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II Nanosciences and Biomedical Nanotecnologies Symposium
*II Simpósio Nacional de Nanociência e Nanotecnologia Biomédi*ca

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Lisboa - Universidade Lusófona

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Programa

Open Session | *Sessão de abertura*

Pres. do Conselho de Admin. do INFARMED, Dr. Eurico Castro Alves
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1st Session |*Sessão 1*
Nanomedicine: past and future | *Nanomedicina: passado e futuro*

Chairman | Moderador - **João Nuno Moreira**
Speakers | *Prelectores*
Manuela Gaspar
Ana Paula Pêgo
Eliana Souto

1st Session (continuation) | *Sessão 1 (continuação)*
Nanomedicine: past and future | *Nanomedicina: presente e futuro*

Chairman | Moderador - **Helena Florindo**
Speakers | *Prelectores*
Philippe Maincent
Maria Fuente
Ana Grenha

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Biomedical nanotechnologies | *Nanotecnologias biomédicas*

Chairman | Moderador - **António Almeida**
Speakers | *Prelectores*
Carlos Viegas
Ronald Neufeld
Cristiana Paulo

3st Session | *Sessão 3*
Nanochemistry and nanophysics | *Nanoquímica e nanofísica*

Chairman | Moderador - **Teresa Neves Petersen**
Speakers | *Prelectores*
João Rodrigues
Luís Melo
José Farinha

Open Session

From Left to Right / Da esquerda para a direita:
Eurico Castro
Luís Monteiro Rodrigues
Manuel de A. Damásio
Isabel Vitória Figueiredo

1st Session | Sessão 1

Nanomedicine: past and future | *Nanomedicina: passado e futuro*

Chairman / *Moderador*

João Nuno Moreira

Resumé / *Currículo Resumido*

João Nuno Moreira received his B.Sc. in Pharmaceutical of Sciences, M.Sc. in Cellular Biology and Ph.D. in Pharmaceutical Technology from the University of Coimbra.
At present, he is Assistant Professor at the Faculty of Pharmacy and Principal Investigator at the Department of Vectors and Gene Therapy, Center for Neuroscience and Cell Biology, University of Coimbra.
Scientific activity is focused on the design of lipid-based nanosystems for drug and nucleic acid targeted delivery, addressing the impact on the tumor microenvironment, both at the cellular and molecular level. Scientific competences include formulation and characterization of lipid nanovesicles, in vitro mechanistic studies, in vitro cytotoxicity, biodistribution, and therapeutic testing in animal models of human disease. Has experience in coordination of funded research projects as principal investigator and participates as co-investigator in several funded research projects, within academia as well as in collaboration with pharmaceutical industry. Over the years has supervised Ph. D. students, Master students, Research Fellows or undergraduate students.
Has a member of the MIT-Portugal program, has lectured at MIT on the course on Principles and Pratice in Drug Development (2010 - 2012).
Co-author of several publications in peer-reviewed journals, 3 book chapters and 3 patents requests (one of them has been granted in US - U.S. Patent No. 8,231,895). Has been collaborating with national (Portuguese Foundation for Science and Technology-FCT) and international funding agencies as reviewer. Member of the Comission for the Evaluation of Medicines (INFARMED, since 2010).

C.01 - Liposomes technology platform for clinical applications

Lipossomas com aplicações clínicas

Speaker / *Prelector*

Maria Manuela Gaspar

Resumé / *Currículo Resumido*

Maria Manuela Gaspar has completed her PhD in Pharmaceutical Technology in 2005. She is a researcher at the iMed.UL, Research Institute for Medicines and Pharmaceutical Sciences, Nanomedicine & Drug Delivery Systems Group, Faculdade de Farmácia da Universidade de Lisboa. She has expertise in design, characterization and biological evaluation of colloidal carriers, in particular liposomes, for low and high molecular weight molecules with therapeutic applications in infectious, inflammatory, parasitic and cancer diseases. She is co-author of scientific publications including book chapters, papers in peer-reviewed journals, communications in scientific meetings and co-inventor of patents.

Abstract / *Resumo da Comunicação*

Since the first description of vesiculated phospholipid systems in 1965 by Alec Bangham, termed “bangosomes”, almost 50 years of research allowed to transform those vesicles from membrane models into successful drug delivery systems. Many technological advances are on the basis of this evolution and nowadays liposomes are used in diverse areas to deliver antibiotics, anti-cancer, anti-inflammatory, anti-parasitic drugs, anesthetics, macromolecules, such as enzymes and oligonucleotides and in vaccines, imaging and even cosmetics. This success is due to the unique properties of liposomes that can efficiently incorporate different kind of molecules irrespectively of molecular weight, electric charge or solubility, can interact with cells, are biodegradable and biocompatible and can be manufactured with different sizes and properties.
The technological platform to produce different kind of liposomes according to the therapeutic objectives is mature nowadays. This fact and the possibility to stabilize and scale up liposomes have attracted the interest of the Pharmaceutical Industry and the clinical acceptance.
Several liposomal products are in the market, many others in advanced clinical trials and there is the expectation that new and more sophisticated liposomal products will be available in the near future.
Different aspects of the advances in liposome technology and examples of liposomal products development will be discussed.

1st Session | Sessão 1
Nanomedicine: past and future | *Nanomedicina: passado e futuro*

C.02 - The nanotechnology applied to the field of regenerative medicine: a big contribute at the nanoscale
A nanotecnologia aplicada à medicina regenerativa: um grande contributo para a nanoescala

Speaker / *Prelector*

Ana Paula Pêgo

Resumé / *Currículo Resumido*

Ana Pêgo got her Ph.D. in Polymer Chemistry and Biomaterials from the University of Twente, the Netherlands, in 2002. In 2003 she became a researcher at INEB where she is a Principal Investigator since 2012. She is the Co-Coordinator of the NEWTherapies Group and leader of the Biomaterials for Neurosciences team. By using nanomedicine strategies the Biomaterials for Neurosciences team aims at providing in situ and in a targeted manner the required signals to promote nervous tissue regeneration.
She has been appointed the Scientific Director of the Bioimaging Centre for Biomaterials and Regenerative Therapies of INEB in 2010 and she is an Invited Auxiliary Professor at the Faculty of Engineering of the University of Porto (FEUP) and Affiliated Professor at Instituto de Ciências Biomédicas Abel Salazar (ICBAS) of the University of Porto.

Abstract / *Resumo da Comunicação*

Regenerative medicine can be defined as the science of persuading the body to heal itself by providing, in situ, the molecular signals, cells and structures that can promote tissue regeneration.
Regenerative Medicine approaches are based on the development and/or manipulation of molecules, cells, tissues or organs towards the repair, replacement or support of body parts that suffered a lesion.
The progress achieved in this new area of medicine has also profited at large of the new knowledge of the properties of the tissues at the nanoscale as well as of the design of new biomaterials also with defined nanostructures.
In this talk I will illustrate, based on examples of work being conducted in my team, "small" contributions supported by nanotechnology to the area of regenerative medicine.

C.03 - Perspectives in solid lipid nanoparticles development
Perspectivas no desenvolvimento de nanopartículas sólidas lipídicas

Speaker / *Prelector*

Slamovira Doktorovova

Resumé / *Currículo Resumido*

Slavomira Doktorovova holds a Master degree in Pharmacy, received from Comenius University in Bratislava, Slovakia. Currently she is a PhD student at Institute for Biotechnology and Bioengineering - Centre of Genetics and Biotechnology, University of Trás-os-montes e Alto Douro (IBB-CGB/UTAD) in Vila Real, Portugal. Her current research tasks focus on targeted drug delivery by means of lipid carriers (solid lipid nanoparticles).

Abstract / *Resumo da Comunicação*

Solid lipid nanoparticles (SLN) were developed in the beginning of the 1990-ties, simultaneously but independently by laboratories in Germany and Italy. SLN are colloidal drug carriers consisting of lipid cores that are solid at room and body temperature and stabilized by suitable surfactant(s) layer. Material used in traditional pharmaceutical forms like fatty acids, triglycerides, phospholipids, polysorbates, or poloxamers are used; therefore the resulting drug carrier has pre-requisites to be safe.
Twenty years after patenting SLN, more than 350 individual formulations of various drugs exist; plus many reports dealing with drug-free SLN, concerning their optimal formulation design, optimal production parameters, physico-chemical properties and interaction with cells in culture and animal tissues. Several patents held by pharmaceutical companies concerning SLN are filed. SLN technology is already implemented in some cosmetic products.
SLN were developed primarily with the aim of conferring improvement in cancer therapy. There is already evidence of capacity of SLN improving drug internalization by (cancer) cells and there are some reports on reversal of resistance to chemotherapeutic drugs. The effect of SLN per se is often addressed only marginally in SLN reports, but the safety of the carrier itself and its contribution to the observed effects in vitro will be highly important if SLN are to move from pre-clinical to clinical phases of research, leading to potential marketing authorization.
We have collected information about SLN interaction with (mostly human) cells in culture from the scientific reports available up to date (over 120 reports) and analyzed their impact on i) cell viability in function of used dose, ii) cellular membrane integrity and iii) sub-lethal effects (oxidative stress, genotoxicity). We have indicated possible problems with current methods for evaluation of interaction with cells, and the lack of use of uniform methodology across the available reports.

1st Session (cont.) | Sessão 1(cont.)
Nanomedicine: past and future | *Nanomedicina: passado e futuro*

C.04 - Oral delivery of insulin
A administração oral de insulina

Chairman / *Moderador*

Helena Florindo

Resumé / *Currículo Resumido*

Helena F. Florindo has completed her PhD in 2008 and since then she is an assistant professor at Department of Galenic Pharmacy and Pharmaceutical Technology, School of Pharmacy, University of Lisbon.
She is currently principal investigator of internationally evaluated research projects which falls within the pharmaceutical sciences, and has recently established a research team with six PhD students and two MsC students, who has been mainly involved in the development of nanotechnology-based immunotherapeutic strategies to overcome tumor immunosupressive properties by specific immune cell targeting.
Helena is co-author of peer-reviewed international papers, book chapters and most of her research work has been communicated in the form of oral communications and posters at important international meetings.
Since 2009, she is member of veterinary medicines evaluation commitee of the Direcção Geral de Alimentação e Veterinária, Ministério da Agricultura.

Speaker / *Prelector*

Philippe Maicent

Resumé / *Currículo Resumido*

Philippe Maincent has completed his PhD in 1981. He is Full Professor of Pharmaceutical Technology and the director of the research group (EA Cithéfor; about 20 researchers). He has published more than 100 papers in reputed journals and serving as an editorial board member of repute. He is the associate editor Europe to "Drug development and Industrial Pharmacy". He is the author of 3 patents and about 200 presentations at international symposia. He served as an external assessor at the French Drug Agency (ANSM). He was vice-president of the marketing authorization committee and also president of the quality working groups (pharmaceutical and generic).

Abstract / *Resumo da Comunicação*

Since its discovery, insulin remains the major treatment for type 1 diabetes and many type 2 diabetic patients, insulin being administered parenterally.
The oral route of insulin delivery, being the most convenient, would also be the most physiological, taking advantage of the portal-hepatic route of absorption and adequate insulin delivery avoiding peripheral hyperinsulinemia while potentially preventing adverse effects of weight gain and hypoglycemia. However, insulin is less absorbed by the intestinal mucosa and is rapidly degraded enzymatically in the gastro-intestinal tract.
Several strategies to overcome these problems will be described. Among them, polymeric biodegradable and biocompatible nanoparticles elicit a long-term anti-diabetic effect after oral delivery in diabetic animals, by protecting insulin against degradation and by facilitating the uptake of insulin associated or not to the nanoparticles through a paracellular, or a transcellular pathway. Short-term formulations under clinical development will also be discussed.

1st Session (cont.) | Sessão 1(cont.)

