

Optimization of an integrated system for the quantitative measurements of nanoparticles cellular uptake and cellular localization

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To approach a reliable development of nanoparticle (NP) for theranostics purposes it is crucial to standardize the processes of NP-synthesis and determine the dynamic of interaction NP-cell. In this study an integrated in sylico/in vitro multi-step system has been developed to: a) characterize physicochemical features of neo-synthesized NP; b) quantify the uptake of NP in cells; c) determine their sub-cellular localization; d) evaluate possible alterations of endosomal-vesicular system. In the first step the polymeric matrix of biocompatible poly(methylmethacrylate) (PMMA) NP was covalently bound to the fluorescent dye Rhodamine B (RhB). The reliability of the synthesis was verified by determining size, monodispersity, amount of RhB for NP and ζ potential for each single batch produced. This analysis is essential to guarantee a quantification of NP uptake in cells. In the second step quantitative analyses of fluorescent signal during NP incubation in a mouse breast cancer cell line (4T1 cells) has been developed by the combination of flow cytometry and plate fluorimetry experiments. This approach enabled us to quantify the mean intensity of RhB and, as a consequence, to determine the concentration of NP for each single cell. The third step was focused on the evaluation of subcellular localization of NP at different time-points. To this aim, a combined approach was developed by first visualizing 4T1 cells through confocal microscopy and then processing serial sections at different z-planes to obtain a 3-D reconstruction of the whole cell volume. These experiments enabled us to determine the dynamic of internalization and the cellular accumulation of NP. In the last step the interaction between NP and the main sub-cellular structures (plasma membrane, cytoskeleton, mitochondria, Golgi apparatus, endosomes, early-late lysosomes) was investigated by coupling observational results (immunolabeling, confocal microscopy, 3-D reconstruction) to the quantification of fluorescent signals by a dedicated software (TissueQuest) for the cell segmentation. Our specific results revealed that:1) 4T1 cells incorporated 103-104 NP in few hours; 2) NP migrate to the perinuclear area but do not penetrate into the nucleus;3)NP interact with the lysosomes from 24 to 72 hours after incubation. However, the main output emerging from this study is the optimization of a method easily transferable to other research areas such as brain endothelial cells and/or neurons.

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Acknowledgement / funding:
This study has been supported by a grant of the Italian Association for Cancer Research (AIRC) ‘‘5 per mille’’

Highlighting someMAGNIFYCO project results: iron oxide nanocubes as heat mediators for combining hyperthermia treatment with drug delivery

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In the last 4 years, within the MAGNIFYCO consortium, eleven European groups have been working onthe development ofmagneticnanomaterialsthatcan act as drug "nanocontainers" for dual cancer treatmentscombining hyperthermia and controlled drug release(www.magnifyco.eu).

Within the project aims, the consortium had to identify efficient heat mediators based on superparamagnetic nanocrystals. A wide variety of iron-based nanocrystals were studied. We found out that among the different types of iron oxide nanocrystals synthesized by non-hydrolytic colloidal methods, nanocubes of 19-24 nm edge have very high specific absorption rate (SAR) values thus making them promising as heat agents under alternating magnetic field. In vitro and in vivo studies, in our case ovarian cancer was the selected tumor model,have been carried out within the consortium and important conclusions can be drawn on the heat-abilityof iron oxide nanocubes,not only on the system itself but also when confined in living cells or in a tumor. Procedures to specifically functionalize these nanoobjects with stimuli-responsive polymer shell for drug delivery purposes or with specific antibody fragments (AFRA) to target ovarian cancer cells have been also intensively investigated. Besides the partial success achieved in some of those studies,much can be learned from our experience.

An overview of these results will be presented.

Nanotechnology Approaches for Cardiovascular Stents

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Nanotechnology entails nanoscale tools, materials and processes for the effective treatment of cardiovascular disease which constitutes a social and economic burden for the western societies. Until now, the vascular stents are the landmarks of re-opening of stenotic arteries in clinical practice. The most commonly used drug eluting stents (DES) have as drawbacks the late stent thrombosis and the in-stent restenosis caused mainly by the delayed endothelialisation owing to the polymers or the uncontrolled release of anti-proliferative drugs from DES surface. Thus, there is a need for new drug delivery nanosystems that enable the controlled release of drugs at the specific atherosclerotic sites.

In this talk, an overview of the recent advances in nanomedicine that has provided novel insights to atherosclerosis treatment will be given in parallel with nanotechnology strategies to advance stents.

A wide spectrum of drug delivery nanosystems ranging from biodegradable nanoporous drug delivery platform in multi-layer configuration up to drug loaded nanoparticles embedded in electrospun biodegradable polymeric matrices will be presented as nanotechnology enabled solutions. The nanoparticulate scaffolds may release their therapeutic payloads in a controllable manner and promote tissue regeneration simultaneously. By finding the balance between the efficacy and toxicity of nanotechnology enabled systems, new frontiers in atherosclerosis treatment will emerge.

pH Sensitive Laponite/Doxorubicin/Alginate Nanohybrids with Improved Anticancer Efficacy

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Although doxorubicin (Dox) has been widely used in the treatment of different types of cancers,[1] its successful application is limited by drug resistance mechanisms which are often associated with ion-trapping inside acidic compartments, over-expression of efflux pumps, activation of detoxifying or DNA repair mechanisms, etc..[2] To maintain the desirable therapeutic efficacy of free Dox, a large dosage or an increased number of injections may be needed, which may lead to adverse side effects in normal tissues, especially in the heart and the kidneys.[3] As a biocompatible natural polymer, alginate (AG) has been widely studied as carrier for drug release or encapsulation of cells, due to easy drug loading and simple administration procedure. Laponite (LP, 25 nm in diameter and 1 nm in thickness) can establish strong interactions with guest compounds.[4] The charges on its surface are pH dependent.[5] In the present work, Laponite and alginate were used to prepare nano-sized materials (nanohybrids) for delivery of the anticancer drug, doxorubicin. The objective was to develop a drug delivery system with enhanced efficacy and minimal adverse side effects. Briefly, Dox was loaded onto Laponite through strong electrostatic interactions to get Dox-loaded LP complexes. After, alginate was coated onto the Dox-loaded LP complexes (LP/Dox/AG nanohybrids). The results demonstrate that the nanohybrids have high encapsulation efficiency (81±11%), are sensitive to pH and display a sustained drug release behavior (almost of zero order in 21 days). Cell culture experiments indicate that the LP/Dox/AG nanohybrids can be effectively internalized by CAL-72 cells (an osteosarcoma cell line), and exhibit a remarkable higher cytotoxicity to cancer cells than the free Dox. The nanohybrids use the acidic environment of the endo-lysosomes to release the drug, simultaneously helping to disrupt the endo-lysosomes through the proton-sponge effect and diminishing endo-lysosome Dox trapping. Furthermore, as the nanohybrid carriers are able of sustained drug delivery, those that remain in the cytoplasm and still contain Dox are expected to exert a prolonged anticancer activity. The merits of Laponite/alginate nanohybrids, such as biocompatibility, high loading capacity, and stimulus responsive release of cationic chemotherapeutic drugs make them excellent platforms for drug delivery.

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Acknowledgement / funding:

This research was supported by Fundação para a Ciência e a Tecnologia (FCT) with Portuguese Government funds (from the CQM Strategic Project PEst-OE/QUI/UI0674/2011, from the NMR and MS Portuguese Networks - PTNMR-2013, RNEM-2013, and, partially, from the Project PTDC/CTM-NAN/116788/2010 and the project PTDC/CTM-NAN/112428/2009). FCT is also acknowledged for the Science 2008 Programme (Y. Li) and the Ph.D. grant SFRH/BD/88721/2012 (M. Gonçalves). The support of VidaMar Resorts is also gratefully acknowledged.

In vivo fate of Avidin-Nucleic-Acid-Nanoassemblies as a novel theranostic tool

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This study describes the pre-formulation studies and the body-cell distribution and clearance, after intravenous administration in mice, of a dually fluorescent biodegradable poly-avidin nanoassembly based on the Avidin-Nucleic-Acid-Nano-ASsembly (ANANAS) platform (Morpurgo, Radu et al. 2004; Pignatto, Realdon et al. 2010; Morpurgo, Facchin et al. 2012), as a potential advancement of classic avidin/biotin-based targeted delivery(Schetters 1999; Lesch, Kaikkonen et al. 2010).The ANANAS formulation here optimized possesses many of the necessary requirements for nanobased theranostic tools, such as free circulation in the bloodstream, safety, multifunctionality and high composition definition: the assembly circulates freely in the bloodstream, it is slowly captured by filter organs, it is efficiently cleared within 24-48h and is poorly immunogenic. In general, the formulation displays more favourable pharmacokinetics than its parent monomeric avidin.The results suggest that the ANANAS platform is a promising tool for diagnostic purposes for future translational aims. In addition, the assembly shows a time-dependent cell penetration capability, suggesting it may also function as a NP-dependent drug delivery tool. The ease of preparation together with the possibility to fine tune the surface composition makes it also an ideal candidate to understand if and how nanoparticle composition affect its localization.

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Acknowledgement / funding:

This work was funded by the University of Padova Progetto di ATeneo 2007 CPDA072372.

Nano-porphyrin drugs for clinical applications in dermatological photodynamic therapy

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Photodynamic therapy has recently became a good treatment option for actinic keratosis and basal cell carcinoma [1]. The photodynamic therapy (PDT) consists in the administration of a non toxic photosensitizer (PS) drug and after a certain period of time in which the drug is accumulated in the tumor it is irradiated with visible light, usually a long wavelength red light [2]. Improvement and development of the photosensitizing drugs, led to the development of new drugs on the market, those porphyrins-based remaining on the top, due to their compatibility with the human body, and their revolutionary photophysical and photochemical properties [3,4].In this paper will be presented some porphyrin nano-structures [(tetra-methoxy-phenyl-porphyrin (TMOPP) and tetra-sulphonated porphyrin (TSPP)] used in thetreatment skin tumors, as actinic keratosis (AK) and basal cell carcinoma (BCC), which are the most common applications of topical-PDT within dermatology today. Some correlations between molecular structure, photophysical, photochemical properties and clinical results on cell cultures, animals and human volunteers, will be discussed, too. The in vitro tests showed cells having lower viability, lower proliferation capacity, and high apoptosis/necrosis percentages, so an active destruction of the cells. Cell viability before and after PDT treatment was evaluated by optical phase contrast, fluorescence microscopy and atomic force microscopy. Some data about the lipid peroxides level (measured as thiobarbituric reactive substances) and protein carbonyls (indices of oxidative effects produced on susceptible biomolecules), show an increased value in tumor tissues 24 h after treatment. The levels of thiol groups and total antioxidant capacity have been determined int umors, too, their decreasing values being the effect of the strong tumoral oxidative process.

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Acknowledgement / funding:

This work was supported by a grant of the Romanian National Authority for Scientific Research, project number 11035/2007 and CNDI-UEFISCDI, project number222/2012.

Nanotechnologies in drug delivery – An industrial perspective

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The design and manufacture of objects in the 10-1000 nm range has opened access to new Drug Delivery tools operating at the cellular and molecular levels, referred to as nanomedicines (1-3). While they were primarily envisaged for Life Cycle Management, to avoid the combination of the risks associated to a new Drug Delivery System to the risks associated to a new drug, the scope of nanomedicines is currently broadening with the development of translational sciences (4). At the same time, the variety of applications of nanomedicines in terms of route of administration, raw materials, expected biopharmaceutical perfomances, etc, leads to a multiciplicity of cases in terms of quality management (5). The objective of this presentation is to describe the multi-dimensional context of nanomedicines, to identify some of the methodological gaps that may slow down or prevent their evolution and to clarify some principles of nanomedicines quality design to facilitate the collaborations between the public Research Institution and the Pharmaceutical Industry.

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Industry and Nanotechnology: The point of view of SME Companies

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Nanotechnology has now fully established as the new frontier of innovation, basic pillar across all fields of technological application. Since the end of previous millenium much has been written, published and discussed to define and designing possible scenarios and promising improvements, based on the new approach to the very small, even in the field of medicine: the concept of Nanomedicine increasingly took its real shape. Research was in the universities but many bigger investments were required to bring ideas to the patient, and thus to the market. Similar to what already happened in biotech few years before, from academic ideas many start-ups born with different kinds of entrepreneurship behind them: well before the slow and sly reactions of big industries, SMEs tried to develop the field of Nanomedicine. However, the enthusiastic expectations at the beginning of 2000s had to deal with the necessary gradualness of maturation required by innovative ideas, especally in so sensitive field of Medicine, both at level of institutions and regulatory bodies, at large-scale industry and the public itself. Furthermore many capitalization processes have undergone a marked slowdown for drastic reductions in investments due to the economic crisis started in 2008.
As other institution in the rest of the world, the European Community has never stopped, however, to stimulate research in the field, and in recent years we are witnessing in Europe to a real implementation of Nanomedicine, with approved products and many projects in clinical development.
The talk will cover the themes common to many SME who have decided to work in the field of nanotechnology by bringing the experience of Nanovector, a company founded in 2002 with mission to operate in the field of nanomedicine through the development of its colloidal lipid carriers applied as systems of drug delivery.
Following a synthesis of strategies applied and experiences of collaborative networks, focusing on issues of intellectual property and technology transfer, and by analyzing mistakes and success stories, we'll try to describe the scenario of perspectives and developments expected nowadays by SME in light of Horizon2020.