Nanomedicine: past and future | *Nanomedicina: passado e futuro*

C.05 - Targeted nano-oncologicals to battle cancer

Nanoprodutos direccionados ao tratamento do cancro

Speaker / *Prelecto*

María de la Fuente Freire

Resumé / Currículo Resumido

María de la Fuente obtained her PhD degree in 2006 at the University of Santiago de Compostela (Spain), in the field of nanomedicine and nanotechnology. She was a visiting scientist at the University of Angers (France), the University of Valladolid (Spain), the University of Kuopio (Finland), and the Institute of Biomedical Research "Alberto Sols" in Madrid (Spain). She worked as a post-doctoral researcher at the UCL-School of Pharmacy, London (UK) (2007-2010), and at the Nanobiofar group, University of Santiago de Compostela (2010-2012). She currently holds a research position ("Miguel Servet" program) at the Health Research Institute - Clinical University Hospital of Santiago de Compostela (Spain), Translational Medical Oncology group. María is the author of more than 20 scientific articles published in internationally recognized journals and book chapters, two international patents, and a number of presentations at international congresses. Her research is focussed on the engineering, development, characterization, and preclinical evaluation of novel nanocarriers for the delivery of APIs, biotech drugs and gene therapies to target cancer cells and improve the outcome of metastatic disease.

Abstract / *Resumo da Comunicação*

Despite the enormous amount of research efforts devoted to the development of new oncological therapies, the advances made are still limited in metastatic disease due to the complex pathways and multiple cellular components involved in cancer dissemination. Poor patient survival is directly attributable to widespread metastasis, drug resistance and the lack of effective treatment strategies. Therefore, the development of new strategies aimed to identify novel therapeutic targets and overcome the mechanisms of cancer resistance and dissemination is urgently needed.

The field of nanotechnology applied to the delivery of bioactive molecules is having increasing impact in the development of new oncological therapies. Indeed, currently there are 10-marketed anticancer nanomedicines and a few dozens under clinical development. Nanocarriers can be prepared out of a wide array of materials, including safe biomaterials that are degradable by normal means to the host and be specifically designed for effective association a wide variety of molecules and biomolecules: from small hydrophobic drugs to hydrophilic macromolecules, contrast agents and radionuclides, alone or in combination. They can be fine-tuned to modulate their plasma circulation times and biodistribution, so that they can overcome specific biological barriers, have an improved penetration of drugs in the tumour-surrounding environment, and accumulate in the tumour by passive and active-driven mechanisms. Active-driven mechanisms involve their functionalization with one or more targeting ligands to specific cell biomarkers, for preferential interaction/accumulation with cells of interest.

Although up until now the design of drug nanocarriers has largely relied on achieving an improved delivery of cytotoxic drugs into the cancer cells, currently nanotechnology is expected to revolutionize cancer treatment. Nanocarriers can deliver multiple combinatory and targeted therapies, based on gene therapies and novel biomolecules, to increase the efficacy of currently available anticancer treatments. Moreover, nanocarriers can simultaneously address other cell types involved in tumour progression, as those forming part of the tumour microenvironment.

C.06 - Polysaccharide-based nanoparticles: useful tools in transmucosal nano drug delivery

Nanopartículas baseadas em polissacáridos: ferramentas úteis para administração transmucosal de fármacos

Speaker / *Prelecto*

Ana Grenha

Resumé / *Currículo Resumido*

Ana Grenha has completed her PhD in Pharmacy in 2007. She is Assistant Professor of Pharmaceutical Technology at the University of Algarve and Principal Investigator of the Bioencapsulation Group in the Centre for Molecular and Structural Biomedicine (CBME), which integrates the Associate Laboratory Institute for Biotechnology and Bioengineering (IBB). She has published around 20 papers in reputed journals and several book chapters. She is Section Editor of Journal of Pharmacy and Bioallied Sciences and has more than 50 presentations at international symposia.

Abstract / *Resumo da Comunicação*

Polysaccharides have been used very frequently in the design of drug delivery systems and, particularly, nanoparticles. Their structural flexibility, low cost, high availability and propensity for biocompatibility and biodegradability, due to the natural original, are the main reasons for their preferential selection. Chitosan, alginate, hyaluronic acid and dextran are among the most used polysaccharides in the preparation of nanocarriers. However, the universe of polysaccharides is wider and includes other materials that might exhibit potential as well. Carrageenan, locust bean gum, fucoidan, chondroitin sulfate and pullulan are some of the examples.

In this talk, several formulations of nanoparticles based on these "not so explored" polysaccharides are reported for an application in nasal and/or lung transmucosal protein delivery. BSA and/or insulin are used as model proteins. The complete characterisation of the nanocarriers, which includes the determination of morphology, size, zeta potential, production yield, encapsulation efficiency, loading capacity and release profile, will be shortly presented. The main focus of the presentation will rely on biocompatibility studies performed on Calu-3 and A549 cells (respiratory cell models), resulting from the exposure to the nanoparticle formulations. These studies comprise a typical cytotoxicity assay (MTT), as well as the determination of both the inflammatory response (cytokine release, namely IL-6 and IL-8) and the integrity of the epithelial barrier, by means of the measurement of transepithelial electrical resistance, when applicable.

National funding from the Portuguese Foundation of Science and Technology is acknowledged (project PTDC/SAU-FCF/100291/2008 and PEst-OE/EQB/LA0023/2011).

2st Session | Sessão 2
Biomedical nanotechnologies | *Nanotecnologias Biomédicas*

Chairman / *Moderador*

António J. Almeida

Resumé / *Currículo Resumido*

António J. Almeida is currently Full Professor of Pharmaceutical Technology at the University of Lisbon, Faculty of Pharmacy and the leader of the NanoDDS Research Group of the Research Institute for Medicines and Pharmaceutical Sciences (iMed.UL).

He has been working since 1990 in nanoparticulate carriers for vaccine delivery by the mucosal routes, using nano/microparticles produced with PLGA, PCL and solid lipids. He has started his research work in this field during his PhD studies at the University of Aston, Birmingham, UK (1990-1993), where he published some pioneer works on nasal immunisation with antigen-adsorbed PLGA microspheres and described the uptake and translocation of microspheres at the rat's nasal mucosa. His research group has also focused in lipid nanoparticles (LN) for protein delivery, having worked as a Visiting Scientist at the Free University of Berlin, Germany (1995/1996).

A new approach to the production of an anti-streptococcal vaccine has been developed, particularly against equine strangles (Streptococcus equi), demonstrating that association of antigens with polymeric microspheres induces full protection against experimental infection.

He has worked as a Visiting Professor at the Center for Research in Molecular Medicine and Chronic Diseases (CIMUS), University of Santiago de Compostela, Spain (2012).

Since 1996 he has been a quality expert involved in the evaluation of new medicines, both at national (INFARMED) and international (European Medicines Agency) levels.

He has published several book chapters, papers in reputed journals, patents and presentations at international symposia. He has served as an editorial board member of J. Biomed. Nanotechnol., J. Microencapsul. and J. Drug Deliv. Sci. Technol.

C.07 - Development of a new approach to the periodontal regeneration *Desenvolvimento de uma nova abordagem para a regeneração periodontal*

Speaker / *Prelector*

Carlos Viegas

Resumé / *Currículo Resumido*

1991 - Degree in Veterinary Medicine from the Faculty of Veterinary Medicine - Technical University of Lisbon, Portugal.

1997 - Master Science in Animal Experimentation at the Medicine School - Coimbra University.

2004 - European PhD title in Veterinary Sciences at the Faculty of Veterinary Medicine - Complutense University of Madrid.

2005 - Specialist Degree in Dentistry and Maxillo-Facial Surgery at the University Complutense of Madrid.

2010 - President of the Portuguese Society of Veterinary Dentistry (SPMEDVE).

2013 - Vice-President and Pedagogic Council President of the School of Agriculture and Veterinary Sciences, Head of the Veterinary Sciences Department - UTAD.Researcher from 3 B´s research (ICVS-UMinho) group with several published publications in international journals in the area of periodontal disease genomics and periodontal regeneration.

Abstract / *Resumo da Comunicação*

Periodontitis is highly prevalent in humans. As the current therapies are often inefficient, even bone grafts or growth/differentiation factors, Tissue Engineering (TE) could be an alternative. Thus, a tissue-engineered double layer scaffold (DLS) based on starch+poly-caprolactone (SPCL) enriched with adipose stem cells (ASCs) was developed for periodontal regeneration. A SPCL membrane which aims at acting as GTR barrier, and a wet-spun fibre mesh without and with osteoconductive silanol groups were combined to obtain the DLS. DLS was characterized by Fourier Transmission Infra-red (FTIR), scanning electron microscopy (SEM), mechanical and degradation tests. Canine ASCs were seeded/cultured onto the scaffolds and then characterized by MTS, DNA quantification, SEM, PCR and ALP quantification. The same cells were subcutaneously transplanted in mice and assessed the host response.

Ultimately, DLS were implanted in a mandibular rodent defect and compared to collagen commercial membranes. After 8 weeks, new bone formation was quantified by the Donath technique.

Functionalization with silanol groups was confirmed by FTIR. DLS exhibited adequate tensile strength and degradability and provided a good support for canine ASCs adhesion and proliferation. SPCL-DLS-Si revealed higher expression of osteoblast genes. These cells also did not induce any immunogenic reaction in the host. Histomorphometry revealed that SPCL-DLS-Si induced higher bone formation compared to collagen.

SPCL-DLS-Si bioactive matrix with the canine ASCs revealed good potential to be used in periodontal and *bone* TE strategies, and could also be proposed in non-autologous canine preclinical studies before human clinical applications.

2st Session | Sessão 2
Biomedical nanotechnologies | *Nanotecnologias Biomédicas*

C.08 - The path from milli through micro to nano: Does size matter in the encapsulation and delivery of bioactive therapeutics?

Transição da escala micro para nano: o tamanho importa no encapsulação e administração de fármacos?

Speaker / *Prelector*

Ronald J. Neufeld

Resumé / *Currículo Resumido*

Ronald Neufeld completed his PhD in 1980 after working for several years in Industrial Engineering and Process Development. He served as a Professor and Associate Dean in the Engineering Faculty of McGill University (1980-1997), and as Department Head and Professor of Chemical Engineering at Queen's University (1997 to present). He has published 140 papers in refereed international journals and serves on the editorial board of several international journals. His research involves the development of nano and microparticulate systems for the encapsulation, protection and controlled delivery of therapeutic cells, biopharmaceuticals including insulin, enzymes, and other bioactives.

Abstract / *Resumo da Comunicação*

Encapsulation for the controlled delivery of bioactives and therapeutics generally involves the formulation of spherical particulate materials. Control of design parameters such as matrix polymer, drug loading, and particle diameter can ensure that therapeutic objectives are met. Objectives may include drug targeting, controlled delivery, drug stabilization, and reduction in drug toxicity.

The path toward developing drug loaded nanoparticulates will be described involving use of natural, synthetic and semi-synthetic polymers. Alternative delivery vehicles will also be described including use of natural fibers and nanofibers, implantable polymeric cylinders, and the incorporation of particulate drug vehicles into natural polymer based dressings and hydrogels. A case study will be presented involving the development of nanoparticulate insulin for oral delivery.

C.09 - Antifungal nanoparticle conjugates

Conjugados de nanopartículas e fármacos antifúngicos

Speaker / *Prelector*

Cristiana Paulo

Resumé / *Currículo Resumido*

Cristiana Paulo has recently completed her PhD in Bioengineering Systems in the MIT Portugal Program. The work was developed at Biocant, under supervision of Dr. Lino Ferreira. The result of her project was a Patent of antifungal nanoparticles for the coating of biomedical devices. The work was published in Biomacromolecules and Biomaterials and has been presented in several international conferences. At the present, she is chief technology officer at Matera, a startup company that produces antimicrobial nanoparticles and coatings for biomedical and environmental applications.

Abstract / *Resumo da Comunicação*

Nosocomial fungal infections are often associated with medical devices. We developed antifungal nanoparticle conjugates that can efficiently kill fungi. For that, we covalently immobilized amphotericin B (AmB), potent antifungal, widely used in clinical practice and effective against a large spectrum of fungi, onto silica nanoparticles (SNP). These antifungal nanoparticle conjugates (SNP-AmB) kill several strains of Candida sp., mainly by contact, and are more fungistatic and fungicidal than 10 nm colloidal silver. SNP-AmB maintain their antifungal activity when they are immobilized on a surface. Coating with SNP-AmB can be used to produce antifungal medical devices. Surfaces coated with SNP-AmB have no cytotoxic effect against human blood cells.

We explored the mechanisms of toxicity of SNP-AmB on human endothelial cells and fibroblasts. The concentrations used were higher than cells would encounter if a device released all the SNP-AmB. Human endothelial cells internalize high amounts of SNP through macropinocytosis while human fibroblasts internalize less SNP. We further show that concentrations of SNP-AmB and SNP up to 400 μg/mL do not substantially affect fibroblasts. In contrast, endothelial cells are sensitive to low concentrations (above 10 μg/mL), in particular of SNP-AmB. This is because endothelial cells internalize high amounts of nanoparticles and their membrane is more sensitive to AmB. Low-moderate concentrations of SNP-AmB (up to 100 μg/mL) induce the production of reactive oxygen species, LDH release, expression of pro-inflammatory cytokines and chemokines and high expression of heat shock proteins. High concentrations of SNP-AmB (above 100 μg/mL) disturb membrane integrity and rapidly kill human endothelial cells.

3st Session | Sessão 3

Nanochemistry and nanophysics | *Nanoquímica e nanofísica*

Chairman / *Moderador*

Teresa Neves Petersen

Resumé / *Currículo Resumido*

Teresa Neves Petersen holds a Diploma (1994) in Biochemical Engineering from the Technical University of Lisbon, Portugal, a master degree from the University of St Andrews, Scotland, and a PhD (2000) in Biophysics from Aalborg University, Denmark. During her PhD she has carried out protein electrostatic and structure/function relationship studies, analyzed large protein structure data sets to derive sequence/structure/function insights (protein data mining), NMR studies and carried out research on the interaction between UV light and proteins. Fluorescence spectroscopy has been a key technology during her studies. During her PhD she worked at the Technical University of Lisbon (IST), University of Trondheim (Magnetic Resonance Center) in Norway, Aalborg University in Denmark, and University of Maryland (Center for Fluorescence Spectroscopy) in USA. As a postdoctoral researcher for 3 years she spent time at Aalborg University and at the Lund Laser Center, Lund University, Sweden where she worked on ultrafast spectroscopy studies on proteins and on the effects of UV light on proteins.

In September 2003 Teresa Petersen became an Associate Professor at Aalborg University.

In 2004 she established the "Ultrafast Biospectroscopy Laser Lab" facilities at Aalborg University, a key laser facility and an associate member of LaserLab Europe. She was the co-founder and Chief Science Advisor of BioNanoPhotonics A/S from 2003-2009, a university owned company that has developed a competitive method for the production of micro-arrays, bio-functionalized nanoparticles and surfaces coated with biomolecules.

In 2011, Teresa Petersen became a Principal Investigator at INL, the International Iberian Nanotechnology Laboratory, leading the BioPhotonics group, where her work is focused on the interaction between light and biomolecules, in particular proteins, and its applications in nanomedicine. Teresa Petersen has established a new laser lab facility at INL, and this lab is also an associate member of LaserLab Europe. A main application is the use of light in order to develop a new photonic cancer therapy. Teresa Petersen has a keen interest on the effect of UV light on key pharmaceutical proteins, at the molecular level. The BioPhotonics group is part of the Nanomedicine Department.

Teresa Petersen is a Program Committee Member since 2004 within the Bios session on SPIE Photonics West meeting (The International Society for Optical Engineering).

Teresa Petersen has published 62 peer-reviewed papers and 5 patents.

C.10 - Developing the nanochemistry and nanomaterials areas at the University of Madeira

Desenvolvimento das áreas Nanoquímica e Nanomateriais na Universidade da Madeira

Speaker / *Prelector*

João Rodrigues

Resumé / *Currículo Resumido*

João Rodrigues got the Ph.D. on Inorganic Chemistry from the University of Lisbon in 1999. Since 1997, he is professor at UMa - University of Madeira, where he is responsible for the areas of general chemistry, inorganic/organometallic chemistry, nanochemistry and nanomaterials. He is the Head of Centro de Química da Madeira, Leader of the Molecular Materials Research Group, Director of the Master in Nanochemistry and Nanomaterials and member of the governing body (General Council) of UMa.

His scientific work has been devoted to the development of potential useful molecular materials, namely dendritic (hyperbranched), polymeric metal-containing systems and nanoparticles, having in view their potential use as electronic and/or biomedical nanomaterials. He is author/co-author of 34 peer-reviewed articles (h=12), 1 book chapter, 9 proceeding papers, 15 invited lectures in international conferences and 50 other oral presentations.

Abstract / *Resumo da Comunicação*

Nanosciences and nanotechnologies are topics of great economical and social impact. These areas of knowledge are transversal to other sciences and are not exclusively important for the most developed and rich countries in the world, like USA and Germany (Fig.1). Indeed, their contribute to increase the quality of life and the economy of undeveloped countries, as well as those from the outermost regions of Europe (like Madeira Island), deserves a particular attention. As a matter of fact, nanotechnologies are expected to help overcoming global asymmetries, create more local jobs and be an opportunity for the creation of new knowledge-based enterprises.

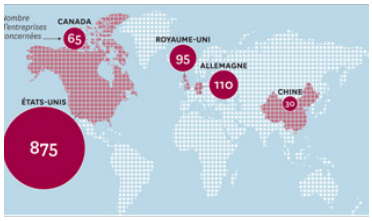

Figura 1 - Most important countries in the Nano field
(Credits: Courrier International, nº:1183/06.07.2013)

In this communication, the strategy under development at the University of Madeira (UMa) in the Nano field, the main achievements, the importance of the field for the UMa internationalization strategic plan, the ongoing research projects, and the new Master in Nanochemistry and Nanomaterials will be presented.

Acknowledgements: FCT- Fundação para a Ciência e a Tecnologia (Pluriannual base funding of CQM, PEst-OE/QUI/UI0674/2011-ext. 2013), and Santander bank (Invited Chair in Nanotechnology).

3st Session | Sessão 3

Nanochemistry and nanophysics | *Nanoquímica e nanofísica*

C.11 - Scanning probe microscopy: a nanotechnology tool in life sciences

Microscopia de varrimento por sonda: uma ferramenta da nanotecnologia nas ciências da vida

Speaker / *Prelector*

Luís Melo

Resumé / *Currículo Resumido*

Luís Viseu Melo has completed his PhD in Engineering Physics at IST in 1996. He is a Professor at the Physics Department of IST, where he co-leads the first SPM laboratory installed in Portugal. He has co-authored more than 50 papers in international publications. He is Vice-President of the OECD Working Party on Nanotechnology and a member of the EC High Level Group on Nanotechnology.

Abstract / *Resuno da Comunicação*

Richard Feynman, in 1959, pointed out for the first time the potential of being able to work at the scale of the biological processes in cells. The Scanning Probe Microscopy family of techniques provides this possibility. In these techniques a narrow probe scans the sample surface and one interaction between the tip and the surface is monitored. This can be the tunneling current (STM, Scanning Tunnelling Microscopy), the Van der Waals force (AFM, Atomic Force Microscopy, the most widely used), magnetic force (MFM, Magnetic Force Microscopy) or others. These instruments also allow for the modification of the surface of the sample and for manipulation at the nanoscale.

The STM was awarded the Nobel Prize for Physics in 1986 (H. Rohrer and G. Binnig) shortly after its invention in 1981, and meanwhile the family of techniques has evolved. It is now widespread, and new applications are being found every day.

C.12 - Highly luminescent nanomaterials

Nanomateriais altamente luminescentes

Speaker / *Prelector*

José Paulo Farinha

Resumé / *Currículo Resumido*

PhD in Chemical Engineering (Gaspar Martinho, IST, 1990)
Postdoc in Polymer and Colloid Chemistry (M. A. Winnik, University of Toronto, 1997-1999)
Professor of Physical Chemistry Materials and Nanosciences at IST
Research associate at CQFM-IST and IN:Institute of Nanoscience and Nanotechnology (AL) Over 70 scientific publications and 1000 citations in peer-reviewed journals (h factor 21), several national and international patents, 100 communications in scientific meetings and 20 invited orals.

Abstract / *Resumo da Comunicação*

Luminescent nanomaterials are used in many applications, from advanced imaging techniques to biodiagnostic, etc.
The ability to increase the brightness and photostability of these materials have direct impact on their performance, lowering the limit of detection in diagnostic applications and allowing their use in demanding laser scanning imaging techniques.
Here, we will show the use of different approaches to achieve highly fluorescent materials, using polymers, silica nanoparticles and hybrid metal and semiconductor nanoparticles.
Keywords: Nanoparticles, Imaging, Diagnostics

Panel Comunications | *Comunicações em Posters*

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P.02 **MICRO/NANOFLUIDICS WITH JELLY CHIPS: A "LEARN-BY-DOING" APPROACH.** Joana Brito, Vânia Tavares and Hugo Ferreira.

P.03 **SYNTHESIS AND CHARACTERIZATION OF S-NITROSO-OLIGO-CHITOSAN AS NO-DONOR**. Nuno Martinho, Marie Socha, Catarina Pinto Reis, Pierre Leroy, Philippe Maincent and Stéphane Gibaud.

P.04 **NOVEL DOSAGE FORM FOR ORAL CANDIDIASIS BASED ON ENCAPSULATION TECHNIQUES: LONG-TERM EFFICACY TESTS OF DIFFERENT CARRIER SYSTEMS**. Luís Vasques Roque, Patrícia Rijo, Marina Baptista and Catarina Pinto Reis.

P.05 **RATIONAL DEVELOPMENT OF LIPID-BASED DELIVERY SYSTEMS FOR NYSTATIN – A BIOPHYSICAL STUDY**. Andreia dos Santos, Joaquim M. T. Marquês, Ana S. Viana, Rodrigo R. F. de Almeida and Liana C. Silva.

P.06 **NOVEL DOSAGE FORM FOR ORAL CANDIDIASIS BASED ON ENCAPSULATION TECHNIQUES: THE INFLUENCE OF DIFFERENT CARRIER SYSTEMS.** Luís Vasques Roque, Patrícia Rijo, Marina Baptista and Catarina Pinto Reis.

P.07 **DIFFUSION AND STABILITY STUDIES OF DRUG LOADED BACTERIAL CELLULOSE MEMBRANES.** Joana Portugal Mota, Nuno Hélder Silva, Inês Eduardo, Carmen S.R. Freire, Armando J.D. Silvestre, Carlos Pascoal Neto, Catarina Rosado.

P.08 **NOVEL ANTIBIOTHERAPY USING NATURAL PRODUCTS.** Patrícia Rijo, Sara Figueiredo, Fátima Simões, Catarina Reis, Catarina Silva and Célia Faustino.

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P.011 **SURFACE ANALYSIS OF NANOPARTICULATE SYSTEMS OF PLGA WITH AZELAIC ACID FOR ACNE'S TREATMENT**. Ana Gomes, Lia Ascensão and Catarina Reis.

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P.014 **ENCAPSULATION OF PLECTRANTHUS MADAGASCARIENSIS EXTRACT INTO POLYMERIC BEADS**. Diogo Matias, Marisa Nicolai, Catarina Silva, Catarina Reis, M. Fátima Simões and Patrícia Rijo.

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P.016 **NANOTECHNOLOGY AS POTENTIAL THERAPY FOR ALOPECIA**. Ana Rebelo, Patrícia Rijo, Marisa Nicolai, Ana Monteiro and Catarina Reis.

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P.029 **INEXISTENCE OF NANOPRODUCT REGULAMENTATION (GMP): WHAT ALTERNATIVES WE HAVE?** Bruno Gouveia, Tânia Gonçalo and Catarina Reis.