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European legislation on nanotechnology

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Nanoparticles provide promising new methodologies to deliver pharmacological agents into special regions of our body, cross the cell membranes, or to avoid interactions with membrane transporters and metabolic enzymes. As in the case of all innovative medical approaches, establishing efficient regulation which promotes the treatments of diseases but prevents the (mis)use of insufficiently characterized new agents or protocols has been a difficult task. The European Medicines Agency (EMA) has a basic guideline for the evaluation of commercial human applications of nanomedicines (1), but a continuous development of new protocols requires a regular up-date of such guidelines. The Committee for Advanced Therapies (CAT) of the EMA is a special task force to regulate all advanced therapies, including cell-based and gene-therapy approaches. This Committee is continuously working on proper solutions and suggestions, how the regulation for advance therapies and nanomedicines in certain cases may be combined, and how to perform the proper evaluation and approval of specific new medicines, which may involve both technologies.

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Realistic solutions for the future of Nanomedicine: To boldly go where no one has gone before

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The current model of the pharmaceutical (Pharma) industry has not worked for a long time and the sector could now be described as (focusing on) reduction of work force, closing down research sites, vertical integration and acquisition of biotechnology companies and numerous licensing deals. Nanomedicine has the promise of offering new therapeutic applications and new solutions to the pharma industry. It has the potential to offer competitive advantage to companies and for the next few decades as more and more researchers seek to pursue promising research areas, it could change many processes and applications. However, success to date for companies in nanomedicine has not been realized as initially hoped. In addition, the challenge does not end in the laboratory as a successful therapeutic application and market introduction can only be achieved through a successful patenting process. Indeed, the patent system can offer biotechnology companies and researchers major advantage in obtaining licensing revenues, and leverage in deals and mergers. One of the major keys is to recognize promising areas of research and projects that yield patents and lead to potential clinical trials and real efficacy in the clinic as early as possible. Other factors include patent filing, technology transfer and a good strategy. In this talk, important and critical issues related to a successful nanomedicine venture are presented and discussed. In addition, the comparative patenting systems and landscape both in the U.S. and Europe with respect to realistic solutions for effective technology transfer and commercialization such as an open innovation model and possible future advances in personalized nanomedicine and innovative clinical practices are discussed.

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Akcnowledgement / funding
Biotrends International, LLC.

Open innovation in Nanomedicine: challenges and achievements towards a real personalized medicine

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In the last decade the applications of nanotechnologies to life science (nanobiotechnology) and in particular to medical unmet needs (nanomedicine) presented a fast growth in terms of new findings, witnessed by an increasing number of filed patents and published papers. Most of the process had its centre of mass on academia, with a high creativity level but a certain lack of focus on real translatability towards clinical applications. As a consequence, a second stage of activities has been setup in the last years by the reference group at EU level – the European technology Platform on Nanomedicine (ETPN) – mostly focusing on federating all nanomedicine stakeholders (academia, small and large industries, clinicians, regulatory bodies, patients' associations) and conceiving an innovative and sustainable technology transfer process in a sustainable perspective of open innovation. In the lecture the effort and the achievements in policy making at European level will be presented, also zooming on some research-to-market success cases.

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Acknowledgement / funding
European Technology Platform on Nanomedicine
Fondazione Don Carlo Gnocchi ONLUS
European Priject CSA „Nanomed 2020"
and the Organizing Committee of the NPMED Congress

POSTER SESSION

SESSION 1 – P1

Charge Transport in Peptides as a Useful Tool for the Development of Biosensors to Probe the Onset of Amyloidosis-like Diseases

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The focus of this work is on the numerical investigation of the charge transport properties of the de novo-designed alpha3-peptide, as well as its variants 5Q-alpha3and 7Q-alpha3. Their charge transport properties are investigated within a tight-binding model Hamiltonian, using Dyson's equation together with a transfer-matrix treatment to solve a time independent quantum Schrödinger equation. The input parameters (amino acid vertical ionization and di peptide hopping energy) were obtained by performing ab initio calculations within the Density Functional Theory (DFT).
The alpha3-peptide is a 21-residue peptide with three repeats of the seven-residue (heptad) sequence Leu-Glu-Thr-Leu-Ala-Lys-Ala, which forms an alpha-helical bundle structure through hydrophobic interaction between Leu residues. The 5Q-alpha3 and 7Q-alpha3 peptide is obtained by Ala → Gln substitution at the 5th and 7th position, respectively, of the alpha3-peptide amino acid sequence. The alpha3-peptide and its 5Q-alpha3 variant has the ability to form fibrous assemblies that are observed by transmission electron microscopy and atomic force microscopy, whereas the 7Q-alpha3 does not.
We investigate if the biased alpha3 polypeptide and its variants can be identified by charge transport measurements through current-voltage (Ix V) curves, as a pattern to characterize their fibrous assemblies. From their Ix V profiles, we found that the alpha3 peptide, which presents the most fibrous assemblies, shows the smaller current saturation, whereas the 5Q-alpha3 variant, which forms fibrous assemblies more attenuated than those of thealpha3 peptide, has a current saturation higher than alpha3,but smaller than 7Q-alpha3. Finally, the 7Q-alpha3 variant does not form fibrils and shows the highest current saturation, suggesting that charge transport in peptides can turn to be a useful tool for the development of biosensors to probe the onset of amyloidosis-like diseases. If the secondary structure of the peptides is considered, the number of charge transport channels should increase due to hydrogen bonding related to the secondary structure, further increasing saturation currents, but not specifically enough to change the order I(alpha3)of the charge transport in proteins and polypeptides should stimulate experimental and engineering technological developments.

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Acknowledgement / funding:

This work received financial support from the Brazilian Research Agencies CAPES (PROCAD and Rede NanoBioTec), CNPq (INCT-Nano(Bio)Simes and Casadinho) and FAPERN/CNPq (Pronex).

SESSION 1 – P2

Quantum Biochemistry Analysis of Statins Complexed with HMGR Enzyme

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Hundreds of millions of adults have high cholesterol, which has generated a billionaire market of drugs. Patents covering the leading statins have expired recently,pressuring the development of new drugs. Statins act by inhibiting the HMG-CoA reductase in the process of converting HMG-CoA to mevalonate, a committed stepin the biosynthesis of cholesterol. It is observed in clinical trials that this action decreases by 20 to 60% the low density protein (LDL) cholesterol levels, reducingcoronary events by up to one-third over a five years period.

Quantum chemistry methods are being used for simulation of molecular systems with up to hundreds or even thousands of atoms (in the last case, usingsupercomputers). A detailed understanding of the ligand pathway actions leading to its bonding to HMGR residues in the binding pocket at the quantum biochemistrylevel of description is important and depends on the evaluation of the contributions of each amino acid residue to the total binding energy, allowing for the design ofnew ligand derivatives.In this work, we take full advantage of the published crystallographic data of HMGR complexed with statins to perform computer simulations within an ab initioquantum mechanical approach, based on the DFT and in the framework of the molecular fractionation with conjugate caps (MFCC) strategy, to investigate thedetails of the binding interaction of the statins atorvastatin, cerivastatin, rosuvastatin, fluvastatin, mevastatin and simvastatin to the HMGR enzyme. The purpose is toelucidate why statins have differences in their efficiency to reduce cholesterol levels by obtaining and comparing the interaction energy between the HMGR residuesand ligand atoms. The binding pocket size radius (r), defined as the distance to the centroid of the ligand) used to estimate interaction energies was varied from 0.25to 1.2 nm, and a profile of the interaction energy was obtained for each HMGR-statin complex. The present work reinforces the role of computational simulations atthe quantum level as a valuable tool to understand and develop new drugs. The binding energy analysis of statins complexed with HMGR presented here has agood correlation with the thermodynamic studies, and the clinical trial data, previously released.

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Acknowledgement / funding:

This work received financial support from the Brazilian Research Agencies CAPES (PROCAD and Rede NanoBioTec), CNPq (INCT-Nano(Bio)Simes andCasadinho) and FAPERN/CNPq (Pronex).

SESSION 1 – P3

NMR protein-ligand interaction studies under non-homogeneous conditions for biomaterial generation: a model for artificial lectin-carbohydrate recognition

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Smart biomaterials for tissue regeneration need to incorporate molecules able to interact with specific cellular adhesion or morphogenic proteins of the extracellularmatrix (ECM). NMR binding studies allow obtaining structural information essential for the comprehension of biological processes and, nowadays, high-resolutionmagic-angle-spinning (HR-MAS) NMR spectroscopy is a well-established tool for the study of heterogeneous systems. Here we present the generation of amodel-system used to explore the possibility to reveal interactions between two molecular entities, one of which linked to a solid support, to mimic a bioactivespecies immobilized on a biomaterial surface. The carbohydrate recognition processes that take place in the ECM have a pivotal role in promoting cell adhesion anddifferentiation and, thus, tissue regeneration. We prepared a pseudo-receptor, that mimics lectin binding site, coupling a Tryptophan residue to a Sepharose resin,and we characterized its interaction with a panel of different monosaccharides. The results obtained support the theory according to which lectins bind carbohydratesexploiting the CH-π interactions occurring in their active site. Moreover the NMR exploited approach here described can be generally applied when the interactingspecies do not have the same solubility properties in physiological conditions and, in particular, can be exploited for the analysis and characterization of molecularrecognition events occurring at biomaterial surface.

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Acknowledgement / funding:
We gratefully acknowledge FONDAZIONE CARIPLO, project 2008/3175 for financial support

SESSION 1 – P4

Biocompatibility of functionalized porous Si-based nanoparticles

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The use of porous silica nanoparticles (PSN) as drug delivery vehicles offers new perspectives in cancer therapy. The size and shape of the particles are easily tunable. The high pore volume and surface area allow for a high drug load. Due to the flexibility of the platform and the vast possibilities for further functionalizations, PSN may offer targeted delivery and controlled release a, b, c. Further, surface tailoring allows detailed engineering to circumvent unwanted biological interactions, facilitate bioavailability and cellular uptake. The aim of this study was to define the biocompatibility of the PSN and to settle a suitable experimental protocol to functionalize PSN (size up to 450 nm). Because

PSN are particularly susceptible to air or water oxidation, to stabilize PSN we first performed a partial chemical oxidation of the nanoparticles with Piranha Solution(H2SO4/H2O2 2:1)d and tested their effect on human epidermoid cancer cells (H1355) and human peripheral blood lymphocytes (PBL).
We then settled a PSN functionalization procedure. After oxidation, the PSN surface was treated with the chemical linker APTES [(3-aminopropyl)triethoxysilane] in order to introduce a free functional ammino groupe. The functionalization of PSN before and after APTES treatment was examined using Fourier Transform Infrared Spectroscopy (FT-IR). The increase in intensity and slight change in the position of the peaks indicated that the PSN surface was functionalized and thus ready forthe subsequent covalent binding of fluorochromes, peptides or drugs. The results obtained for biocompatibility of PSN-treated cells, evaluated by MTT assay after 48and 72 h treatment showed substantially no cytotoxic effect on both PBL and H1355 cell lines, on the contrary a slight increase of vitality was observed already at 48h incubation. To evaluate instead the cellular uptake of the nanoparticles, APTES-functionalized PSN were reacted with TRITC (tetramethylrhodamineisothiocyanate) and after removal of TRITC excess incubated with H1355 for 24 h. Analysis by confocal laser scanner microscopy revealed the presence of a large amount of PSN inside the cells and a smaller amount also into nucleous. These preliminary data highlighted that PSN are suitable vectors for cellular delivery and might provide further insights for clinical applications in cancer therapies.

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Acknowledgement / funding:
Programma Operativo Nazionale Ricerca e Competitività 2007-2013 - PON01_02782

SESSION 1 – P5

New applications of Surface Plasmon Resonance (SPR) for the analysis of nanoparticles protein corona

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A great interest is currently devoted to the development of nanoparticles (NPs) for biomedical purposes, designed to improve pharmacokinetic profiles of their cargos(imaging probes or drugs) and to enhance specific targeting at the disease site.

Surface Plasmon Resonance (SPR), widely used for the analysis of biomolecular interactions, represents a technique of choice for rapid and quantitative analysis of interactions between functionalized NPs and their putative biological targets. We recently showed that SPR can be also useful to analyze the protein "corona", i.e. the protein layer which coats NPs once they come in contact with biological fluids, and which markedly affects NPs pharmacokinetic and pharmacodynamics properties. Formation of protein corona must be carefully considered when developing new NPs. Proteomic assays, although very informative, are not suitable for arapid screen of different NPs; we thus developed a new SPR-based approach to investigate the adsorption of relevant proteins onto NPs in a short time and with a relatively high-throughput. For this, we incubate NPs in the biological fluid of interest, isolate NPs and flow them onto chip surfaces immobilizing the antibodies against the selected protein. For the first proof-of-principle studies we looked at NPs adsorption of human serum albumin, because of its effects on NPs circulation time, and apolipoprotein E, because of its possible involvement in blood-brain barrier passage of NPs. Our preliminary data suggest the feasibility of the SPR approach to detect the adsorption of the selected proteins on NPs and to estimate the strength of this adsorption. Moreover, analysis carried out with NPs preincubated in biological fluids for different times allow to study the "labile corona", formed at initial time points and characterized by a fast exchange rate with free proteins, and the "hard corona", due to a more stable layer of proteins. We envisage that the analysis for other adsorbed proteins might allow to predict NPs behaviors such as cell uptake or interaction with the immune system. Moreover, SPR may allow systematic screenings of the behavior of different NPs (in size, charge and composition), after incubation for different periods of time indifferent biological fluids.
This novel application of SPR sensors may be very convenient for a rapid and informative analysis of protein corona, and potentially very useful for the characterization, screening and development of biomedical NPs.

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SESSION 1 – P6

Functionalization of liposomes with Epigallocathechin gallate and their effect on aggregation of the Aβ1-42 peptide

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Alzheimer's disease (AD) is characterized by the abundance of intra neuronal fibrillary tangles and the extracellular deposit of the amyloid β-peptide (Aβ) as amyloid plaques. An effective therapeutic approach

might be to interfere with the Aβ aggregation using potent anti-amyloidogenic and fibril-destabilizing molecules. A major polyphenolic component in green tea, Epigallocatechin gallate (EGCG) shows neuroprotective activities, antioxidative and iron chelating action, preventing significantly the Aβ fibrillogenesis. Since EGCG is poorly soluble in lipophilic media, its delivery across the biological membrane is limited. Moreover, in the case of its application as interfering agent of the Aβ aggregation kinetics, EGCG delivery to the brain represents a complex problem, due to the presence of the blood-brain barrier, that prevents unwanted substances to enter the brain.
Engineered nanomaterials, objects with dimensions of 1-100 nm, are providing interesting biomedical tools potentially able to solve these problems, thanks to their physico-chemical features and to the possibility of multi-functionalization, allowing to confer them different features at the same time.
In this context, we prepared and characterized liposomes (LIP) surface decorated with a chemically-modified EGCG and their effect on the Aβ42 aggregation has
been investigated. Ester derivatives of EGCG with palmitoyl (EGCG-C16) have been synthesized by a conventional chemical method. Palmitoyl chloride (1.5 EQ)was dropped in ethyl acetate including EGCG (1 EQ) and triethylamine (1.3 EQ). Reaction mixture was stirred for 24h at 0°C. After removed the solvent, the residuewas purified by silica gel chromatography to give the EGCG-C16. LIP constituted by a matrix of sphingomyelin and cholesterol (1:1, M/M) added with EGCG-C16(5.4 μM, 21.8 μM or 109 μM) were prepared by freeze-thawing procedure (diameter 80%). The results showed that the properties of EGCG was maintained also after incorporation in LIP. Moreover, after incorporation into LIP the palmitoyl ester derivatives of EGCG displayed lower cell toxicity respect to non-incorporated one, as assessed by MTT on endothelial cell line. The functionalization of EGCG-LIP with molecules able to enhance their cellular uptake and crossing the blood-brain barrier in vitro will be the next step of this work.

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Acknowledgement / funding:

This investigation was carried out within the project Regione Lombardia, Fondo per la promozione di accordi istituzionali, Progetto no. 4779 'Network Enabled Drug Design(NEDD)'

SESSION 1 – P7

Solid Lipid Nanoparticles: a strategy to overcome the blood-brain barrier

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Diagnosis and therapy of brain diseases are often compromised by the difficulty to cross the blood brain barrier (BBB). Recently, the emerging field of nanotechnology has generated new promises to solve this problem. Nanoparticles (NPs) have several advantages in terms of biocompatibility, non-immunogenicity, non-toxicity and they can be functionalized to carry imaging agents and/or drugs, and to enhance the

blood circulation residence time. Finally, the NPs surface can be modified with specific ligands in order to achieve site-specific delivery and successful penetration of the BBB. The objective of present investigation was to study the effect of surface characteristics of solid lipid nanoparticles (SLN) covalently coupled with the monomer of ApoE-residues (141-150) on cellular uptake in brain capillary endothelial cells. Radiolabelled and fluorescent (fluoroprobe strictly associated to SLN) have been used to evaluate the transcellular transport in in vitro BBB model based on human cerebral microvascular endothelial cells (hCMEC/D3). SLN made of tripalmitin, loaded with different fluorescent dyes (Bodipy, Tritc and Texas Red) and functionalized with phosphatidic acid (Aβ ligands) and DSPE-PEG(2000)-Maleimide have been investigated. SLN uptake was monitored byconfocal-laser-scanning microscopy and quantified by radiochemical techniques. The peptide mediated an efficient cellular uptake of SLN. SLN without surface-located peptide displayed less membrane accumulation and cellular uptake. In order to assess the ability of ApoE-SLN to enhance their transcellular transport, we studied the permeability through an in vitro BBB model. With respect to the un-functionalized SLN, the ApoE-SLN significantly enhanced their cellular uptake and permeability through the cell monolayer (PE = 0.6 • 10-5 cm/min vs PE = 6.95 • 10-5 cm/min, respectively; Student's t-test, p value

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Acknowledgement / funding:

The research leading to these results received funding from the European Community's Seventh Framework Program (FP7/2007-2013) under grant agreement n° 212043 (NAD). We thank Pierre-Olivier Couraud for providing the hCMEC/D3 cells.

SESSION 1 - P8

Biosynthesis of copper nanoparticles using leave extract of Syzygium cumini and assessment of its antibacterial activity against Pseudomonas aeruginosa

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In the present study the biosynthesis of copper nanoparticles (bsCuNPs) using leave extract of Syzygium cumini have been reported. The synthesized bsCuNPs was examined for the antibacterial potential against Pseudomonas aeruginosa and their plausible mechanism. The structural, optical and thermal properties of theas synthesized bsCuNPs were investigated in details. It was noteworthy that bsCuNPs showed the promising antibacterial activity in a dose- dependent manner. Results also showed that the protection of histidine (a ROS quencher) against ROS clearly suggested the implication of ROS in antibacterial activity of bsCuNPs. Itis encouraging to conclude that bsCuNPs abides the potential of its applications in biomedicine for management of diseases caused by multi drug resistant (MDR)strains of P. aeruginosa. This study shows the possibility of using indigenous microbial bioresource for the environmental friendlily and economic biosynthesis of medically important bsCuNPs.

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Acknowledgement / funding:
Financial support for this work through the Centre of Excellence in Materials Science (Nanomaterials), Department of Applied Physics, Z.H. College of Engg. & Tech., Aligarh Muslim University, Aligarh-202002, Uttar Pradesh, India is greatly acknowledged. We also thank to technical support of Mr. Khateeb Ahmad, Lab Assistant.

SESSION 1 – P9

Synthesis and chemical-physical characterization of PEGylated gold nanoparticles for biological applications

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Gold nanoparticles show a huge potential in several biomedical applications, such as therapy, diagnostive imaging, treatment, and prevention of diseases [1]. In particular, their surface modification plays an important role to modulate particle size and shape, which are fundamental parameters for the optimization of the system solubility, long-term stability, biocompatibility and attachment of selective and biologically active functional groups [2]. Here, we describe the synthesis and the PEGylation process of spherical gold nanoparticles as well as their preliminary conjugation with proper antibodies, for use as

possible tool in the cancer diagnosis and prognosis. The pristine and the functionalized GNPs were characterized in terms of morphology and structure. In particular, Dynamic Light Scattering (DLS), Transmission Electron Microscopy (TEM) (Figure 1) and X Rays Diffractometry (XRD) were adopted in order to evaluate the particles shape, size, size Distribution, and crystalphase. Stable and monodisperse spherical gold nanoparticles were easily obtained with a diameter of 20 nm by means of Turkevich approach [3].
Thermo-gravimetric analyses were used to estimate the degree of the polymer functionalization, which occurs with short reaction times. In the case of derivatised systems, the particle diameters increase of 2-6 nm. Citotoxicity tests on SKBR3 human breast cancer cell line were also performed to evaluate the biocompatibility of the derivatised GNPs.

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Acknowledgement / funding:
We would like to acknowledge financial support from MIUR, PRIN 2010-11 project entitled “Nanomed” (prot. 2010FPTBSH_009).

SESSION 1 – P10

TARGETING IN VITRO OF CANCER ANTIGEN 125 WITH ANTIBODY-COATED GOLD NANORODS

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Objectives: Epithelial ovarian cancer (EOC) is the most lethal gynaecological malignancy and is counted among the most common causes of cancer-related deaths in women. Cancer Antigen 125 (CA-125) is the most widely used biomarker for confirmation of diagnosis and management of ovarian cancer. It is a large molecular weight transmembrane glycoprotein that is over expressed in many carcinomas including EOC [1-2]. Gold nanorods (GNRs) provide an attractive nanomaterial platform for biomedical research. They exhibit two distinct surface plasmon resonance (SPR) bands, a weak transverse SPR band at ~ 520 nm and an intense longitudinal SPR band which can be tuned from visible to near infrared (650 - 900 nm) regions by increasing their aspect ratios. The longitudinal band is the basis for their in vivo applications, as NIR light only have minimal absorption by skin and tissue, leading to minimal tissue invasion and deeper (up to 10 cm) tissue penetration [3-5]. In this study, we report the preparation and evaluation of GNRs functionalized with antibodies against CA-125 for the targeting of cells over expressing CA-125.Experimental Section: The design of the probe consists of pegylation of GNRs by a heterobifunctional polyethylene glycol (PEG) in order to replace the toxic surfactant, CTAB (hexadecyltrimethyl-ammonium chloride), and ensure the colloidal stability. The resulting pegylated gold nanorods were further conjugated with amonoclonal antibody against CA-125, via carbodiimide (EDC) coupling agent, to provide localized targeting functionality. In vitro uptake of antibody-coated GNRswas evaluated in HeLa cells via silver staining, spectrophotometric and dark field microscopy studies. Toxicity of these particles was also calculated via MTT assay.Exposition of antibody-coated GNRs to plasma samples (healthy volunteers) and

then selective targeting on cells was carried out to understand if the particles keep their targeting capabilities after plasma exposition.
Results: Selective targeting and accumulation of antibody coated-GNRs was observed in vitro and after plasma exposition. Furthermore, MTT data indicate that antibody-coated GNRs did not induce any significant variation of cell viability and, thus , they are suitable for biological applications.
Conclusions: The developed antibody-coated GNRs are nontoxic and an excellent candidate for in vivo targeted delivery of drugs, non-invasive imaging based on localized hyperthermia and photo-thermal therapies.

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Acknowledgement / funding:
This work has been partially supported by the Projects of the Health Board of the Tuscan Region "NANOTREAT"

SESSION 1 – P11

One step synthesis of PEGylated gold nanoparticles for biomedical applications

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Colloidal gold nanoparticles are expected to find various biomedical applications owing to their special properties such as: amenability of synthesis and functionalization, extensive thermal stability, less toxicity and ease of detection [1]. In this regard the development of new biosynthesis methods of colloidal gold nanoparticles is essential. Since poly(ethylene glycol) (PEG) is one of the most versatile biopolymer, environmentally benign and already used in the pharmaceutical and biomedical industries, much of the research interest has been focused on developing new methods of PEGylation [2]. We report a novel, easy and green preparation method yielding stable and biocompatible gold nanoparticles by exploiting PEG with the chain length ranging from 200 to 20000 ethylene glycol units. Surprisingly, Au3+ can be smoothly reduced to gold nanoparticles in a few minutes by employing PEG, thus being able to act as both reducing agent and stabilized of colloidal gold nanoparticles. The presence of NaOH in the preparation method represents a key element in the successful synthesis of colloidal gold nanoparticles since the OH groups generated in the solution enhance the speed of chemical reduction of gold ions, as in the case of PEGylated silver nanoparticles[3]. The as-obtained PEGylated gold colloids exhibit a narrow absorption peak around

523 nm (± 10 nm) with a full width at half maximum of about 52 (± 5), indicating the successful synthesis of spherical PEG-coated gold nanoparticles with a narrow size distribution, which is further proved by transmission electron spectroscopy images. Zeta potential measurements show a decrease in the surface charge of the PEGylated gold nanoparticles by increasing the PEG chain length, not affecting their stability, thus inducing a passage from Coulombian repulsion to a steric one. An enhancement of the vibrational signal of PEG molecules enveloping the gold nanoparticles is detected by employing a 532 nm laser line, demonstrating the promising potential of this type of nanoparticles as effective Raman tags.