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P01. Wavefront shaping methodologies to improve light concentration inside biological tissues

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Introduction: Although light has long being used in biomedicine, scattering always limited its application. Superficial (tenths of micron for human epidermis) phototherapy is already a well-established application, requiring intrusive methods to deliver light when the target is deeper inside the body. Several work teams have developed different methods to achieve beam shaping, being the two most common using a Spatial Light Modulator (SLM) [1] and using a Deformable Mirror [2]. This study aims the development of new methodologies to concentrate light inside biological media in a non-invasive way, one of the last frontiers in laser biomedical applications. Preliminary tests with unpolished glass and low level induced aberrations have already take place using the deformable mirror to shape the wavefront.
Objectives: The main objective to be pursued is to develop a new methodology based in phase shaping to concentrate light inside turbid biological media. The aimed application is to give means to the development of multifunctional nanoparticles which phototherapeutic potential can be improved by their in-depth activation. In the present phase of the work, the objective is to determine the constrains to be taken in consideration and to analyse the early performance of the methodology being developed.
Methods: The first step was to test different configurations for the system to choose the one, which produced the best results in the beam wavefront shaping. Though a laser with wavelength around 800 nm (near-infrared) will be used in the future due to low absorption in the biological tissues for this wavelength, for practical reasons, a 635 nm (visible) laser was used in the initial tests reported in this work. We used an Oko Technologies 15 mm 37-channel Micro Machined Deformable Mirror working together with an Oko Technologies uEye wavefront sensor equipped with a 700 spots Shack-Hartmann mask both integrated by FrontSurfer software supplied by OKO to shape the beam. The collimated beam was first reflected to the deformable mirror and focused into the unpolished glass. A lens placed after the glass creates an image of the focal point in the wavefront sensor; then, the FrontSurfer software gives the mirror new improved configurations towards obtaining a plane wave. In our tests, the corrective loop was stopped after 5000 iterations.
Results: To the present date only preliminary results, testing the system with unpolished glass, were achieved. Wavefront improvements of 39% in peak-to-valey (43% in RMS) and 46% regarding Optical Power were achieved after the iterative wavefront shaping.

	Peak-to-valley	RMS	Optical Power
Before	1.914	0.413	1.331
After	1.371	0.289	0.912

Table 1 – Results before and after wavefront shaping.

Conclusions: With the achieved results we conclude that, though we're still in an early stage of development, the setup works and gives us an optimistic perspective of success to the work ahead.
Acknowledgements: This work was partially supported by national funding by the FCT - Portuguese Fundação para a Ciência e Tecnologia through the project PTDC/BBB-BMD/0611/2012.
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P02. Micro/nanofluidics with jelly chips: a "Learn-by-Doing" approach

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Introduction: Micro/nanotechnology fields are in fast development and micro/nanofluidics are a strong driving force [1]. The motivation behind this work is the teaching of nontrivial concepts related with micro/nanofluidics. Using unexpensive, non-toxic and easy materials is possible to apply a "Learning-by-Doing" approach to reach a young and lay public [2].
Objectives: The main goal of this work is to provide an interactive way of learning about micro/nanofluidics concepts including soft-lithography and several concepts such as pressure-driven flow, laminar flow and turbulent flow. The aim is not only to understand the theoretics but also to visualize and to understand its meanings. Another important goal is to introduce other thematic areas into this innovative form of teaching such as chemistry.
Methods: The work is divided into three fundamental steps. The first one is the manufacturing of a basic jelly chip starting with the production of a round plastic mold with a simple channel shape. The base of the mold is formed by plastic dish and the channel is formed with wooden sticks used for mixing coffee. Then a mixture of water and jelly is poured into the described mold to solidify. Afterwards, the jelly chip is ready to be stripped from the plastic mold and used. The second step consists of the demonstration of micro/nanofluidics concepts previously discussed. Therefore, two distinct chips are made: one with a simple channel with just one inlet hole and one outlet hole and another one with a channel forming a Y or a T, meaning two inlet holes and one outlet hole. For the third step a chip with two parallel channels is made and inside each channel a pH indicator strip is placed. Solutions containing food dyes are used during experiments for easier visualization.
Results: Using the jelly chip it is possible to observe several phenomena. In the case of the pressure-driven flow using the simplest channel, the blocking of the outlet hole of the channel stops flow. This experiment illustrates the fact that the air remaining on the channel exerts pressure and that higher pressure gradients are needed at ever-smaller scales. Injecting two different colored fluids in each of the Y channel's inlets it is possible to observe that fluids mix with difficulty. This experiment illustrates several concepts such as laminar and turbulent flow s, and diffusion at the interface of the two fluids. Finally, the jelly chip with two parallel channels enables the identification of two unknown fluids' pH values and illustrates potential applications of micro/nanofluidics as reactors and sensors.
Conclusions: As it is known, the "Learning-by-Doing" approach is one of the most powerful methods of teaching and captivating both young and lay people. It is also a way to simplify what otherwise could be extremely hard to visualize and understand. Thus, micro/nanofluidics with jelly chips as it was described could be easily implemented as a high school protocol for encouraging academic training.
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P03. Synthesis and characterization of S-nitroso-oligo-chitosan as NO-donor

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Introduction: Nitric oxide (NO) is an endogenous modulator involved in several physiological intra- and extracellular pathways, especially vasorelaxation. NO is present in different amounts and duration within different parts of the body and therefore the controlled release of optimal amounts is necessary for an effective treatment [1]. S-nitroglutathione (GSNO), an endogenous NO-bearing molecule, is expected to have highly therapeutic value but the high susceptibility to decomposition makes its use highly challenging. To address this issue, polymers and polymeric particles have arisen as an interesting alternative to incorporate NO-bearing molecules due to improved stability and altered biodistribution [3].
Objectives: The goal of this work was to synthesize a NO-bearing polymer, S-nitroso-oligo-chitosan (SNOC), with different amounts of NO – namely SNOC/G1 (less NO amounts) and SNOC/G2 (higher NO amounts). Both polymers were characterized for their NO content and SNOC/G1 was analyzed for its stability at different pH conditions.
Methods: 1. Synthesis of GSNO: GSNO was synthesized in solution and used without further purification by reaction with $NaNO_2$ (1:1 mol/mol) in acidic conditions and adjusted to pH 7.4 after 1h. **2. Synthesis of SNOC G1 and G2:** The synthesis comprises two/three synthetic steps. First, thiolation of chitosan was achieved through linkage of GSH to amine groups via 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride/N-Hydroxysuccinimide system. Thereafter, the polymer was nitrosated with $NaNO_2$ to obtain SNOC/G1. Since the thiol polymer bears disulfide bonds we performed the nitrosation of all thiol groups by their prior reduction with $NaBH_4$ to obtain SNOC/G2. **3. Purification and Analysis:** Thiol polymer was purified by dialysis (MW cutoff 3.5 kDa) followed by freeze-drying. The thiol content was determined using Ellman's reagent and NO was quantified by UV spectrophotometry (λ = 334 nm) and Griess & Saville method (λ = 570 nm). **4. Stability test:** The stability of SNOC/G1 was compared to GSNO in different pH (pH 1.2, 6.8 and 7.4) at 37ºC using fluorimetry (λ_{ex}=375 nm, λ_{em}=415 nm).
Results: The conjugated polymer displayed an amount of 241.84 and 449.88 µmol/g of polymer immobilized as free thiols and disulfide bonds respectively. High nitrosation yields were obtained and SNOC/G2 had no (expected) disulfide bonds between polymer and GSH as in SNOC/G1. This corresponded to a degree of substitution of 9% in relation to initial amino groups of bulk chitosan or to approximately 3 molecules of S-NO groups per molecule of SNOC/G2. Nevertheless, SNOC/G1 was less stable than GSNO and no pH-dependent effect was observed. This rapid release may be due to the proximity between thiol groups therefore promoting formation of disulfide bonds with release of free GSH, which in turn may promote more disulfide bonds.
Conclusions: There is a great potential for SNOC polymer as a mean to deliver NO. We synthesized a new NO-carrier based on a biodegradable polymer, chitosan. Although the stability of SNOC/G1 showed a short life, further studies on SNOC/G2 should be performed. This also constitutes a platform to use the polymer to produce nanoparticles.
References: [1] Clinical Science (2000) **98**: 507-20. [2] Circulation (2001) **104**: 2263-2265. [3] J. Biomed. Mater. Res. A (2010) **92**: 1233-1243.

P04. Novel dosage form for oral candidiasis based on encapsulation techniques: long-term efficacy tests of different carrier systems

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Introduction: Nystatin (Nys) is an antifungal substance usually used in the treatment of oral lesions such as oral candidiasis. Briefly, Nys binds to ergosterol and forms pores in the fungal membrane that lead to K^+ leakage and death of the fungus. It is generally not absorbed orally and thus not indicated for invasive fungal infections. The recommended dose for oropharyngeal candidiasis is 500,000 to 1,000,000 units 3 to 5 times daily as oral suspension or tablets (dissolved in the mouth) for 1 to 2 weeks [1]. Common side effects include metallic taste, dry mouth, anorexia and nausea.
Objectives: In this study, Nys will be encapsulated into alginate macroparticles, microparticles and nanoparticles, and then these different carrier systems will be incorporated in toothpaste. The aim of this study is to improve the time residence of Nys in the mouth and thus improve the drug bioavailability using an innovative formulation and easily integrated into patient routine.
Methods: Macroparticles were prepared by extrusion/external gelation method meanwhile microparticles and nanoparticles were prepared by emulsification/internal gelation method [2]. No preservatives were used in toothpaste. The encapsulation efficiency (EE,%) was measured on day one (first day after encapsulation) by spectrophotometric method at 216 nm for the macroparticles and 267 nm for the microparticles and the nanoparticles. The susceptibility testing (efficacy test) was performed along the time and results were evaluated by the capacity of inhibition of *Candida Albicans* in Muller Hinton culture medium at 37±0.5ºC with an incubation time of 24 hours and in duplicate [3-5].
Results: EE was 89.6±2.7%, 63.1±8.8%, 85.7±0.9% for macro-, micro- and nanoparticles, respectively. In the susceptibility test, data showed values around ± 1.7 cm for macro-, ± 2 cm for micro- and ± 1.5 cm for nanoparticles after the 1st month of Nys encapsulation. After the 3rd month of encapsulation, lower values were obtained: ± 1 cm for macro-, ± 1.6 cm for micro- and ± 1.1 cm for nanoparticles. Free Nys (non-encapsulated Nys) had ±1.7 cm after the 1st month and ±1.3 cm after the 3rd month of encapsulation.
Conclusions: Despite a lower EE than macroparticles, this study showed that microparticles allowed a higher fungal inhibition and efficiency along the time when compared with the macro- and nanoparticles and even with Nys. Other stability studies are undergoing.
References: [1] [www.infarmed.pt/infomed/download_ficheiro.php?med_id=5846&tipo_doc=rcm [2] Biotechnol. Bioeng. (1998) **57**: 438-446. [3] Microbiologia: Conceitos e Aplicações, 2ed. São Paulo: Makron Books, [4] Microbiologia, 1ed. São Paulo: McGraw-Hill, vol.1, 1980. [5] Mycostantin (in dailymed.nlm.nih.gov/dailymed/archives/fdaDrugInfo.cfm?archiveid=5812).