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Acknowledgement / funding:
This research was supported by CNCSIS-UEFISCDU, project number PN-II-ID-PCE-2011-3-0954.

SESSION 1 – P12

Nanoparticles mediated chiral interaction between cyclodextrins and pharmacetical compounds

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We report a new, very simple, fast and accurate method of chiral recognition of propranolol enantiomers based on selective attachement of propranolol-β-cyclodextrin complexes onto colloidal silver nanoparticles surface. By taking advantage of the unique plasmonic properties of silver nanoparticles, the chiral separation of propranolol enantiomers was successfully studied and proved by Surface-Enhanced Raman Spectroscopy (SERS). The quantum chemistry calculations of native cyclodextrin - propranolol enantiomers complexes have been used as a further proof of the proposed interaction mechanism. It has been shown (experimentally and theoretically) that β-cyclodextrin (compared with the other two classes of native cyclodextrins α and γ) had the best chiral recognition ability for propranolol enantiomers, hence producing the largest difference in the SERS spectra of propranolol enantiomers - native cyclodextrin complexes [1]. The crucial role of this new chiral separation method is played by the colloidal silver nanoparticles. More precisely, the chiral recognition mechanism is based on the formation of different classes of inclusion complexes of propranolol and cyclodextrin and their selective attachment onto the silver nanoparticles. The plasmonic properties of the nanoparticles allowed the acquisition of specific SERS signals for the two propranolol enetiomers. It has been shown that, in the specific case of Rand S propranolol enantiomers, the naphthalene ring of R-propranolol fits better into the β-cyclodextrin cavity. For the other two classes of native cyclodextrinsγ-cyclodextrin gives only a partial enantiomeric separation whereas α -cyclodextrin shows no enantio-selectivity. Computational chemistry based on DFT served as a tool for elucidating the underlying mechanism of molecular interactions responsible for chiral discrimination by giving important clues related to the evolution of the Raman peaks. The influence of several factors (nature and concentration of chiral auxiliary, selector/selectand, ratio, pH, interaction time, etc.) over the obtained SERS spectra was also successfully assessed [2].

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Acknowledgement / funding:
This work was supported by CNCSIS-UEFISCDI project number PNII-RU TE 259/2010

SESSION 1 – P13

Haemocompatibility Assessment of Biomedical Membranes

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Biomedical membranes are widely used in medical devices that save lives. This work involves using a rheometrical technique as a measure of surface haemocompatibility of different membranes namely Polyethersulfone (PES), Polypropylene (PP) and Polypropylene oxide (PPO) A&B. The hemocompatibility result based on rheometrical techniques was performed on 300 microns gap shows that blood clots formed on PP surface has a the highest fractal dimension Df = 1.96 ie. more compacted clot formed compared with other types of membranes, where as the clotting times were comparable.
Keywords: rheology, biocompatibility, blood coagulation, membranes.

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Acknowledgement / funding:
This work was supported by Haemair® (UK) and Almergheb University (Libya).

SESSION 1 – P14

Zinc oxide nanoparticles as Adjuvant Therapy for streptozotocin-induced Types- 2 diabetic rats treated with Glimepiride

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The objective of this study was to evaluate the role of Zinc oxide nanoparticles and/or Glimepiride (a second-generation sulfonylurea drug) in treating Type-2 diabeticrats, non-insulin dependent diabetes mellitus (NIDDM). In the present study, zinc oxide nanoparticles were evaluated for antidiabetic effects

and safety. Materials &methods: Zinc oxide nanoparticles (1, 5 and 10 mg/kg) were tested with or without Glimepiride for antidiabetic activity in streptozotocin-induced Types-2 diabeticrats. Cytotoxicity, hemolysis, acute and subacute toxicity tests, and mechanism-of-action studies were performed of zinc oxide nanoparticles. Results: Oraladministration of zinc oxide nanoparticles alone resulted in significant antidiabetic effects - that is, improved glucose tolerance, higher serum insulin (59%), reducedblood glucose (35%), reduced nonesterified fatty acids (38%) and reduced triglycerides (39%) while addition of Glimepiride significantly improve these parameterswhen compared with zinc oxide nanoparticles alone. Zinc oxide nanoparticles were systemically absorbed resulting in elevated zinc levels in the liver, adiposetissue and pancreas. Increased insulin secretion and superoxide dismutase activity were also seen in rat insulinoma cells. Conclusion: Zinc oxide nanoparticles are apromising antidiabetic agent along with standard antidiabetic agents.

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Acknowledgement / funding:
We are grateful to Professor Mahmoud Ahmed Omara (Department of chemistry, faculty of science, Kafer Elsheik, Egypt) for his help in obtaining the Zinc oxide nanoparticles free of charge.

SESSION 1 – P15

What NMR spectroscopy can say you about your nanoparticles and liposomes

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NMR spectroscopy is not yet extensively exploited in order to perform nanoparticle and liposome characterization. Nevertheless, the combination of high resolutionliquid state with HR-MAS (High Resolution Magic Angle Spinning) NMR techniques can provide structural information, at atomic level, about their composition, bothfrom a qualitative and a quantitative point of view, their surface decoration with specific molecular entities, their interaction with bio-molecular targets of interest.

Some examples of these applications will be reported in this communication.

Acknowledgement / funding:
The research leading to these results has received funding from the European Community's Seventh Framework Programme (FP7/2007-2013) under grantagreement no.212043 (NAD) and from Regione Lombardia, Fondo per la promozione di accordi istituzionali, Progetto no. 4779 'Network Enabled Drug Design(NEDD)'.

SESSION 1 – P16

Gold nanoparticles decorated with glycomimetics as molecular tools and diagnostic-therapeutic agents

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The sodium-glucose co-trasporter SGLT-1 and the sodium glutamine co-transporter have been described being involved in the protection-repair action of the intestinal epithelium, and thus may represent ideal targets for the development of a therapeutic approach aimed at the prevention and/or treatment of gastrointestinalmucositis induced by chemotherapy. We recently identified a glucose derivative named BLF501, exerting anti-inflammatory activity at a molar concentration fiveorders of magnitude lower than glucose (1). In parallel studies SGLT-1 has been described to be involved in anti-inflammatory and anti-apoptotic signaling, in therepair of plasma membrane integrity and tight junction (TJ) integrity (2). These findings led us to investigate glucose and ligand BLF501 protective efficacy in an invitro model of chemotherapy-induced injury of enterocytes. Indeed, glucose showed a clear protective activity in this model, and this effect was accompanied byinhibition of doxorubicin (DXR)-induced release of reactive oxygen species (ROS). Results obtained in preliminary in vitro experiments with SGLT-1 activators leadus to assume that the SGLT-1 receptor activation is indispensable for protecting the small intestine from mucosal damage induced by DXR. In animal gastrointestinalmucositis (GIM) models treatment with oral glutamine prevents mucosal injury and improves intestinal recovery following chemotherapic injury. These findingsdescribe an hypothetical mechanism by which GLN prevents intestinal epithelial damage during chemotherapy (3).With these preliminary data in hand, and with the above mention rational, our research topic within this project consists in the study and evaluation of possiblemultivalent and synergic effects operated by glucose-like ligands (BLF501) in combination with glutamine and/or glutamine-like compounds in the preservationand/or recovery of the intestinal epithelium damaged by the administration of chemotherapeutic agents. To this aim we designed and synthesized gold nanoparticles(4) decorated with BLF501, glutamine and combination of both.

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Acknowledgement / funding:
We greatly acknowledge COST action CM1102 for supporting the STSM of Mr. Giuseppe D'Orazio at the CIC biomaGUNE research centre.

SESSION 1 – P17

Polymeric nanoparticles for brain delivery: an in vivo study

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New promising approach for drug systemic delivery to the central nervous system is the use of nanosized carriers. Considering that optimization and further validation of these systems is needed, the design of novel fluorescent polymeric nanoparticles to target brain tissues and follow their in vivo distribution is proposedin this study. As far as polymeric nanoparticles are concerned, two innovative fluorescent nanospheres were designed: ethylcyanoacrylate-made nanospherescoated with polysorbate 80 and human serum albumin-made nanospheres. They were prepared by emulsion polymerization method and by coacervation methodand chemical cross-linking with glutaraldehyde, respectively. Nanospheres were characterized in terms of dimensional analysis, polydispersity and Zeta potential,morphology, encapsulation efficacy and loading capacity. Ethylcyanoacrylate- and albumin-made nanospheres were produced with good yields (65% and 80%respectively). Both nanospheres were suitable for the intraperitoneal administration (mean diameter ≤300 nm; PDI 0.2), had a sphere-like shape and a goodencapsulation efficacy (≈98%). Intracerebrally injected ethylcyanoacrylate- and albumin-made nanospheres in the nucleus basalis magnocellularis of anesthetizedrats didn't induce glial mediated inflammatory response. Differently from albumin-made nanospheres that remained in loco 24 hours and one week after theintracerebral administration, ethylcyanoacrylate-made nanospheres mobilized from the injection site and distributed unilaterally in the injected hemibrain. Preliminaryexperiments demonstrated that, one week after injection, ethylcyanoacrylate-made fluorescent nanospheres were detected within microglial and neuronal cells andin blood vessels.
Systemically administered ethylcyanoacrylate- and albumin-made nanospheres to C57BL/6 mice were able to cross the blood brain barrier and a subchronic treatment of two weeks with both preparations had no side effects nor induce locomotor or cognitive impairment as compared to vehicle treated mice.
In conclusion our in vivo study demonstrated the ability to overcome the blood brain barrier of these nanovectors, that may provide innovative drug delivery systemsfor Alzheimer's disease treatment and therapy.

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Acknowledgement / funding:

Supported by University of Florence.

SESSION 1 – P18

How dendrimers can interphere with copper trafficking

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Dendrimers are macromolecules with controlled size and functionalization.
These macromolecules, with sizes in the range of 1-10 nm depending on the decoration, are characterized by unique properties. One of the most important properties is the ability to solubilize highly stable peptide aggregates, like the amyloid fibrils, that can not even be attacked by proteases and are hallmarks of neurodegeneration.
The amyloid fibrils contain a large amount of Zn, Fe and Cu. The Cu content is correlated with the high content of Cu in synaptic region, especially when neurodegeneration occurs, and Cu-amyloid peptide interactions are the object of in vitro and in vivo studies [1].
Recently [2], the interactions between copper (Cu) and poly-propylene imine (PPI) dendrimers have been investigated in detail, showing the ability of PPI-G4 to loadup to 18 Cu ions per dendrimer at high pH (~10) and at relatively low Cu concentration (0.01 M). This new property may be useful to sequestrate free and toxic Cuions that are released by neurons because of pathological unfolding of metallo-proteins in degenerating cells. Also, supramolecular interactions in dendrimers,allowing the formation of dendrimeric fibrils, are strongly affected by transition metal ions and by their counterions [3].
In this contribution, a first attempt to describe the interactions between copper and PPI dendrimers is described. Simple mechanical models are designed, in order toprovide reasonable coordination sites for Cu in PPI-G4. Calculations based on density functional theory are then performed to understand the balance betweenmechanical tensions in the dendrimer and the coordination chemistry of Cu [4].

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Acknowledgement / funding:
The project PolyDen (SUPSI-CH) is acknowledged.

SESSION 1 – P19

Nanostructed Hydroxyapatite for regenerative medicine

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The promising trends in biotechnology and tissue engineering are based on development of advanced materials with biomimetic features created by designing andtailoring of specific surface properties such as the enhancement of the surface affinity to selective adhesion and proliferation of different cell strains, improvement ofbiological response and tissue compatibility.[1] It has been widely described the ability of bioceramics such as hydroxyapatite (HA) to form a bonding with thesurrounding bone tissue.[2] Since inorganic materials such as hydroxyapatite possess a paucity of reactive functional groups, biomolecular modification of thesematerials is still challenging.An efficient method for the direct and covalent decoration of granules of nanostructured apatite with a sample monosaccharide is presented (Scheme); thehydroxyapatite material was directly functionalised with a short azido-containing spacer arm, to which α-propargyl glucopyranoside has been chemoselectivelyligated by Huisgen-type cycloaddition. The "glycosylated" hydroxypatite was characterised by its ability to interact with glucose recognising lectins.

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Acknowledgement / funding:

We gratefully acknowledge MIUR, under project FIRB RBPO68JL9 and FONDAZIONE CARIPLO, project 2008/3175 for financial support.

SESSION 2 – P20

Multi-walled Carbon Nanotubes as Drug Delivery Carriers for Mitoxantrone, an Antineoplastic Drug

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Drug delivery system is considered a valuable strategy of administering a pharmaceutical compound to achieve a therapeutic effect. Carbon nanotubes (CNTs) have been introduced recently as a novel carrier system for delivery of anticancer agents. In our work will discuss the efficacy of Mitoxantrone (an antineoplastic drug,MTX [1]) absorbed on multiwalled-CNT (MWCNTs) on human breast cancer cell line, MDA 231. MWCNTs were were purified and oxidized through a well known method [2]. MTX was adsorbed on oxidized MWCNTs by electrostatic interaction with CNT carboxylic group and MTX amine groups. The adduct has been characterized through Raman Spectroscopy, Thermo Gravimetric Analysis (TGA), and TEM. Trypan blue dye exclusion assay was used to investigate the cytotoxic effect of MTX adsorbed on CNTs. The results showed that MTX loaded on carbon nanotubes (MWCNTs-MTX) produced a reduction in MDA 231 cell viability slightlylower if compared to MTX in solution. Cell viability in presence of MTX loaded on carbon nanotubes was both dose- and time-dependent. These preliminary results showed that MWCNTs-MTX are much effective as the free drug in killing tumor cell line; its physico-chemical and pharmacokinetics properties may support its use as an in-situ neo-adjuvant and/or adjuvant cytotoxic device. Actually, we are studying the combinatory effect of MWCNTs-MTX and some antineoplastic gallium salts, with the purpose of obtain a reduction of therapeutic MTX dose, maintaining drug's efficacy.

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Acknowledgement / funding:

The authors would like to acknowledge financial support from "Project SAL-45" financed by the Regione Lombardia (2010) and by a project financed by theFondazione Alma Mater Ticinensis (2010).

SESSION 2 – P21

Amperometric detection of nitrite by using graphene/poly(methylene blue) nanocomposite electrodes

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It has been determined that there is a strong relationship between death rate from Alzheimer's, Parkinson's, diabetes and the progressive increases in humanexposure to nitrates, nitrites and nitrosamines through processed and preserved foods as well as fertilizers [1]. Nitrite (NO2-) commonly exists in natural environmentand is widely used as additive and corrosion inhibitor in food system. However, it is found that nitrite can react with secondary amine to produce nitrosamine, whichis a strong

carcinogen to human bodies. Nitrosamines are easily generated from nitrites under strong acid conditions, such as in the stomach. Nitrosamines becomehighly reactive at the cellular level, which then alters gene expression and causes DNA damage. Therefore, quantitative detection and determination of nitrite is veryimportant in analytical chemistry. Different methods have been developed to determine nitrite, such as electrochemistry, spectrophotometry, capillaryelectrophoresis, and chemiluminescence methods. Among these methods, electrochemistry is more favorable owing to its high sensitivity, relatively good selectivity,fast response, and low cost.Graphene was initially isolated by Novoselov and Geim with mechanical exfoliation of highly oriented pyrolytic graphite [2]. Graphene demonstrates interestingproperties, including high mobility of charge carriers, unique transport performance, high mechanical strength, and extremely high thermal conductivity [3]. Polymericthin films of methylene blue (MB) introduces highly stable, electroactive, and efficient redox centers and these important surface properties allow largeelectrocatalysis applications [4].Since nitrite oxidation at bare electrodes involve a relatively high overvoltage, the usefulness of these electrodes for nitrite detection is very limited [5]. In the presentstudy, we constructed a novel nitrite biosensor based on the oxidation of nitrite at relatively low potentials by using graphene/poly(MB) nanocomposite electrodes bycombining the unique electronic properties of graphene with the above-mentioned excellent properties of poly(MB). It has been determined that this modifiedelectrode improves the detected concentration of nitrite in acidic solutions and exhibits a good electrocatalytic response to the oxidation of nitrite. Under optimalconditions, modified electrode showed low detection limit, wide linear range, good reproducibility, and stability.

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Acknowledgement / funding:
This work has been supported by Ataturk University (Project: BAP 2011/377)

SESSION 2 – P22

Biocompatibility of tungsten-disulfide-inorganic-nanotubes and fullerene-like-nanoparticles on salivary gland cells

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Multiwall WS2 nanotubes (INT-WS2) and fullerene like nanoparticles (IF-WS2) have many potential medical applications. A venue not yet explored is the exploitation of such nanoparticles on the salivary glands, yet the first step toward such applications is to prove their biocompatibility. Our aim was to test the biocompatibility of INT/IF-WS2 on the A5 and RSC salivary gland cells.
The cells were cultured and subjected after one day to different concentrations of INT-WS2 (0.22, 3.52, and 35.2 μg/ml for the A5 cells) orIF-WS2 (35.2 and 100 μg/ml for the RSC cells), and were compared to control groups. The growth curves, trypan blue exclusion viability test, and carboxy-fluorescein succinimidyl ester (CFSE) proliferation assay were obtained. Furthermore, cells' morphology and interaction with the nanoparticles were observed by light microscopy, scanning electron microscopy (SEM), and energy dispersive x-ray spectroscopy (EDS).

The results showed no significant differences in growth curves, proliferation kinetics, and viability between the groups compared. Moreover, no alterations inthe cells morphology were observed. However, interestingly, the nanoparticles demonstrated the capability to penetrate the cells.
The kinetics, viability and morphology of both types of salivary gland cells were not affected by the nanoparticles, yet further investigation should be done regarding the nanoparticles cell-penetration phenomena observed.

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Acknowledgement / funding:

The electron microscopy studies were conducted at the Irving and Cherna Moskowitz Center for Nano and Bio-Nano Imaging at the Weizmann Institute of Science.

SESSION 2 – P23

IRON CHELATORS AND PHYTOCHEMICAL ACTIVATION OF Nrf2 PROTECT AGAINST CHRONIC IRON OXIDE NANOPARTICLES OVERLOAD INDUCED CARDIOTOXICITY

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Increased production of reactive oxygen species has been implicated in the pathogenesis of iron oxide nanoparticles overload induced cardiotoxicity, and enhancedendogenous antioxidants have been proposed as a mechanism for regulating redox balance. Nuclear factor (erythroid-derived 2)-like 2 (Nrf2) is a transcriptionalregulator of phase II antioxidant enzymes, and activation of Nrf2 has been suggested to be an important step in attenuating oxidative stress associated with ironoxide nanoparticles overload induced cardiotoxicity. A well-defined green tea has been shown to activate Nrf2 and induce phase II enzymes through the antioxidantresponse element. The purpose of these experiments was to determine if treatment of cardiomyocytes with iron chelators (deferiprone, deferoxamine) alone or incombination with phytochemical activation of nrf2 (green tea) induces phase II detoxification enzymes can protect cardiomyocytes from iron oxide nanoparticlesoverload induced cardiotoxicity. Seventy five albino rats are divided into six groups: two control groups (noniron-loaded and iron-loaded) and four iron-loaded groupsclassified as follows: deferiprone group, deferiprone combined with green tea group, deferoxamine group and deferiprone combined with green tea Heart tissue andblood samples were taken for histopathological examination of the heart, determination of total iron-binding capacity, 8-OH-deoxyguanosine (8-OH-dG), myocardiallipid peroxidation and glutathione (GSH) content. Less histopathological cardiac changes and a significant decrease in all biochemical parameters, exceptmyocardial GSH, were observed in the deferiprone group. The addition of green tea improves the biochemical and histopathological changes in comparison to thoserats administered deferoxamine or deferiprone individually.

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Acknowledgement / funding:
Self Funding.

SESSION 2 – P24

The possible Quantum dots nanotoxicity on thyroid hormones and pancreatic function

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Quantum dots (QD) are tiny particles, or "nanoparticles", of a semiconductor material, traditionally chalcogenides (selenides or sulfides) of metals like cadmium orzinc (CdSe or ZnS, for example), which range from 2 to 10 nanometers in diameter (about the width of 50 atoms). Although there is clear evidence that certain CdTequantum dots may act as endocrine disrupters, the authors suggest that further studies are required to come to conclusive findings There is lack of literatureregarding toxicity of QD whether acute or chronic. Studies specifically designed for toxicologic assessment (e.g., dose, duration, frequency of exposure, mechanismsof action) are few. Many of the studies from which QD toxicity information is derived and that have been cited in reference to QD toxicity were performed bynanotechnology researchers rather than toxicologists or health scientists. Potential routes of QD exposure are environmental, workplace, and therapeutic ordiagnostic administration. Workplace exposures (e.g., engineers, researchers, clinicians) may result from inhalation, dermal contact, or ingestion. The aim of thisstudy was to investigate the nanotoxicity assessment of quantum dots on thyroid hormones and pancreatic function. Here, an initial systematic animal toxicity studyof CdSe-ZnS core-shell quantum dots (QD) in healthy albino rats is presented. A pilot study will be conducted to determine LD 50 of fluorescence 540nm, 6nmdiameter, 1mg/ml in toluene QD (sigma Aldrich). One tenth of the determined LD50 dose will be used to induce chronic toxicity to the studied animals. Animals willbe divided into 2 groups: 1. Control group (10 male rats) receiving normal saline orally by gastric tube for 21 week. 2. QD group (10 male rats) receiving thedetermined dose of QD from our pilot study for the same period as the control i.e. 21 weeks. At the end of the period animals will be sacrificed and blood sampleswill be collected for measuring thyroid stimulating hormone (TSH), total thyroxine(total T4), free thyroxine (free T4) and Total triiodothyronine (Total T3) and Freetriiodothyronine (Free T3) as well as insulin levels, glucose level and Hemoglobin A1C from both groups. In addition, oxidative and antioxidant parameters(malondialdehyde [MDA], reduced glutathione [GSH], catalase, and superoxide dismutase [SOD]) were determined. Thyroid and pancreatic samples will be collectedfor microscopic examination. The study is undergoing and waiting for the result.

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Acknowledgement / funding:
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SESSION 2 – P25

Study of the effects of nanoliposomes engineered for the treatment of Alzheimer's disease on the electrical activity of cortical neurons.

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Alzheimer's disease (AD) is one of the most known and worldwide common neurodegenerative disorder. A possible therapeutic approach in order to treat AD patients could be the development of drugs able to target Aβ and manage to limit or even inhibit Aβ accumulation. During the years, different therapeutic strategies have been studied but they offer only modest and short-term benefits. Nano-carries appears to be very promising tool both in diagnostic and therapeutic approaches since they can be functionalized in order to have the ability to cross the blood-brain barrier and to bind Aβ. As a consequence, nanoparticles should improve both qualitatively and quantitatively the transport of drugs directed to the central nervous system, therefore limiting side effects.Once nanoparticles cross the blood-brain barrier, they reach an environment rich of neurons.
In this study we evaluated the effects of nanosized liposomal drug carriers, specifically functionalized (NL) to interact with endothelial BBB cells on primary culture of cortical neurons isolated from neonatal rats.
Biocompatibility studies (LDH and MTT assay) were performed after 48 hours (h) of cell-NL incubation revealing that, at the concentration tested (10 μM), the release of LDH and the mitochiondrial distress were not relevant (0.14±1% and 3.99±0.5%, respectively). Patch clamp experiments were conducted after 4 or 48 h of NL incubations. Data showed that the resting membrane potential (Vm) was significantly depolarized only only after 4h of NL incubation (Vm=-48.2±2.8 in control, Vm=43.3±3.9, $p<0.05$ and Vm=-49.6±5.5 after 4 or 48 h of treatment, respectively, n=10). The currentthresholdrequired to activate thefiringofaction potentials significantly decreased when neurons were incubated with NL, being 41.1±3.3 pA in control and 34±3.1pA after 4 h and reverting to 44.5±8.7 after 48 h of incubation.The frequency of firing in control cells was of 9.89 Hz, after 4 h of incubation it increased by about 80% (17.78, $p<0.001$) and reverted to 10 Hz after 48 h of incubation.
In conclusion we demonstrated that biocompatible nanoliposomes specifically functionalized for AD treatment interacted significantly with cortical neuronsand influenced the electrical activity of the cells.

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Acknowledgement / funding:
The research leading to these results has received funding from the European Community's Seventh Framework Program (FP7/2007-2013) under agreement No. 212043.