P05. Rational development of lipid-based delivery systems for Nystatin – A biophysical study

Andreia dos Santos[1,2*], Joaquim M. T. Marquês[1], Ana S. Viana[1], Rodrigo R. F. de Almeida[2] and Liana C. Silva[1]
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[2] CQB, Faculty of Sciences, University of Lisbon, Portugal

Introduction: Nystatin (Nys) is a naturally occurring polyene antibiotic with a strong antifungal activity, a broad action spectrum and low induction of antibiotic resistance. Nys is a promising alternative to current more commonly used antifungal therapies that are becoming ineffective due to acquired resistance particularly in immunocompromised patients. However, Nys' low solubility and elevated cytotoxicity in mammalian cells have kept it from becoming a viable therapeutic alternative. The systemic usage of this antibiotic is therefore dependent on the development of effective carriers for Nys delivery. A rational design of these carriers might be accomplished through a deeper understanding of Nys molecular mechanism of action. It is widely accepted that Nys interacts with membrane lipids, particularly sterols, and forms ion channels that consequently cause the disruption of cell homeostasis. However, the exact molecular mechanism of action is yet to be solve, particularly, it is still not known if Nys has higher affinity to specific membrane lipids or if channel formation is mediated by membrane biophysical properties [1].
Objectives: This work aims to understand Nys interaction with lipid membranes for the rational development of lipid-based Nys nanoparticles to be used as an alternative therapy for fungal infections.
Methods: Liposomes composed of a fluid lipid and different gel-domain forming lipids (sphingomyelin (SM) or DPPC) were used to understand the role of membrane biophysical properties and/or lipid structure on Nys molecular mechanism of action. The liposomes were characterized by photon correlation spectroscopy and surface charge. Membrane properties and Nys-lipid membrane interaction were studied by steady state and time-resolved fluorescence spectroscopy and Atomic Force Spectroscopy (AFM).
Results: The results show that Nys has a stronger partition towards SM containing membranes, but the formation of Nys active species is favored by the presence of a DPPC gel. This is probably due to Nys' ability to stabilize DPPC-gel domains, as suggested by the increase in surface area of gel domains. In contrast, Nys interaction with SM containing membranes, results in a destabilization of SM-gel domains, which might influence the formation of Nys aggregates in the membrane.
Conclusions: The interaction between Nys and the membrane could be modulated by the lipid composition of the gel domains, suggesting that both the type of lipid and the gel nature of the membrane play an important role in Nys molecular mechanism of action. This information is vital for the development of delivery systems that effectively encapsulate Nys and display specificity towards fungal cells.
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P06. Novel dosage form for oral candidiasis based on encapsulation techniques: the influence of different carrier systems

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Introduction: Nystatin (Nys) is an antifungal substance widely used in the treatment of oral lesions such as oral candidiasis. However, it is generally not absorbed orally and thus not indicated for invasive fungal infections. The recommended dose for oropharyngeal candidiasis is 500,000 to 1,000,000 units 3 to 5 times daily as oral suspension or tablets (dissolved in the mouth) for 1 to 2 weeks [1]. Common side effects include metallic taste, dry mouth, anorexia and nausea.
Objectives: In this study, a commercial formulation of Nys will be encapsulated into alginate macro-, micro- and nanoparticles, and then these different carrier systems will be incorporated in toothpaste. The aim of this study is to improve the time residence of Nys in the mouth and thus improve the drug bioavailability.
Methods: Macroparticles were prepared by extrusion/external gelation method meanwhile micro- and nanoparticles were prepared by emulsification/internal gelation method [2]. No preservatives were used in toothpaste. The encapsulation efficiency (EE,%) was measured on day one (first day after encapsulation) by spectrophotometric method at 216 nm for the macroparticles and 220 nm for the microparticles and nanoparticles. The susceptibility testing (efficacy test after 24 h) was tested along the time and results were evaluated by the capacity of inhibition of *Candida Albicans* in Muller Hinton culture medium at 37±0.5ºC with an incubation time of 24 hours and in duplicate [3-5].
Results: EE was 87.2±0.9%, 78.4±0.7%, 75.6±8.5% for macro-, micro- and nanoparticles, respectively. In the susceptibility test, data showed values around ± 2.4 cm, ± 2.7 cm and ± 2.5 cm for the macro-, micro- and nanoparticles. Free Nys showed an inhibition halo around ± 2.5 cm.
Conclusions: Microparticles allowed a higher fungal inhibition when compared with other carriers. Further studies will include the evaluation of this effect along the time.
References: [1] www.infarmed.pt/infomed/download_ficheiro.php?med_id=5846&tipo_doc=rcm [2] Biotechnol. Bioeng. (1998) **57**: 438-446. [3] Microbiologia: Conceitos e Aplicações, 2ed. São Paulo: Makron Books, [4] Microbiologia, 1ed. São Paulo: McGraw-Hill, vol.1, 1980. [5] Mycostantin (in dailymed.nlm.nih.gov/dailymed/archives/fdaDrugInfo.cfm?archiveid=5812).

P07. Diffusion and stability studies of drug loaded bacterial cellulose membranes

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Introduction: Bacteria cellulose (BC) is produced by some bacteria like *Gluconacetobacter sacchari* presenting unique physical and mechanical properties, arising from its exceptional tri-dimensional and branched structure, as well as high purity and biocompatibility. Substantial interest has developed on this material, particularly in products for the regeneration of damaged or diseased skin. Previous studies confirmed that BC could be successfully applied to modulate the bioavailability of drugs [1] and, hence, be used in the production of systems that have, simultaneously, the ability to absorb exudates and adhere to irregular skin surfaces.
Objectives: This study assessed the efficacy and stability of BC membranes as the basis for systems for topical and transdermal drug delivery.
Methods: Caffeine and diclofenac Na were used as high and low aqueous solubility model drugs, respectively. A drug loading process in BC membranes was developed for both molecules, and the drug distribution was evaluated by electronic microscopy. Each system was also compared with conventional formulations already in the market, like gels or patches. *In vitro* diffusion studies with Franz cells were conducted, using human epidermal membranes. Equivalent doses of the different formulations were placed in the donor compartment of the diffusion cells. The multiple steady-state fluxes of caffeine and diclofenac in each formulation system were determined.
In parallel, stability studies were conducted at 40°C/75% RH, room temperature/60% RH and 40°C/0% RH. Drug release was measured before and after 3 months storage, for both drugs.
Results: Diffusion studies showed that the permeation rate of the drugs in BC membranes was similar to that obtained with the conventional systems. No differences on drug release profiles were observed after 3 months storage (all conditions) for both drugs, showing that the formulations were storage stable under heat and/or humidity.
Conclusions: Drug loaded BC membranes proved to be an effective and stable drug delivery system for high and low aqueous solubility drugs. Further studies will be conducted to establish applicability of this technology to other drugs, as well as impregnation of silver nanoparticles with antimicrobial activity for wound healing.
References: [1] Int. J. Pharm. (2012) **435**: 83-87.

P08. Novel antibiotherapy using natural products

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Introduction: Antimicrobial resistance has turned into a public health threat as a consequence of the rapidly emergence and spread of drug-resistant pathogens that cannot be treated with currently available antibiotics [1]. Recently our team has isolated a royleanone compound, 7α-acetoxy-6β-hydroxyroyleanone, from *Plectranthus grandidentatus* which has shown potent *in vitro* antibacterial activity against methicilin- and vancomicin-resistant bacterial strains [2,3] and was also active against a multidrug resistant strain of *Mycobacterium tuberculosis* [4]. In an attempt to obtain more potent and selective inhibitors based on the natural royleanone structural motif, two strategies are being pursuit: i) introducing appropriate substitutions at the C6 and/or C12 hydroxyl functions by esterification reactions; ii) and another strategy based on encapsulation technology which involves encapsulation of the antimicrobial drug in appropriate drug delivery systems, namely polymeric (alginate) particles, simultaneously improving the pharmacokinetic profile while minimizing the royleanone cytotoxic side effects.
Objectives: This study aimed at the development of more potent and selective derivatives of natural antimicrobial royleanone by i) esterification reaction with amino acids, such as *N*-acetyl-L-cysteine, in an attempt to mimic natural antimicrobial peptides [5]; and ii) encapsulation of the novel derivatives in alginate particles for efficient drug delivery.
Methods: The novel antimicrobial agent developed was prepared by esterification of the royleanone lead with *N*-acetyl-L-cysteine, according to established amino acid and peptide synthesis procedures already employed by the research team in the chemical synthesis of membrane-intercalating, amino acid-based antimicrobial amphiphiles [6]. The new derivative was prepared and characterized by spectroscopic methods and encapsulated in alginate particles by extrusion/external gelation method.
Results: A novel amino acid derivative of natural antimicrobial royleanone has been prepared and successfully encapsulated into alginate particles.
Conclusions: The hydrophilic nature of alginate particles combined with the polymer bioadhesive effect makes alginate a very promising vehicle for efficient delivery of the novel royleanone derivative to infected cells in intracellular compartments. Alginate particles loaded with antimicrobial royleanone derivatives may also be applied as anti-biofilm coating in an attempt to reduce microbial adhesion and colonization onto medical devices. This strategy will be exploited to target the novel natural product-based antibiotics to biofilm interfaces and towards intracellular infection at adequate therapeutic levels, based on the solid expertise of the research team on polymeric carriers for drug delivery [7,8].
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P09. Chitosan-based nanoparticles are biocompatible with respiratory epithelial cells *in vitro*

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Introduction: The adequacy of nanoparticles (NP) for systemic delivery of therapeutic macromolecules has been demonstrated in many occasions, providing increased molecule stability along with the possibility of achieving controlled release and/or the targeting of specific sites [1].The lung has long been proposed as a systemic route of administration, but pulmonary delivery of nanoparticles is hindered by inadequate aerodynamic properties, which however might be overcome using microencapsulation strategies [2]. Importantly, any drug delivery system must comply with safety requirements, which first evaluation might be addressed by several *in vitro* assays in relevant cell lines. In fact, it has been said that epithelial cells *in vitro* can be more sensitive to toxicological insult than the epithelium *in situ*.
Objectives: The aim of this work was to evaluate the biocompatibility of respirable powder formulations, consisting of mannitol microspheres containing chitosan/carrageenan/ tripolyphosphate (CS/CRG/TPP) NP, in human respiratory epithelial cell lines.
Methods: CS/CRG/TPP NP were prepared by polyelectrolyte complexation [3], while microparticles consisting of NP:Mannitol (20/80, w/w) were obtained by spray-drying (laboratory-scale mini spray-dryer, Buchi® Mini Spray Dryer, B-290, Buchi, Switzerland), according to a previously developed protocol. The biocompatibility profile of CS/CRG/TPP NP and microencapsulated NP, as well as that of the raw materials involved in nanoparticle production, was determined *in vitro* in Calu-3 and A549 cells by several assays. The cytotoxic behavior of materials/formulations was determined by the MTT test (Calu-3 and A549 cells) at 3 and 24h. The transepithelial electrical resistance (TER) was monitored during 24h after NP exposure (Calu-3 cells) and the inflammatory response generated by the exposure (Calu-3 cells) was quantified by measuring the levels of IL-6 and IL-8 existing in cell supernatants.
Results: CS/CRG/TPP NP (4/1/1, w/w; size: 208 ± 11 nm; zeta potential: +50 ± 2 mV) were efficiently microencapsulated using mannitol as microsphere matrix material, resulting in final adequate aerodynamic diameters for systemic lung delivery (2.76 µm). The exposure of both cell lines to NP and microencapsulated NP (1 mg NP/mL) revealed absence of overt toxicity for up to 24h, with cell viabilities of 90-100%. Additionally, while TER remained practically unaltered in the presence of nanoparticles, no significant inflammatory response was generated by the exposure to the carriers, as IL-6 and IL-8 levels remained similar to control (unexposed cells).
Conclusions: CS/CRG/TPP nanoparticles and microencapsulated nanoparticles demonstrated absent or low cytotoxicity when in contact with cell lines of human origin from the bronchial and alveolar regions of the pulmonary tract. In this manner, this *in vitro* behaviour is an encouraging indicator of the biocompatibility of the tested carriers as lung delivery systems.
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P010. *Locust Bean* Gum-based nanoparticles as antigens carriers

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Introduction: Polymeric nanoparticulate systems have been presented as promising tools to successfully meet the challenge of delivering biopharmaceuticals by oral route [1]. Nanoparticles can be obtained by different methods, although the most accepted are those avoiding the toxicity of organic solvents and aggressive preparation conditions, like ionic interaction [2]. Natural polymers are frequently used in the production of drug delivery systems since they observe more easily the requisites of biocompatibility and biodegradability demanded in biomedical field [3]. Locust bean gum (LBG) is a neutral polysaccharide (galactomannan) very abundant in the Portuguese region of Algarve. Its content in mannose units makes it a very attractive material to specifically target intestinal M-cells, located in the Peyer's patches, which over-express mannose receptors, making LBG-based nanoparticles a promising delivery system for oral immunization [4].
Objectives: The objective of this work is to design LBG-based nanoparticles for oral immunization, which effectively encapsulate different model antigens.
Methods: LBG derivatives were synthesized and characterized by FTIR and SEC[3]. LBG-based nanoparticles were obtained by ionic complexation between negatively charged LBG derivatives and either chitosan (CS) or positively charged LBG derivatives. An antigenic complex from *Salmonella* Enteritidis (HE) and ovalbumin (OVA) were used as model antigens. Nanoparticle size and zeta potential were determined by photon correlation spectroscopy and laser Doppler anemometry, respectively. The encapsulation efficiency of model antigens and the release profile in SGF and SIF were determined by the microBCA protein assay.
Results: Three charged LBG derivatives, one cationic (amine) and two anionic (sulphate and carboxylate), were synthesized and the functionalization was confirmed by FTIR. LBG based nanoparticles were successfully obtained, displaying sizes between 180 and 370 nm, and zeta potential between +9 and +48 mV. HE and OVA were successfully encapsulated in the CS-sulphated LBG (1:2, w/w) nanoparticles with an efficacy of 32% and 26%, respectively. Release profile of both model antigens in SGF and SIF demonstrates a high protection of the encapsulated antigens (max. release of 40% OVA in SGF).
Conclusions: LBG derivatives were effectively synthesized and nanoparticles with adequate physicochemical properties were obtained. HE antigenic complex and OVA were successfully encapsulated into the nanocarrier system and the *in vitro* release profile demonstrates the suitability of the system for oral administration.
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P011. Surface analysis of nanoparticulate systems of PLGA with Azelaic Acid for acne's treatment