SESSION 2 – P26

Influence of iron oxide nanoparticles PEGylation on endothelial cells and macrophages in vitro

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Nanotechnology develops nanoparticles (NPs) for treatments of human diseases. The understanding of potential toxicity of NPs is needed before considering clinicalapplications1.Magnetic iron oxide nanoparticles (IONPs) are a promising tool for drug delivery and diagnostics agents2.
Particle size and surface modification lead to different responses in terms of cell nonspecific or receptor-mediated uptake3. The surface modification of NPs with a polyethylene glycol (PEG) corona provides mainly the benefits of protection from rapid degradation and aggregation and reduction in toxicity4. In this context, we evaluated the effects of PEGylation of IONPs on cultured endothelial cells (HUVECs) and macrophages (RAW264.7), taken as models of cells exposed to NPs after systemic administration5. Highly uniform magnetite nanocrystals coated with oleylamine surfactant were synthesized by solvo-thermal decomposition in organic solvents and transferred to the water phase by mixing with an amphiphilic polymer, resulting in highly stable and dispersible IONPs. NPs were characterized by TEM, DLS and zeta potential. We tested the biocompatibility on HUVECs and macrophages for 1 and 24 h at 20/50/100 μg/ml. On HUVECs, we measured the nitric oxide (NO) production by Griess method, the levels of endothelial-NO-synthase (eNOS) phosphorylation and the presence of nitrotyrosine residues. Cells viability and apoptosis-mediated cell death were also carried out. Since macrophages play an important role in the inflammatory process in response to particles, activation of macrophages were investigated evaluating the production of NO, TNF-α, IL-1 and COX-2.
Surfactant-coated Fe3O4 nanocrystals had a mean diameter of 8.2 ± 1.4 nm; IONPs surface was saturated with PEG molecules (PEG-IONPs). The treatment with PEG-IONPs affected HUVECs viability in a dose dependent manner within 24 hours. The NO production was increased after 1h at the higher concentration tested. Preliminary data shows that eNOS activation and the presence of nitro-tyrosine residues were time dependent. The macrophages treatment with PEG-IONPs induced a dose-dependent decrease in viability, but not NO production. Preliminary data showed the activation of inflammation markers already after one hour of treatment. These results suggest that PEG-IONPs, in a given concentration range, have a reduced risk of affecting vascular homeostasis and inflammatory response, rendering them potentially suitable for the future in vivo tests

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Acknowledgement / funding:
This work was supported by grants from FAR 2011, FAR 2012

SESSION 3 – P27

CAN IN VITRO CELL UPTAKE PREDICT TARGETED-NP BIODISTRIBUTION? EFFECT OF SERUM

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To evaluate if in vitro uptake by hCMEC/D3 can be used for prediction of formulation brain targeting potential. Mono- and dual-decorated liposomes (dd-LIPs) were prepared, by surface-immobilization of a monoclonal antibody (MAb) against transferrin receptor (TfR) and/or a peptide analogue of apolipoprotein E3 (APOe) -to target the LDLr - and investigated for blood brain barrier (BBB) targeting efficiency. For LIP preparation lipids [DSPC:Chol:PEG-Lipid:DSPE-PEG-Maleimide (2:1:0.16:0.001 mol/mol/mol)] were hydrated with PBS, pH 7.4, and the resulting LIP dispersions size was decreasedby probe sonication. For MAb attachment thiolation was performed using Traut's reagent. The reaction was carried out for 1,5 h in the dark and excess Trauts reagent was isolated after 3 washes with PBS using Amicon Ultra-15. For LIP uptake, FITC-dextran-labeled vesicles were incubated with hCMEC/D3 cells (200nmoles liposomal lipid/106 cells) in medium (containing 5, 10, 20 or 50% (v/v) FCS) at 37°C, for 60 min, then washed in ice-cold PBS (x3), detached from plates, re-suspended in PBS and assayed by FI (after cell lysis in 2% Triton X-100). Cell auto fluorescence was always subtracted.
All LIP dispersions had mean diameters between 100-150 nm and were monodisperse. The in-vitro uptake by hCMEC/D3 cells was significantly affected by ligand decoration with peptide or MAb, the dd-LIPs exerting an additive targeting effect, when uptake was evaluated in cell culture medium supplemented with 5% FCS. However, the uptake of the various formulations was affected differently when uptake was evaluated in presence of increased FCS concentrations. Indeed, although uptake of mono-MAb-LIPs as well as dd-LIPs was not drastically affected by FCS concentration (only a slight 15-20% reduction in LIP uptake was measured for dd-LIP when FCS was increased from 10 – 50%) the mono-peptide-LIP uptake was reduced more than 50%, suggesting that perhaps protein absorption (from FCS proteins) blocks the peptide-receptor interaction. Interestingly, a similar reduced brain targeting was noticed for mono-peptide-LIP (compared to the other LIPS) in a recently completed in vivo study [1]. This observation is in good agreement with recently published effects of serum proteins on the targeting ability of transferrin-decorated liposomes [2]. hCMEC/D3 cell uptake studies performed in presence of 20-50% FCS may be a good predictive tool to screen the brain targetimg potential of ligand targeted NPs.

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Acknowledgement / funding:
[1]Funding from the EC FP7/2007-2013, under grant agreements n° 212043
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SESSION 3 – P28

PEGYLATED NANOLIPOSOME INTERACTIONS WITH IN VITRO BBB MODEL: EFFECT OF VESICLE PHYSICOCHEMICAL CHARACTERISTICS

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To evaluate the effect of PEG-nanoliposome (NL) size and surface charge on their interaction with hCMEC/D3cells.PEG-NLs, encaspulating FITC-dextran as an aqueous space probe and Rhodamine- lipid as membrane probe), consisting of DSPC/Chol and 8mol% PEG2000,were prepared by thin film hydration followed by extrusion through appropriate pore size filters (50, 100, 200 and 400 nm) to give vesicle diameters between 100 –400 nm. Liposomes were characterized for size and surface charge by DLS and their FITC and lipid content was measured by FI and Stewart assay, respectively.NL uptake by hCMEC/D3 after 1 h incubation of 200nmoles of lipid/106 cells was studied as previously described [1]. Transport through cell monolayers was also evaluated by measuring FITC and Rhodamine, in order to evaluate the integrity of liposomes in receiving compartments. [1]. Effect of vesicle charge was evaluated by replacing 5, 10 or 15% of DSPC with DSPG in the NLs. Actual measured NL sizes were where 104-108, 127-134, 209-231 and 326-389. Cell uptake experiment results indicate that NLs can be categorized in two classes, those with diameters 200nm. When liposome size was within the first category (15 mV (vesicle containing 15 mol% DSPG). From transport studies (NLs sizes were100-450 nm) it was seen that only when NL diameter was > 220nm, %transported FITC and Rhodamine labels decreased significantly. Experiments carried out with PEG-NLs with diameters between 100-450 nm, prove that the hCMEC/D3 cellular model of BBB, is not affected by NL characteristics when their size is.

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Acknowledgement / funding:
The research leading to these results has received funding from the European Community's Seventh Framework Programme (FP7/2007-2013) under grant agreements n° 212043

SESSION 3 – P29

NEW TYPE OF CURCUMIN DECORATED NPS. EFFECT ON AB-AGGREGATION

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Purpose: In order to target amyloid-beta (Aβ) peptide (one of the state-of-the-art methodologies currently investigated for AD therapy and diagnosis) a novelnon-planar DPS-PEG-curcumin and the corresponding DPS-PEG-curcumin surface decorated nanoliposomes (DNLs), were developed. The effect of DNLs on Aβ1-42 aggregation was tested in vitro.Material and Methods: For the synthesis of DPS-PEG2000-curcumin, we used commercially available DSPE-PEG2000-maleimide which was reacted with4-methoxytrityl-thiol (Mmt-SH) in presence of DIPEA to the corresponding DSPE-PEG2000-S-Mmt. Removal of the Mmt-group in presence of 1% trifluoroacteic acid(TFA)/triethylsilane (TES) (95:5) gave the unprotected DSPE-PEG2000-SH, which was further reacted with curcumin (Cur) to give the desired DPS-PEG2000-Cur.SUV-type nanoliposomes consisting of DSPC/Chol (2:1) containing 8% DSPE-PEG2000-OMe and further incorporating 10% DPS-PEG2000-Cur were prepared viathin film method followed by probe sonication, giving the corresponding DPS-PEG2000-Cur DNLs. The effect of DPS-PEG2000-Cur DNLs on Aβ aggregation wasstudied by the ThioflavinT (ThT) assay, which was performed on Aβ1-42 peptides, de-seeded one day before the experiment by subjection to a previously reportedage-reversal protocol.1 In general a mixture Aβ1-42, ThT and DPS-PEG2000-Cur DNLs were incubated and FI measurements were taken at several time points.The peptide aggregation was also carried out in absence of NLs (control-1) or in presence of plain NLs without DPS-PEG-Cur on their surface (control-2).Results: The size of the novel DNLs was measured at 120,0±3,677 nm (PDI: 0,24) and their zeta potential at -6,07±0,47 mV. In vitro aggregation experiements ofDNLs showed that DPS-PEG2000-curcumin DNLs are able to substantially inhibit Aβ1-42 aggregation (in vitro), while the control NLs had no effect.Conclusion: DPS-PEG2000-

curcumin DNLs substantially lowered the degree of Abeta-peptide aggregation and can are currently being further explored, in vitro andin vivo, for their potential in treatment and diagnosis of Alzheimer's disease.

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Acknowledgement / funding:
The research leading to these results has received funding from the European Community's Seventh Framework Programme (FP7/2007-2013) under the agreementno 212043.

SESSION 3 – P30

'Sink effect' of dually-decorated nanoliposomes on Abeta42 clearance

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Plaques containing β-amyloid (Aβ) peptides are one of the hallmarks of Alzheimer's disease (AD) and the reduction of Aβ is considered a primary therapeutic target. Studies in mouse models of AD have indicated that lowering Aβ levels in the brain can minimize the neurodegeneration. Recently many strategies have been employed to reduce Aβ brain levels and one of them is based on the 'sink effect' hypothesis, that is the peripheral administration of Aβ binding agents able to reduce Aβ brain amount by sequestering it in the plasma. De Mattos et al. reported that peripheral administration of a monoclonal anti- Aβ antibody resulted in an increase of Aβ in the plasma despite minimal entry of the antibody into the brain, suggesting the equilibrium of Aβ between the brain and plasma. We have previously demonstrated that liposomes (LIP) embedding acidic phospholipids have high binding affinity to Aβ42. This study investigated the potential effect of dually-decorated LIP on the Aβ exchange across an in vitro model of the blood-brain barrier (BBB). In particular, we examined the effect of dual-radiolabelled-LIP composed of sphingomyelin/cholesterol/phosphatidic acid (47.5/47.5/5 mol%) and surface decorated with human ApoE-derived peptide (a.a. 141-150) (mAPO-E) on Aβ42transcytosis across the BBB. The clearance of the soluble low molecular weight aggregates Aβ42 from the basolateral to the apical side was followed by ELISA assay, whereas the LIP distribution was followed by radioactivity counting. We observed that these LIP significantly enhanced the cellular uptake of Aβ42 (3-fold)from the basolateral side, compared to the amount of Aβ42 uptaken from the cells in the absence of LIP. Moreover, the LIP treatment strongly enhanced the basolateral-to-apical transcytosis of Aβ42 across the BBB (+298%), compared to transcytosis of Aβ42 alone. Moreover, the rate of LIP-mediated Aβ42 clearance was time- and lipid dose-dependent. Finally, the presence of Aβ42 in the basolateral side of the BBB significantly increases the LIP ability to cross the BBB (+19%).The integrity of the cell monolayer was tested in the all experiments used by measuring transendothelial electrical resistance (TEER), paracellular permeability of[14C]-sucrose, transcellular permeability of [3H]-propranolol and the cell viability. LIP and Aβ42 treatment did not affect the BBB functional and bioelectrical properties. This study provide rationale for the use of Aβ binding LIP as a treatment strategy in AD.

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Acknowledgement / funding:
This investigation was carried out within deframe of the FP7 project NAD, G.A. CP-IP 212043-2

SESSION 3 - P31

Inhibition of Amyloid beta 42 oligomerization by APOE-labelled liposomes: a fluorescence study

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Alzheimer disease (AD) is the most common form of senile dementia. Investigation of AD brains has revealed the presence of protein aggregates forming deposits known as amyloid plaques The main constituent of such deposits is a peptide called Amyloid Beta (AB).[1] AB exists in two allotypes: the 40-residues-long AB40(more abundant) and the 42-residues-long AB42.[2] Due to the hydrophobic nature of its Ile-41 and Ala-42 residues, AB42 is more prone to hydrophobicity-driven self-assembly than AB40. AD is caused by the accumulation of an excess of AB42.[3] Although AB42 fibrils were initially indicated as the source of neuronal degeneration in AD,[4] this hypothesis is challenged by the low correlation between the degree of dementia and the extent of amyloid plaques. Recent studies indicate that the most neurotoxic peptide aggregates are soluble oligomers of AB42.[5] In spite of this, our insight on the mechanisms of AB42 self-assembly is incomplete and systematic studies on the potential of nanoparticles in hindering such pathogenic process are still embryonic.
Here, a bulk characterization of the first steps of AB42 self-assembly is afforded by using fluorescein-isothiocyanate (FITC) labeled synthetic peptide and exploiting the fact that oligomerization provides a solvent-shielded environment to the fluorophore, enhancing its quantum yield. The extent of oligomerization and its kinetics are probed in a wide range of physiologically relevant AB42 concentrations. The chemical affinity of FITC-AB42 for liposomes embedding acidic phospholipids(phosphatidic acid, PA or phosphatidyl-inositol, PI), already demonstrated to bind with high affinity the AB42 peptide, is assessed by exploiting the FITC quantum yield depression in lipophilic environment. Finally, the efficacy of PA-embedded liposomes further surface-decorated with human apolipoprotein-E-derived peptide(APOE) in inhibiting FITC-AB42 peptides aggregation is tested. Data show unambiguously that AB42 oligomerization takes place in hours in pseudo-physiological conditions at concentrations as low as

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Acknowledgement / funding:
This research received funding from the European Community's Seventh Framework Programme (FP7/2007–2013) under grant agreement 212043 (NAD).