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Introduction: Although nanotechnology is a relatively recent technology in scientific research, the development of its central concepts happened over a longer period of time. The significant advances in this technology lead to the development of new diagnosis and therapy methods for several diseases [1]. Poly-D,L-lactide/glycolide (PLGA) copolymer is a worldwide known polymer and is applied to prepare nanoparticles because of the biodegradation and biocompatibility profiles [2]. Azelaic acid (AzA) loaded nanoparticles had been investigated to apply in the treatment of acne vulgaris [2]. Despite these nanoparticles being already investigated in terms of efficacy and safety [2] there is a need to better understand their surface characteristics and modifications concerning the preparation conditions and the loading procedure.
Objectives: The purpose of this study was to evaluate the surface characteristics of a new nanoparticulate system of PLGA with AzA for the treatment of acne in terms of size and morphology by scanning electron microscopy (SEM) analysis.
Methods: Nanoparticles were produced by a modified spontaneous emulsification solvent diffusion method [3]. AzA-loaded and empty nanoparticles of PLGA morphology and size were analyzed by SEM analysis and by photon spectroscopy. The analysis was performed before and after the centrifugation procedure.
Results: Concerning the batches that were assessed before the centrifugation procedure, the empty nanoparticles of PLGA formed a continuous film. There were visible nanoparticles with an asymmetric shape aggregated and suspended in filaments of PLGA. AzA-loaded nanoparticles showed a regular and sharp surface. The AzA-loaded nanoparticles mean size was 378.63 ± 60.86 nm (0.09 ± 0.03, P.I.). Regarding the analysis after the centrifugation procedure, the empty nanoparticles demonstrated an increase of their outer volume (≥2 µm) but maintained their asymmetric shape. AzA-loaded nanoparticles of PLGA maintained their nanosize similar to the batches before the centrifugation procedure but an asymmetric shape was observed. The sample had a homogeneous size distribution.
Conclusions: PLGA nanoparticulate systems morphology and size distribution were influenced by the presence of the drug and the time of analysis regarding the preparation procedure. Further studies will include transmission electron microscopy (TEM).
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P012. Influence of cationic solid lipid nanoparticles on oxidative stress induction

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Introduction: Solid lipid nanoparticles (SLN) are submicron particles with size in the range of 50-1000 nm and composed of physiological, biodegradable and biocompatible lipids that remain solid at body and room temperature. Although these particles present all pre-requisites to be considered safe drug carries, being expected to present low or even none toxicity, their use in medicine requires more evidence of their safety. Oxidative stress is one of the most reported damage associated to nanoparticles, but in SLN it has not been widely studied. The few performed studies have revealed some contradictory result which led us to study the possibility of oxidative stress induction by a set of previously developed and characterized cSLN [1].
Objectives: To study the possibility of oxidative stress induction by a set of cationic SLN (cSLN) in HepG2, a human hepatocellular carcinoma cell line, and if so, if such toxicity is caused by the cationic lipid used in the formulations (cetyltrimethylammonium bromide, CTAB).
Methods: Cellular viability was determined by Trypan Blue exclusion method, the formation of reactive oxygen species was ascertained using DCFH-DA assay, and the alteration of antioxidant enzymes' activities (SOD, GR, GPx) was determined by spectrophotometric assays [2,3,4].
Results: After exposure to cSLN, DCFH-DA assays revealed great increase of ROS production, the activity of SOD was increased and the activity of GR was largely decreased (apoptosis initiation). These results indicate that these nanoparticles cause, indeed, oxidative stress in this cell line, and indicate CTAB as one, but not the only, reason of such toxicity.
Conclusions: This set of cSLN induced oxidative stress in HepG2, thus their use as drug carriers must raise questions and be addressed with caution. However, a hypothesis has been reported in which it is indicated that oxidative stress induction may be advantageous in treatment of cancer. Nevertheless, our results were contradictory and more investigations must be carried out.
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P015. Antioxidant *Plectranthus Ecklonii* extract beads

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Introduction: Currently there is a great interest in delivery systems for bioactive compounds with the purpose of increasing the drug bioavailability and its targeted delivery [1,2]. *Plectranthus ecklonii* is a medicinal plant from the Lamiaceae family that is traditionally used to treat stomach problems, nausea, vomiting, meningitis [3] and skin diseases [4]. Beads are used as stabilizing drug carrier where our team has large experience using the alginate polymer for its biocompatible, biodegradable and non-toxic properties. The use of new *P. ecklonii* extracts in alginate beads will increase the bioavailability of active compounds formulated in a tea-containing microparticles with antioxidant properties.
Objectives: This study aims to prepare *P. ecklonii* extract in alginate beads and evaluate the encapsulation efficiency of the extract after encapsulation process.
Methods: The *P. ecklonii* extract (0.5% w/v) was obtained using a conventional microwave method. The extract main components were quantified by HPLC-DAD and the antioxidant activity of this extract was determined by the DPPH method [5]. The antioxidant extract was encapsulated in alginate polymer using the extrusion/external gelation method. The encapsulation efficiency was evaluated by direct and indirect methods.
Results: Rosmarinic acid (RA) was identified as the main component of this antioxidant extract and was quantified in a concentration of 0.59 mM. The antioxidant activity of this extract regarding its ability to scavenge free radicals showed an IC_{50} value of 129.5 µg/mL. The encapsulation efficiency of the RA of *P. ecklonii* extract in alginate was estimated as 99.9% and 92.6% for direct and indirect method, respectively.
Conclusions: *P. ecklonii* extract-loaded alginate beads were sucessfully prepared. This formulation may be applied as a tea form. This strategy will further applied as nanoparticles and with other bioactive plant extracts.
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P016. Nanotechnology as potential therapy for alopecia

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Introduction: Androgenetic alopecia is a condition characterized by progressive hair thinning and scalp hair follicles miniaturization, having dihydrotestosterone (DHT) as the primary contributor. [1] Finasteride is a type II 5α - reductase inhibitor which inhibits the conversion of testosterone to DHT, reducing DHT in the serum and scalp [1, 2]. This drug significantly increases hair count and improves its appearance through this mechanism [1, 2]. However, it is described that oral finasteride treatment (1mg/day) increases the risk of sexual dysfunction [2]. To the best of our knowledge, there have been no reports of topical formulation of finasteride. Herein, nanoparticles may have a great potential for topical drug delivery since they already showed an improvement of the absorption of poorly soluble drugs, allowed drug targeting, reduced side effects and also increased the drug bioavailability [3].
Objectives: Our purpose was to study the influence of two different polymers, Poly (D,L-lactic-co-glycolic acid) (PLGA) and Polycaprolactone (PCL) on the encapsulation efficiency (EE, %) of finasteride. The amount of free drug in the supernatant was measured by using HPLC analysis.
Methods: Finasteride-loaded PCL and PLGA nanoparticles were prepared by a modified technique of the solvent displacement method and by modified-spontaneous emulsification solvent diffusion method, respectively. Two calibration curves were used for PCL and PLGA. HPLC analysis was performed with UV detection at 210 nm. The analysis was carried out on a RP-18 column with an injection volume of 20 µL. It was an isocratic elution with a 1 mL/min flow. The mobile phase was water: acetonitrile (64:36, v/v). Both, mobile phase and samples were filtered with a millipore filter. Determinations were carried out at room temperature (20-30) and in triplicate.
Results: Calibration curves were obtained and the equations were: for PLGA: y=57.202x - 713.3, R^2 =0.9911 and for PCL: y=45.21x - 369.85, R^2=0.9887. These equations allowed the calculation of the two polymers encapsulation efficiency, resulting in 97.4% for PLGA and 15.5% for PCL. The low encapsulation efficiency for PCL was probably due to the high affinity of the drug for the organic solvents used during the nanoparticle preparation and this may cause a diffusion of finasteride away from the polymer matrix [4].
Conclusions: We conclude that PLGA nanoparticles have greater EE than PCL nanoparticles. It could be attributed to the physicochemical characteristics of the drug and polymers. Finasteride is practically insoluble in water, but has high solubility in organic solvents. In PCL nanoparticle preparation, a great quantity of acetone was used. Acetone may be responsible of the drug diffusion. As finasteride has a great solubility in the outer phase, the amount of the drug in the dispersion medium should be higher. [4] In PLGA nanoparticle method, the amount of organic solvents used was much lower and thus this polymer will be selected for future studies as encapsulant material. This formulation is now under development in order to test whether finasteride nanoparticles are indeed effective for alopecia treatment with lower drug side effects.
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P013. *Lavandula pedunculata* and *Lavandula stoechas* extracts encapsulated into polymeric beads

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Introduction: Two wild Portuguese lavanders, *Lavandula stoechas* subsp. *luisieri* (Rozeira) Rozeira (Syn: *L. luisieri; L. stoechas* var. *luisieri*) and *Lavandula pedunculata* (Mill.) Cav. (Syn: *L. pedunculata* subsp. *sampaiana; L. stoechas* subsp. *pedunculata*) were collected during the flowering stage in the Center and Southwestern regions of Portugal, respectively, and their antioxidant profile was established. We were able to determinate that both *L. stoechas* subsp. *luisieri* and *L. pedunculata* methanol extracts displayed a strong antioxidant activity, being as actives as the control used (Vitamin E). These extracts may be applied both as anti-aging and as anti-inflammatory agents since both physiological processes are related to the excess of free oxidants molecules [1]
Encapsulation can contribute to the stabilization of these compounds and are promising drug carriers, because they can enhance the skin penetration of drugs, deliver the entrapped drugs across cell membranes, and improve sample stability and bioavailability [2]. One of the approaches is the use of encapsulation as efficient alternatives to increase the antioxidant effect of products, representing a potential approach to therapeutic applications that can be apply on dermatology and cosmetic industry.
Objectives: This study assessed the influence of encapsulation of methanol extracts from *Lavandula pedunculata* and *Lavandula luisieri* to increase the antioxidant activity and her bioavailability.
Methods: Two wild Portuguese lavanders *Lavandula pedunculata* (Mill.) Cav. and *Lavandula stoechas* subsp. *luisieri* (Rozeira) Rozeira (leaves and flowers) were collected during the flowering stage, air-dried and extracted with methanol. The antioxidant profile of both was established. Encapsulation was performed according to extrusion external gelation method.
Results: Both plants' methanol extracts displayed a strong anti-oxidant activity, being as active as in the control used (Vitamin E). These extracts may be applied both as anti-aging and as anti-inflammatory agents since both physiological processes are related to the excess of free oxidants molecules [1]. The encapsulation of the extracts was successfully executed.
Conclusions: The two lavanders extracts encapsulated can offer a potential system for targeted delivery to the skin surface, especially for highly lipophilic molecules present in extracts of plants.
References: [1] Ind. & Eng. Chem. Research (2011) **4**: 2088-2097. [2] Drug Target Insights (2007) **2**:147-157.

P014. Encapsulation of *Plectranthus Madagascariensis* extract into polymeric beads

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Introduction: *Plectranthus* genus belonging to Lamiaceae family has showed potent antimicrobial, antioxidant and anticancer activities [1-3]. Previous studies of our team indicate that aqueous of *P. madagascariensis* extracts present potent antimicrobial activity even comparable to reference antibiotics [3]. The long-term stability of these extracts seems to be a major limitation on its application as pharmaceutical ingredients. However, particle encapsulation methodology has been used to enhance stability and bioavailability of several pharmaceutical preparations. Particularly, as encapsulation in calcium alginate beads is a simple, effective and low cost encapsulation method and as alginate is considered as a biocompatible, biodegradable and non-toxic polymer, this methodology can be to be applied to herbal extracts formulations [4].
Objectives: This study intended to prepare and evaluate the encapsulation efficiency of *P. madagascariensis* aqueous extracts into calcium alginate beads.
Methods: Acetonic and several aqueous extracts of *P. madagascariensis* were prepared by infusion, decoction, maceration and microwave techniques. Extraction yields were evaluated after freeze-drying or evaporation of the extract samples. The well diffusion method was used to select the more potent antibacterial *P. madagascariensis extract*. Beads of more active extract were prepared by different extrusion techniques and its encapsulation efficiency was evaluated by HPLC-DAD.
Results: The acetonic maceration method gave both the highest yield of plant extract and the most active extract against *Staphylococcus aureus, Enterococcus faecelis and Bacillus subtilis* (21-36 mm as inhibition zone). The elected *P. madagascariensis* acetonic extract was successfully encapsulated into alginate beads and the encapsulation was positively preformed.
Conclusions: This preliminary study showed the suitability of this technology to increase the stability of natural extracts with potential pharmacologic effects. Further studies should evaluate the controlled release, long-term stability and the biologic activities of *P. madagascariensis* extracts after the encapsulation process.
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P017. Development of pharmaceutical forms for topical finasteride administration

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Introduction: Over the past years there have been a variety of trials evaluating the use of topical finasteride addressing different pharmaceutical formulations, treatment durations, and application frequencies but none of those trials have been successfully performed to date. In this study, we developed several pharmaceutical dosage forms for finasteride. A shampoo is a formulation intended for washing the hair, keeping it in good condition and can be used as a vehicle for topical administration [1]. Lotion is an aqueous liquid for external application without friction. Moreover, it presents additional advantages such as easier application and removal and lower skin irritation. It can be applied over a large area of skin surface and form a thin film, which can lead to high drug concentrations, after water evaporation, what can explain its greater activity [2]. Two or more substances completely dissolved constitute solutions and generally it ensures a rapid action and easy administration. Low stability is a disadvantage [2]. Shampoo formulation may be the first choice of the patient but we expect that lotion and solution may increase the drug time contact with the scalp.
Objectives: Development of three distinct pharmaceutical formulas to finasteride topical administration: shampoo, lotion and solution.
Methods: In formulation of shampoo, Rewomid DC 212 S was added to methylparaben until obtained a consistence of smooth paste. Then, this mixture was added to lauryl ether sodium sulfate and menthol essence. A critic acid solution was added to the amount of water remaining. All previous phases were carefully mixed and glycerol was then incorporated. To the adjustment of the viscosity, Rewomid DC 212 S was added. The lotion contains water, isopropilic alcohol, propylene glycol and microcrystalline cellulose. All the compounds were blended under stirring. Solution was obtained by mixing ethanol 96%, propylene glycol and water. The pH of all formulations was adjusted. Viscosity of the shampoo was measured using Brookfield viscosimeter (S4, 20 rpm and room temperature).
Results: All formulations were clear with a homogeneous aspect. They were colorless and exhibit smooth texture. The shampoo has a pleasant smell; however, lotion and solution have a strong alcohol odor. All formulations exhibited a good spreadability. The shampoo viscosity was 2180 mPas. Finasteride was easily incorporated into those formulations.
Conclusions: Topical formulations were successfully developed. This study is still in development and some important issues are missing. *In vitro* drug release, permeation and toxicity studies using human volunteers are already scheduled.
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P018. Influence of particle size on functional parameters of nano and macro-emulsion sunscreens

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Introduction: Nanotechnology applies to various sectors of Science, and in the cosmetics area, investments have enhanced the idea that nanotechnology products provide real benefits to consumers. Excessive exposure to sunlight can cause side effects such as erythema and skin cancer, thus sunscreens are used in preventing such effects. The ethylhexyl methoxycinnamate and benzophenone-3 are chemical UV filters which absorbs UVB and UVA radiation, respectively, both are widely used in sunscreens products. The increasing consumer demand and advances in knowledge about the production and stability of dispersed systems enable the development of differentiated vehicles, such as nanoemulsions, which besides the inherent stability, show pleasant sensorial aspect, and high spread ability. An efficient method for obtaining nanoemulsion, employing low energy of emulsification is the phase inversion temperature (PIT) based on the change in solubility of ethoxylated nonionic surfactants with temperature variation [1].
Objectives: In this study, sunscreen formulations composed by the same raw material were developed as nano and macroemulsion and both systems were characterized physicochemically and functionally.
Methods: Nanoemulsion, containing ethylhexyl methoxycinnamate (7.5% w/w) and benzophenone-3 (2.0% w/w), was developed by the method of PIT. Macroemulsion was obtained by traditional method broadly described elsewhere. Determination of particle size and size distribution were performed by means of acoustic spectrometer and the determination of *in vitro* photoprotective efficacy was achieved by diffuse reflectance spectrophotometer with integrating sphere [2].
Results: When formulating a product for cosmetic applications, several demands are important, for example, a pleasing appearance, long stability, right consistency (rheology) that can give an agreeable feeling during application, and to provide long term beneficial effects to the skin properties. Achieved nanoemulsion showed bluish brightness aspect, less apparent consistency than macroemulsion, stability longer than 48 hours (22.0 ± 2.0°C) and bimodal particle size distribution with average (mean) sizes around 10 nm (61%) and 4.5 µm (39%). Macroemulsion showed milky aspect, higher consistency than nanoemulsion, instability after 48 hours (22.0 ± 2.0°C) and bimodal particle size distribution with average (mean) size around 202 nm (9%) and 10.4 µm (91%). Average *in vitro* SPF was 8.0 ± 0.9 for nanoemulsion and 12.0 ± 3.0 for macroemulsion. The mean values of UVA/UVB ratio were 0.304 for nanoemulsion and 0.282 for macroemulsion. Finally, average values of critical wavelength were 345.0 ± 0.4 and 344.0 ± 0.8 for nanoemulsion and macroemulsion, respectively.
Conclusions: In this study, results demonstrated that evaluated sunscreens, nano and macro emulsions, have different appearance and particle sizes distribution. Results obtained from *in vitro* sun protection assessment showed that effectiveness profile of sunscreen formulations remained apparently similar and, according to the literature, systems did not reached broad-spectrum.
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P019. Nanoformulations of triazene prodrugs with specific affinity to human melanoma

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Introduction: Melanoma is a common and aggressive malignancy that has been increasing in incidence and mortality during the past 2 decades. Alkylating agents, triazenes, have been used to treat patients; however their lack of specificity and severe toxic side effects pointers to development of new therapies. Tyrosinase is an enzyme located in melanocytes within the melanosome and its activity is up-regulated in melanoma cells, highlighting the use of this enzyme as an excellent target for the specific delivery of chemotherapeutic agents. The synthesis of new triazene prodrugs (TPD), with close structural resemblances with natural tyrosinase substrates, able to be converted into its cytotoxic form, only in melanoma cells by the action of tyrosinase, was the strategy followed in the present work. In addition, the incorporation of these new molecules in liposomes, constituted a complementary approach. The rational for associating these TPD to liposomes is based on the achievement of a nanoformulation that may circumvent the use of toxic solvents for TPD solubilization, protection of molecules from premature degradation, increased internalization in melanoma cell lines, and improvement of their *in vivo* profile that may lead to an alternative strategy against malignant melanoma.
Objectives: Two different TPD were selected in the present work for incorporation in liposomes: the 4-ethoxycarbonylphenyl and the 5-fenoxypentanoic acid derivatives hereafter designed as TPD1 and TPD2 presenting an octanol/water partition coefficient (logP) of 3.2 and 4.3, respectively.
Methods: The incorporation parameters for both TPD were evaluated and the influence of different lipid compositions was compared. The stability of nanoformulations incorporating these two TPD, in human plasma, was assessed and the hydrolysis rates compared with each prodrug in the free form. Cytotoxic effect of both TPD in free and liposomal forms against a human melanoma cell line was performed.
Results: The incorporation parameters of TPD2 in liposomes were not affected either by the rigidity or by the presence of negatively charged phospholipids. However for TPD1, incorporation efficiencies higher than 90% were only achieved for PC liposomes, a neutral and fluid phospholipid. These results evidence the higher hydrophobic character of TPD2 in comparison with TPD1 and consequently higher affinity for the lipid bilayers of liposomes.
The incorporation parameters observed for TPD2 were correlated with the higher stability of these nanoformulations in presence of human plasma and the respective half-lives: more than 160 h whereas, for TPD1 this value was lower than 30 h. In the presence of a human melanoma cell line, the MNT1, TPD1 in the liposomal form demonstrated a higher and faster cytotoxic effect. Forty-eight h after incubation, TPD1 and TPD2 liposomes showed an IC50 of 16 and >125µM. Nevertheless, TPD2 in free form presented an IC50 of 31 µM. These results confirm that TPD2 was not released from liposomes during this incubation period, preventing the premature hydrolysis of the prodrug and consequently their cytoxic effect.
Conclusions: These very encouraging results lead us to the establishment of a murine melanoma model, which will be used to select the most promising compound.

P020. Polymeric-lipid hybrid nanoparticles as drug delivery platforms for skin treatments: characterization and stability studies

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Introduction: Currently, nanocarriers' application to the medical field increases the answer in diagnostic and treatment of complex diseases [1, 2]. Different materials - either of natural or polymeric nature - are selected collectively to formulate hybrid or sophisticated drug delivery platforms, with promising results and significant advantages, compared to traditional approaches [3]. Betamethasone is a high potency corticosteroid, used for inflammatory skin conditions treatment and also as immunotherapy in several cancers, such as melanoma [4, 5].
Objectives: We have assessed the physical characterization parameters for the lipid-polymeric hybrid nanoparticles (LPH-NP), such as: particle size (PS), zeta potential (ZP), polydispersity index (PI), morphology, encapsulation efficiency (EE) (in the case of the loaded LPH-NP) and formulation's pH. Preliminary stability assay was conducted at room temperature (25ºC) for one week. Betamethasone-21-acetate (BTM) was chosen as the model drug for these studies.
Methods: LPH-NP were prepared by solvent displacement method. The BTM content in the supernatant was measured by UV-visible spectrophotometry at 240 nm (Evolution 600, Thermo Scientific, UK) and the EE was then calculated (Linearity > 0.99, n=3). Morphology was assessed by SEM (JEOL 5200LV SEM, JEOL Ltd., Tokyo, Japan). PS, ZP and PI were determined in Delsa™ Nano C (Coulter®, CA, USA).
Results: Empty and BTM-loaded LPH-NP showed a mean size approximately 300 nm, PI around 0.2 and a negative charge (- 8 mV). EE was around 90%. SEM images revealed a homogenous morphology with a few aggregates of nanoparticles. Preliminary stability study indicated that the nanosystems were stable, with little variations, over one week.
Conclusions: LPH-NP are interesting nanosystems for a targeted delivery to the skin layers, especially for highly lipophilic molecules, such as corticosteroids and anticancer compounds. Further studies will focus on the nanoparticles chemical characterization by FTIR and DSC, drug release by HPLC determination and skin permeation assays.
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P021. Curcumin-loaded solid lipid nanoparticles: development, characterization and *in vitro* testing