SESSION 3 – P32

Surface functionalised Nanoliposomes with cis-glycofused tricyclic Aβ ligands: preparation, stability and Aβ binding ability

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Nanoparticles (NPs) are attractive tools in biomedical applications thanks to their biocompatibility, non-immunogenicity, non-toxicity, biodegradability, high physicalstability, possibility of drug loading and releasing, and high ce functionalization possibilities; in particular, liposomes are being extensively explored for theirpotentialities in the medical field.
This work deals with the preparation of surface functionalised nanoliposomes with cis-glycofused tricyclic compounds, novel Aβ-ligands recently synthesised by ourgroup1. In particular we describe the synthesis of selected ligand properly derivatized for the nanoparticle functionalisation. The preparation of the liposomes, andtheir characterization. In particular we describe their structural characterization, their stability and their ability to interact with Aβ-peptides.
For the functionalisation of nanoliposomes with cis-glycofused tricyclic compounds, a "Cu free click chemistry" method2 was used. For this, a cyclooctyne group wasefficiently introduced in the cis-glycofused tricyclic derivatives, which were efficiently reacted with preformed nanoliposomes bearing azide groups on their surface3,under mild conditions. The size of the derived nanoliposomes was measured at 127,1±0,17 nm (PDI:0,17) and demonstrated high size stability at 4oC and highintegrity (retention of encapsulated calcein) at 37oC in presence of plasma proteins. Structural characterization and interaction studies with Aβ peptide wasperformed by NMR spectroscopy through 1H-NMR and water-LOGSY-NMR experiments.

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Acknowledgement / funding:
NAD - Nanoparticles for therapy and diagnosis of Alzheimer's Disease - 2008-2012, FP7-NMP-2007-LARGE-1-Large-scale integrating project NMP-2007-4.0-4
Substantial innovation in the European medical industry: development of nanotechnology-based systems for in-vivo diagnosis and therapy.

SESSION 3– P33

Polymer-protein conjugation for the development of an enzyme replacement therapy for the cure of Primary Hyperoxaluria Type 1

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The chief target of this project is the development of a new non-invasive treatment for Primary Hyperoxaluria type 1 (PH1) a metabolic disorder caused by inheritedmutations in the AGXT gene encoding liver peroxisomal alanine:glyoxylate aminotransferase (AGT)(1). Since the disease is caused by a single enzyme deficiency,an enzyme administration therapy is a valid therapeutic option. The basic principle of the enzyme administration therapy is to compensate the deficit of an enzymaticactivity providing the organism with the functional recombinant protein, an approach proved to be greatly effective for the treatment of many inherited disordersincluding lysosomal storage diseases.(2)
The curative options currently available for PH1 are pyridoxine administration, effective in a minority of the patients, and liver transplantation, a very invasiveprocedure. Thus, the development of an enzyme administration therapy would be a major improvement with respect to the actual therapeutic management of thepatients. A major obstacle in this direction is represented by the need to deliver AGT in its physiologic sub-cellular compartment, i.e. hepatic peroxisomes. However,since the peroxisomal import occurs on the fully-folded protein thanks to a C-terminal targeting sequence, once AGT reaches the cytosol it is supposed to interactwith the import machinery and acquire the correct intracellular localization.(3)
In our work we designed a nanopharmaceutical preparation based on polyglutamic acid (PGA) polymeric carriers (4). PGA nanocarriers functionalised with pyridyldithiol groups have been conjugated with purified recombinant AGT by creating a disulfide bond with Cys residues on the protein surface5. The conjugation does notaffect the spectroscopic and kinetic properties of the enzyme. Moreover, the conjugated enzyme can be released from the carrier upon treatment with 10 mM GSH, a condition similar to that present in the cell cytosol. Following reduction, AGT recovers over 90% of its initial activity. Treatment of a PH1 cellular model with PGA-AGT conjugates leads to the dose-dependent accumulation of enzymatically active intracellular AGT.
Altogether, the results prove that the formulation of an AGT-nanocarrier conjugate able to deliver the enzyme inside the cell is feasible, thus representing animportant step towards the development of an enzyme administration therapy for PH1.

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Acknowledgement / funding:

This work was supported by Telethon Foundation (GPP10092 grant to C.B.V.) Part of this work was conducted under the international collaboration "cooperint" program of the University of Verona

SESSION 3 – P34

PLGA-nanoparticles enhance the curcumin efficacy against acrolein-induced neurotoxicity : relevance for Alzheimer's disease treatment

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Curcumin is known to possess a pleiotropic activity such as antioxidant, anti-amyloid-β activities and neuroprotective properties. Using relatively high concentration of curcumin, we recently showed the neuroprotective effect of curcumin against acrolein, a by-product of lipid peroxidation. In Alzheimer's brain acrolein was found to be associated with proteins detected in neurofibrillary tangles and dystrophic neuritis surrounding senile plaques. Due to its poor aqueous solubility and oral bioavailability, enhancement of curcumin efficiency represents a pharmacological challenge for its theurapeutic applications. The aim of this work was to encapsulated curcumin in PLGA nanoparticles in order to enhance its neuroprotective effects. For this purpose, curcumin-loaded nanoparticles (Nps-Cur) was prepared by emulsion–diffusion–evaporation method. We have characterized the Nps-Curby dynamic laser light scattering (DLS) and transmission electronic microscopy (TEM) analysis was performed. The entrapment efficiency was estimated by HPLC. Cellular uptake of Nps-Cur and curcumin by SK-N-SH cells incubated for 24 h at 37 °C was observed under a fluorescence microscopy. The nanoformulation was then subject to cellular toxicity induced by acrolein using the LDH and Tox-8 (Resazurin-based) assays to determine cell dead and cell survival respectively. By DLS, we found a narrow size distribution of the Nps and Nps-Cur ranging from 124.9 to 161.6 nm. The polydispersity index obtained from DLS was within the permissible range and remained below 0.3. TEM revealed a regular spherical shape of our prepared Nps and Nps-Cur. The entrapment efficiency was 80% with 15% curcumin-loading. The cellular internalization of Nps-Cur showed a wide distribution in the cytoplasm and within the nucleus. Our results showed that 0.5µM of Nps-Cur can protect neuronal cells challenged with 10 µM of acrolein for 24 hours, while at the same concentration, free curcumin was not able to exhibit a significant neuroprotective effect. Our results provided evidence that encapsulation of curcumin in nanoparticle enhance its efficiency against acrolein toxicity. This confirmed the greatest interest of drug-loaded nanoparticle as a promising strategy for drug delivery.

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Acknowledgement / funding:
Financial supports from "Institut sur la nutrition et les aliments fonctionnels" (INAF) and "Fonds de Recherche Québecois-Nature et Technologies" (FQR-NT) are gratefully acknowledged.

SESSION 3 – P35

Raman and SERS on Human Blood by using different types of nanoparticles

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Surface Enhanced Raman Spectroscopy (SERS) has become in the recent years more and more used in the field of nanomedicine. Functionalizing gold and/or silver nanoparticles can lead to the specific detection of marker biomolecules in body fluids. Very promising results were obtain by using SERS and multivariate analysis on human blood plasma for detecting different type of cancers [1-3].
The SERS technique was also recently used in order to characterize the storage behaviour of human red blood cells [4].
In this paper we present our group results in measuring the SERS signals for both blood plasma and human erythrocyte hemolysates, by using different SERS nanoparticle substrates.
We compared the Raman and SERS spectra for both human blood plasma and red blood cells hemolysates obtained by using nanoparticles made of different metals (Ag and Au) and the influence of coating the nanoparticles with PEG (polyethylene glycol) of different molecular masses, prepared by using an original method developed within our group [5].
The differences between the Raman spectra recorded in the presence and in the absence of nanoparticles allowed us to discriminate the SERS signals form the Raman spectra and to characterize the capability of different type of nanoparticles to enhance the Raman signals.
Spectra were recorded by using excitation beams both in the visible (532 nm) and in the near infrared (785 nm)spectral domnains and we discuss the differences between the spectra obtained as a function of the excitation wavelength.
Based on these results, we also discuss the potential use of the SERS technique in screening various diseases.

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Acknowledgement / funding:
We aknowledge the support of the Univeristy of Medicine and Pharmacy "Iuliu Hatieganu" Cluj-Napoca in performing the researches presented in this paper.

SESSION 3 – P36

Cerium oxide and Iron oxide nanoparticles dramatically reduce the antibacterial activity of Ciprofloxacin against gram positive and gram negative biofilm Bacteria

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Infectious diseases caused by bacteria are of the major healthcare problems worldwide. Biofilm mode of growth that provide protection for bacteria againstantibiotics and host immune cells, and the fast emerging drug-resistant bacteria highlight the need for developing new antibacterial agents. Recently, metal oxidenanoparticles have been suggested as good candidates for the development of antibacterial agents due to their unique chemico-physical properties. Cerium oxide(CeO2) and iron oxide (Fe2O3) nanoparticles have been utilized in many biomedical applications. Here we report the influence of CeO2 and Fe2O3 nanoparticles onthe growth of a panel of gram positive and gram negative bacteria, including drug-resistant strains. Minimum inhibitory concentrations (MICs) of CeO2 and Fe2O3nanoparticles that are required to inhibit bacterial planktonic growth and bacterial biofilm biomass were evaluated, and compared to the MICs of the broad spectrumantibiotic ciprofloxacin. The results indicate that CeO2 and Fe2O3 nanoparticles fail to inhibit bacterial growth and biofilm biomass for all the bacterial strains tested.Moreover, adding CeO2 or Fe2O3 nanoparticles to the broad spectrum antibiotic ciprofloxacin almost abolish it's the antibacterial activity. Therefore, results of thisstudy suggest that CeO2 and Fe2O3 nanoparticles are not good candidates as antibacterial agents.

Acknowledgement / funding:
This Project was supported by Deanship of Research at Jordan University of Science and Technology, Irbid, Jordan

SESSION 3 – P37

Anti-Amyloid β peptide mAb functionalized nanoparticles for Alzheimer's disease

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Alzheimer's disease (AD) represents the most common form of dementia worldwide. This devastating neurodegenerative disorder affects more than 35 millionpeople over the age of 65 years. The neuropathological AD condition is featured by progressive loss of cognitive functions and shows two pathophysiologicalhallmarks in the brain: senile plaques, essentially composed by aggregations of Amyloid-β (Aβ) peptide, and neurofibrillary tangles.
The aim of this work was to synthetize nanoparticles (NPs) able to interact with the Aβ peptide in order to use the "sink effect", through Aβ peptide elimination fromthe peripheral circulation, to improve the AD

conditions.First the biotin 1% functionalized NPs were prepared by nanoprecipitation of a polymeric mixture, MePEGCA-co-VB7PEGCA-co-HDCA andHDCA-co-RCA-co-MePEGCA, in a Pluronic F68 solution. After purification by ultracentrifugation, NPs were characterized in size (100 nm), PDI (0.1-0.5), stability(more than 2 weeks) and biotin amount (9.558•1014 molecules of biotin each preparation). Lyophilization was used to estimate the yield of the reaction (25%).In parallel, streptavidin-fluorescein isothiocyanate (SavFITC) was coupled with anti-Aβ monoclonal antibody (anti-Aβ mAb). The conjugate was purified by gelfiltration column using Superdex 200 gel. To demonstrate the success of the purification a semi-native electrophoresis was made and the gel was analyzed byflorescence and coomassie blue. The conjugate's yield (9%) was quantified by spectrofluorimetry and Bradford's dosage.Once obtained biotin 1% NPs and SavFITC – anti-Aβ mAb conjugate, both of them were coupled in a nanoconstruct complex. It was purified by ultracentrifugationand the presence of SavFITC – anti-Aβ mAb conjugate on the biotin 1% functionalized NPs was verified by spectrofluorimetry.Next step will be the evolution of the nanoconstruct, attaching on the Aβ mAb functionalized NPs the anti-CD71 mAb, an antibody directed against transferrin receptor, to give them the possibility to cross the blood-brain barrier and to operate also in the central nervous system's district.

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Acknowledgement / funding:

The research leading to these results has recived founding from the European Community's Seventh Framework Program (FP7/2007-2013) under agreement No. 212043.
The CNRS and the French Ministry of Research are further acknowledgement for financial support.

SESSION 3 – P38

Development of an in vitro co-culture model of the blood-brain barrier: A potential system to study the transport of Alzheimer drugs coupled to nanoparticles?

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Alzheimer's disease (AD) is a progressive degenerative disorder which is correlated with deregulated proteolytic processing of the amyloid precursor protein (APP).The α-secretase ADAM10 cleaves APP into

the neuroprotective fragment APPs-α and simultaneously prevents the formation of Aβ-peptide species1. Acitretin hasbeen identified as a potential inducer of ADAM10 expression in vitro as well as in vivo2-4. Studies in mice have demonstrated that Acitretin not only enhancesADAM10 expression but is also able to overcome the blood-brain barrier (BBB)5. Nevertheless, to study the potential of newly identified α-secretase enhancingdrugs to cross the BBB in vivo is time-consuming and associated with high costs. Thus, the aim was to develop an in vitro co-culture model of the BBB to analyzedrug transport across the BBB based on the enhanced expression of a luciferase-reporter gene which is driven by the ADAM10 promoter in neuronal cells.Primary porcine brain endothelial cells (PBEC) were used to develop a model of the BBB on transwells and were co-cultured with neuronal cells (SH-SY5Y)transfected with reporter construct for ADAM10 promoter activity. The tightness of the barrier was evaluated by microscopy, electrical resistance and permeabilitymeasurements. Acitretin was used as a model compound to study the transport across the barrier via its effect on the ADAM10 promoter activity. Statistical analysis(t-test or one-way ANOVA) was performed using GraphPad Prism.We established an in vitro co-culture model of the BBB with physiological characteristics. Tight junction proteins of PBECs are highly expressed. Microscopicanalyses demonstrate that the endothelial cells grow in a dense monolayer. Cell viability and apoptosis assays indicate that PBECs are not negatively influenced bythe co-culture with SH-SY5Y cells or additional treatment with Acitretin. The expression of AD-related proteins is not altered in SH-SY5Y cells when co-cultured withPBEC. Although the tightness of the barrier is unaltered during drug treatment, the expression of the luciferase protein is enhanced by transported Acitretin.We established an in vitro co-culture model system of primary ECs and neuronal cells capable of indicating ADAM10 gene expression enhancement. This modelallows a screening of drugs or nanoparticle-coupled substances for their ability to cross the BBB and by modification of the reporter to detect effects on variousgenes involved in different brain diseases.

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Acknowledgement / funding:

This work was supported by the Federal Ministry of Education and Research (BMBF) in the framework of the National Genome Research Network (NGFN), FKZ01GS08130.

SESSION 3 - P39

Targeting Cancer with inorganic nanoparticles: from surface engineering to in vitro and in vivo studies

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Inorganic nanoparticles have raised enormous attention in the biomedical field thanks to the size-tunability of their physical properties and to the possibility toengineer their surface with a variety of biomolecules (1-3) and drugs. Thanks to those features, nanocrystals of semiconductor materials and metal oxide, like ironoxides, have been proposed as advanced diagnostic methods and innovative therapeutic approaches to several human diseases, like cancer. A clear definition ofthe interactions of these materials with living systems is fundamental prior to their use on humans.
In this regard we have developed both fluorescent and magnetic nanoparticles for targeting, imaging and treating ovarian cancer, which is one of the mostaggressive types of female tumors. For the targeting study the surface of the nanocrystals was engineered with human Fab fragments against the α-isoform of thefolate receptor which is over-expressed on the membrane of the ovarian cancer cells. In vitro and in vivo studies have been performed in order to assess thetargeting ability of the nanobioconjugate.Furthermore a therapeutic approach to the same type of malignancy is under development through the binding of a chemotherapeutic drug to the surface of themagnetic nanoparticles. Preliminary results will be presented.

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Acknowledgement / funding:
The authors acknowledge financial support from European Union throughthe FP7 project Magnifyco (contract number NMP4-SL-2009-228622).

SESSION 3 –P40

Passage of nucleoside analogues across the Blood-Brain Barrier using squalenoyl nanovectors

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Brain diseases represent a major health concern, due in part to the population aging, but their treatment remains challenging. If many drug candidates may display interesting in vitro activity, most of them do not display in vivo efficiency due to their rapid metabolization and/or inability to translocate the Blood-Brain Barrier (BBB),which hampers their diffusion into the brain after administration (1). A typical example of this paradigme is adenosine. Our goal is to covalently link the squalene tothe adenosine («squalenoylation») (2), in order to obtain stable nanoparticules according to the amphiphilic structure of the compound. This should allow thenanoparticles to cross the BBB by enhancing the intracellular penetration of the nucleoside (3) and its stability in the circulation (4), and finally permit to reach acerebral concentration in adenosine able to have a therapeutic effect.
The adenosine-squalene nanoparticles were obtained by the nanoprecipitation technique, with a mean diameter of 115 nm, a mean polydispersity index of 0.12 anda mean zeta potential of -26 mV. By co-nanoprecipitating the adenosine-squalene with fluorescent probes or radioactive molecules, we obtained labeled nanoparticles, without any significant modification in size or surface charge. An in vitro model of

human BBB (5) (hCMEC/D3 cell line) has been used to study thetoxicity of the nanoparticles in term of cellular viability (MTT test) and barrier properties (TEER measurements, 14C-sucrose). The ability of the nanoparticles to beinternalized by the endothelial cells was observed by confocal microscopy and flow cytometry, and their ability to cross the BBB in vitro was followed by
spectrofluorimetry and radioactivity using a transwell system. FRET nanoparticles have been developed and allowed us to show that the nanoparticles were disassembled after their passage through the cellular monolayer. Finally, the pharmacokinetic and biodistribution of the radiolabelled nanoparticles were studied in-vivo in mice, and compared to the free adenosine behavior. These results show for the first time that the «squalenoylation» technology, which has already been applied to the intravenous administration of anticancer compounds, is competent for the delivery of hydrophilic drugs within the brain sanctuary.

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Acknowledgement / funding:
This project is funded by the ERC Advance Grant Ternanomed allocated to Professor Couvreur. The PhD is also supported by the Région Ile-de-France (grant NerF).

SESSION 3 – P 41

A NOVEL UPTAKE STRATEGY FOR MACROPHAGE-MEDIATED DELIVERY OF GOLD NANOPARTICLES

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Objective: Gold nanoparticles (GNPs) are known to be optimal contrast agents for near-infrared laser photothermal ablation of cancer tissues [1,2]. GNPs targeting of cancer cells is usually pursued by attaching specific molecules on the particles surface and the uptake of GNPs by the mononuclear phagocyte system is generally considered an obstacle to GNPs delivery, removing nanoparticles from blood circulation before they can reach their target.
To date, monocytes/macrophages are also considered as a resource, since those cells can be efficiently extracted, loaded in vitro with GNPs and, after reinjection in the blood flow, be recruited by the tumor and therefore act as a trojan horse for nanoparticles delivery within tumor areas[3,4,5].
The actual challenge is to design GNPs in order to maximize macrophage uptake without compromising cell viability and activities, since loaded cells will have to perform specific functions when reinjected in the blood flow.The surface of pegylated gold nanorods (GNRs) was modified so as to achieve unspecific interactions with cell membranes. Cellular uptake was evaluated by silver staining and spectrophotometric analysis. Cell viability was assessed by MTT test and cell death, due to GNRs mediated laser hyperthermia, was evaluated by arypan blue assay. Macrophage functional characteristics were measured by quantification of reactive oxygen species production after lipopolysaccharide stimulation. Our GNRs were able to massively penetrate both macrophage and different cell lines, which do not have a phagocytic phenotype, without affecting cell viability. Those results indicate that the particles design was appropriate to allow a general uptake mechanism, without requiring a specific macrophage activation. Thefunctional characteristics of macrophages were not significantly altered when cells were uploaded

with GNRs at the dose necessary for performing laserhyperthermia.Cellular vectors are a valuable tool for nanoparticles delivery, allowing to take advantage of the high efficiency of the immune system. This approach bypass the design of complex strategies for tumor targeting and represent a simple method for a safe and efficient delivery of GNRs.
The macrophage-mediated system can be used alone or in combination with other targeting strategy, in order to maximize the tumor loading and therefore the efficiency of the following laser treatment.

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Acknowledgement / funding:
This work has been partially supported by the Projects of the Health Board of the Tuscan Region "NANOTREAT"

SESSION 3 – P42

The toxic effect of Abeta oligomers may be reduced by lipid-based nanoparticles in human ex-vivo models

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The oligomeric Abeta cytotoxicity and the associated biomolecular mechanisms are extremely interesting for the scientific community, as a complete understanding of these phenomena could help to develop therapeutic and effective prevention technologies; in fact new strategies aimed at lowering cerebral load of Abeta are in progress. An attractive strategy is represented by the use of nanoparticles, opportunely engineered for targeting different toxic form of brain Abeta, for the combined diagnosis and therapy of AD (1,2). Both at central and peripheral level, Abeta has several toxic effects. In particular it is able to alter MAPK cell signaling (3,4). Aim of this study was to evaluate the biocompatibility of liposomes, functionalized to sequester Abeta, and to investigate their ability to reduce Abeta toxic effect inhuman fibroblast primary cultures (5). Now, we are also evaluating the capacity of liposomes to sequester the plasma Abeta.
The liposomes (chol/sm) were functionalized with PA, TREG and mApo-PA. They were added to fibroblast cultures from AD and controls, treated with Abeta oligomers. MTT and Neutral Red assays were used to evaluate energetic metabolism and cell viability respectively. Western Blot and phospho-Elisa analyses were performed on cell lysates to evidence modulation in AKT, and p38 and ERK 1/2 activation status.
Our results indicate that the presence of nanoparticles do not reduce cell viability at concentrations used for the experiments. Moreover, liposomes cause a reductionin Abeta-induced toxicity, evaluated in terms

of mitochondrial activity, and a significant modulation of deregulated kinases. These results suggest that liposomes(opportunely functionalized and characterized) could represent a useful tool to antagonize the toxic effect of the Abeta oligomers and potentially reverse the condition of cellular stress. Furthermore, alterations of Kinases-pathway might clarify any molecular mechanisms involved in the pathology, and nanoparticles may be useful to study new potential therapeutic strategies.

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Acknowledgement / funding:
Supported by the EC FP7 Programme reference CP-IP 212043-2 NAD (www.nadproject.eu)

SESSION 3 – P43

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

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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. 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 functionalized with NGF-β for cellular recognition and a fluorescent moiety for intracellular tracking. In PC12 cells cultured with the functionalized 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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Acknowledgement / funding:
This work was partially supported by Fondazione Cassa di Risparmio di Pisa (MDIAB projet, Role of multilayer nanoencapsulation, anti-inflammatory nanostructures, and selective nanoparticle-guided homing in human islet transplantation for the treatment of type 1 diabetes), EC/CNR (MARVENE project, MAgnetic nanopaRticlesFor NerVE RegeNEration, NanosciE+ 2008) and by the Spanish Ministerio de Ciencia e Innovación (project MICINN MAT2010-19326).

SESSION 3 – P 44

Anisotropic gold nanoparticles for detection of DNA hybridization events related with lactose intolerance

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Gold nanoparticles (AuNPs) have been widely used as an efficient probe, due to their strongly enhanced surface plasmon resonance at optical frequencies that makes them excellent scatters and absorbers of visible light. In addition to their optical properties, the ability of AuNPs to achieve a stable immobilization of biomolecules, whilst retaining their bioactivities is a major advantage to apply them as biosensors. Numerous studies have been developed regarding the use of spherical nanoparticles in a variety of fields ranging from biology to medicine, namely in the detection, diagnosis, sensing and therapies procedures, however nextgeneration biosensor platforms will require significant improvements in sensitivity, specificity and parallelism in order to meet the future needs in nanomedicine.Anisotropic nanoparticles, such as, nanotriangles and nanostars are emerging as promising materials in the sensing of DNA sequences [1] and even on thedetection of single nucleotide polymorphisms (SNP) to counter some limitations of the gold nanospheres, mainly in terms of sensitivity [2]. Their shape configurationand higher aspect ratios leads to strong dipole moments and are acutely influenced by local refractive index changes, making their resonance more sensitive to thesurrounding materials. Given the expanded ease of the synthesis procedures and their surface modification it is possible to tune and develop various types ofbiosensor.

Our group demonstrated that gold nanotriangles synthesized by a photocatalytical method [3] are much more sensitive to DNA detection than equivalent sphericalshape particles (size and capping) for hybridization events performed using dark-field optical microscopy and spectroscopy [4]. According to the localized surfaceplasmon resonance (LSPR) phenomena it is possible to distinguish a sensing response (complementarity of targets) with high sensitivity and resolution, regarding ashift of resonance wavelength of LSPR caused by the local refractive index change of an individual nanotriangle.
Moreover, according with a colorimetric method (induced by aggregation) developed by our group for the detection of SNPs using spherical gold nanoparticles [5], asimilar study involving a SNP that is responsible for the lactose intolerance is being currently carried out using gold nanostars. This method is advantageous since itprovides faster, quicker and cheaper alternative to the conventional nutrigenomic test.

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Acknowledgement / funding:

This work has been supported by the Portuguese Foundation for Science and Technology (FCT) together with STAB-Vida Lda. through grant SFRH/BDE/51100/2010 and special acknowledgment to the European Science Foundation ESF (New Approaches to Biochemical Sensing with Plasmonic Nanobiophotonics - Plasmon-Bionanosense, exchange grant 3846 and 4136) for the opportunity given to develop this work in Germany.

BLOCK NOTES