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Introduction: Curcumin has very broad spectrum of biological activities; however, photodegradation, short half-life and low bioavailability have limited its clinical application. Curcumin-loaded solid lipid nanoparticles were studied to overcome these problems. The aim of this study was to optimize the best formulation on curcumin-loaded solid lipid nanoparticles
Objectives: The aim of this study was to optimize a formulation on curcumin-loaded solid lipid nanoparticles. Secondly, the physicochemical properties including particles size (Z-ave), polydispersity index (PdI), zeta potential (ZP), and encapsulation efficiency (EE) were characterized. In addition, the *in vitro* release behaviour of curcumin and interaction with two cancer cell lines *in vitro* was evaluated.
Methods: SLN were produced by high shear homogenization method. Briefly, the lipid phase and curcumin were mixed and heated to a temperature above T_m of the lipid in use. The surfactants were dissolved in water were heated separately. The phases were blended and processed at 8 000 rpm during 10 minutes, at the same temperature. The hot emulsion was poured into equal volume of cold (1-4 °C) water and further processed for 3 minutes. Size distribution (z-ave, PdI) and ZP were determined on a Zetasizer Nano ZS. The drug release of curcumin-loaded solid lipid nanoparticles was determined using dialysis bag technique (cellulose membrane with molecular weight cutoff was 12-14 kDa) in ethanol: water (1:1) receptor medium. 1 mL of curcumin loaded SLN was sealed in a dialysis bag and immersed in 100 mL of preheated receptor medium. The amount of Curcumin released from the nanoparticles was determined by absorbance reading at 422 nm. Cell viabilty was estimated by resazurin assay in MCF-7 and BT-474 cells at concentrations of 0.1 mg/mL and 1.0 mg/mL of curcumin-loaded formulation, at 24, 48 and 72h exposure.
Results: The formulation containing 4.95% Imwitor 900K, 0:25% poloxamer 188, 0.50% of CTAB and.0.05% of curmcumin presented a z-ave of 165±5 nm, PdI was 0,408±0009 and ZP+60±0.51 mV. After 24 hours, the amount of released drug was 22:27%. Viability of MCF-7 and BT-474 cells was improved when treated with curcumin-solid lipid nanoparticles compared to treatment with solid lipid nanoparticles alone.
Conclusions: The release kinetics *in vitro* demonstrated curcumin-loaded solid lipid nanoparticles controlled drug release. These studies confirmed that curcumin-loaded solid lipid nanoparticles could be prepared successfully with high drug entrapment efficiency. However, loading capacity must be improved if curcimin-loaded SLN are to be used for cancer treatment.

P022. Ligand-modified nanoparticles as a multifunctional approach for cancer vaccine

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Introduction: Biodegradable polymeric nanoparticles (NP) are promising vaccine delivery systems due to their capacity to target antigen-presenting cells (APC), provide protection from *in vivo* degradation and a sustained release over time [1, 2]. Also, by co-entraping tumor- associated antigens (TAA) and specific immunoadjuvants in NP, it is possible to stimulate tumor-specific cytotoxic T lymphocytes (CTL) responses and direct the immune response towards the Th1 pathway, which are vital for tumor-therapeutic immunity [3].
Objectives: The aim of this study was to develop a multifunctional nanoparticulate vaccine with cancer immunotherapeutic potential using biodegradable polymeric NP (PLGA/PEG-b- PLGA/ PEG-b-PCL) to deliver melanoma antigens (Melan-A and gp100 epitopes) and the Toll- like receptor ligands (TLRl) CpG oligodeoxynucleotides and Poly(I:C).
Methods: NP were prepared by the double emulsion-solvent evaporation method [4], and their size and zeta potential were characterized by Dynamic Light Scattering and Laser Doppler Velocimetry, respectively. The antigens and adjuvants loadings were determined by MicroBCA™ and Oligreen® assays, respectively. A lectin recognition assay was developed to detect the availability for binding of mannose residues at mannose-functionalized NP surface. Two murine cell lines of APC and primary murine bone marrow-derived dendritic cells (BMDC) were used to study NP internalization patterns by flow cytometry and confocal microscopy. The endocytic pathways and intracellular trafficking followed by NP were also investigated. *In vivo*, C57Bl6 mice challenged with the B16F10 murine melanoma model were used to evaluate the therapeutic potential of NP.
Results: NP showed an average size of 170 nm and successfully co-entrapped more than one adjuvant, along with different TAA. Surprisingly, different internalization patterns were obtained for mannose-functionalized NP by immortalized APC and BMDC. Intracellular trafficking studies demonstrated considerable levels of colocalization with endolysosomal vesicles, with a tendency to accumulate in the endoplasmic reticulum. *In vivo,* the administration of the two melanoma antigens in different mannose-functionalyzed NP containing both TLRl demonstrated to induce the highest B16F10 tumor growth delayment.
Conclusions: These results highlight the versatility of this promising nanoplatform that may deliver cancer antigens to be presented by both the MHC class I and II molecules, and induce a combined activation of Toll-like receptors, predicting the potential for cancer immunotherapy.
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P023. Submicron particles of hyaluronic acid for treatment of osteoarthritis

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Introduction: Hyaluronic acid (HA), also named hyaluronan, is a linear polysaccharide with a high molecular weight (105-107 Da). It is a non-sulfated glycosaminoglycan (GAG) composed of repeating disaccharides, being an excellent bioinert material as having the following characteristics: immune and biocompatible, and biodegradable material [1, 2]. Hyaluronic acid is used in diverse applications in medicine such as viscosupplementation for osteoarthritis treatment [2]. This GAG is found in a synovial fluid, being responsible for supporting the viscoelasticity of biofluids [2]. In general, the concentration of hyaluronan is under 1400-3600µg/g or µg/mL in a synovial fluid. However, the concentration may decrease under inflammatory conditions [2]. The HA can be degraded by enzymes such as hyaluronidase, β-d-glucuronidase and β-N-acetylhexosaminidase [3]. We expect that encapsulation technology could improve the stability of HA. Wall materials for encapsulation may be natural, synthetic or semi-synthetic. In this case, we used poly(D,L-lactide-co-glycolide). PLGA is a widely used copolymer approved by the FDA and European Medicine Agency (EMA) for various medical and pharmaceutical applications, such as drug delivery and tissue engineering applications [4-7].
Objectives: The purpose of the current study was to prepare and characterize PLGA-submicron particles with HA in terms of size, morphology and encapsulation efficiency.
Methods: The PLGA-submicron particles with HA were prepared through the modified-spontaneous emulsification/solvent diffusion method. Briefly, PLGA dissolved in a mixture acetone: ethanol (8:2, v/v) were added to an aqueous phase consisting of poloxamer (PF68) and HA. This emulsion was stirred at 800 rpm during 10 min. Oleic acid was added in order to study its influence on particle size and efficiency encapsulation. Resultant particles were centrifuged at 20000 rpm during 15 min and supernatant was extracted. HA in supernatant was then treated with carbazole and dried in an autoclave. Efficiency encapsulation (EE,%) of HA was determined by spectrophotometry at 280 nm [3].
Results: Mean particle size was 4560.9 ± 3466.3 nm for the submicron particles with oleic acid; 372.5± 270.0 nm for the submicron particles without oleic acid. Particles were spherical with some agglomerates. The EE of HA was 73.6% and 68.2% with and without oleic acid, respectively.
Conclusions: The submicron particles showed a high EE. The presence of some agglomerates may be not considered a disadvantage since it can lead to a slower drug release. Further studies will include *in vitro* release studies and *in vivo* efficacy studies using animal models.
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P024. Development of an analytical method for the determination of hyaluronic acid content in polymeric nanoparticles

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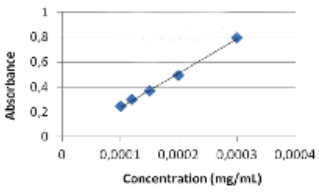
Introduction: Hyaluronic acid (HA), also named hyaluronan, is a linear polysaccharide, with a high molecular weight (105-107 Da) [1, 2]. To quantify it, the large chain generally needs a break-up in small sequences. This reaction may be possible using acid or enzymatic degradations [3].
Objectives: The purpose of the current study was to quantify the hyaluronic acid by conjugation with carbazole using spectrophotometry method.
Methods: First, for conjugation the HA with carbazole is necessary to degrade HA. Acidic degradation was possible through a chemical reaction with sulfuric acid (5 mL of sulfuric acid to 1 mL of HA). HA was added to sulfuric acid solution at -4ºC. After, this mixture was incubated in the boiling bath at 40ºC during 10 min. Then, carbazole was added and then this mixture was incubated in the boiling bath at 40ºC during 15 min. The final solution was dried in an autoclave at 100ºC for an hour. When the solution was at room temperature, it was adjusted to pH 5 with NaOH 1M. The solution was quantified by spectrophotometry at 280 nm [3, 4].
Results: The calibration curve is represented in following figure. The equation was y= 2727.1X – 0.0345 with R^2 of 0.9978. The method was linear.
Conclusions: Our previous experiments showed that direct quantification of HA by spectrophotometry was not possible. This analytical method makes it possible. However, it may be considered a laborious and a very time consuming method and thus some parameters are still under research.



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P025. Optimization of the encapsulation efficiency of a novel oral insulin delivery nanosystem

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Introduction: Oral delivery of insulin may significantly improve the quality of life of diabetes patients who routinely receive insulin by the subcutaneous route. In fact, oral delivery of insulin in diabetes treatment offers many advantages such as higher patient compliance, rapid hepatic insulinization, avoidance of peripheral hyperinsulinemia and other adverse effects [1]. However, the oral delivery of insulin remains a challenge because its oral absorption is limited [2]. Our main goal is to produce small and stable nanoparticles with high load of insulin for further *in vivo* testing. In this study, we used PLGA as core polymer since it shows a good profile to this purpose and according to the FDA it is biocompatible and biodegradable [3].
Objectives: Improvement of the insulin encapsulation efficiency (EE, %) in PLGA nanoparticles using different parameters such as the stirring speed rate, volume of external phase, pH range, type of insulin, type of surfactant and PLGA concentrations.
Methods: Nanoparticles were produced by a water-in-oil-water (W/O/W) multiple emulsion which is a system with a water reservoir phase inside of oil droplets surrounded by an external water phase [4]. First, the polymer was dissolved in a mixture of organic solvents of acetone and ethanol (8:2, v/v) under a magnetic stirring condition. A buffered solution of sodium acetate was added to insulin to adjust the pH to 4.5. After the first emulsion formed, this system was added to the external water phase stabilized with a surfactant agent with or without a salt concentration (NaCl, 0, 2, 5 or 10%). Different parameters were accessed such as the stirring speed (800 to 1400 rpm), external phase (5 or 20 mL), pH conditions, type of insulin (Actrapid®, Insuman® and Novorapid®), type of surfactant (Pluronic®F68 0.1 to 2%; PVA 2%) and PLGA concentrations (75 or 100 mg). The EE was determined in the supernatant (non-encapsulated insulin after centrifugation) using HPLC method under isocratic conditions at a flow rate of 0.8 mL min^{-1} with UV detector at 214 nm (RP-column). The mobile phase was an aqueous solution of Na_2SO_4 anhydrous (0.2 M) adjusted to pH 2.3 with phosphoric acid and acetonitrile (73:27, v/v).
Results: The smallest particle size was 398.6 nm with polydispersity index of 0.19. Another relevant aspect is that the incorporation of a salt decreased the size of the nanoparticles. The higher value of EE of insulin (around 65%) was obtained with Insuman® which is a type of insulin with protamine crystals that may promote the increase of ionic interactions between the polymer and the insulin. However, full characterization of physical-chemical interactions between all species is now required.
Conclusions: This study covers an initial phase of production of insulin nanoparticles with a growing interested in this field. Our previous studies showed EE around 36%. In this study we improved the value EE. Future work will include the bioactivity assessment after encapsulation process.
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P026. New advances in oral delivery of insulin

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Introduction: Diabetes Mellitus is a chronic metabolic disease, which results from the body's failure to produce insulin, and currently requires the person to inject insulin or wear an insulin pump. Besides offering unmatched convenience and ensuring higher patient compliance, orally administered insulin presents several benefits over its injected counterpart. Upon absorption, oral insulin would first pass though the liver before entering systemic circulation – a mode of distribution that might more closely mimic natural insulin physiology and distribution.
Objectives: To produce and test insulin nanoparticles in diabetic rats after oral administration.
Methods: PLGA nanoparticles were produced through the method of spontaneous emulsification by solvent displacement [1, 2]. Particles were characterized in terms of size, zeta potential and encapsulation efficiency (EE, %) by HPLC method. For the *in vivo* study, we followed 3R's principles: 12 *Wistar* rats (male with two months of age and average weight of 300 g) were included in this study. Diabetes was induced with streptozotocin. Twenty-four hours later, all rats presented glycemic values over 300 mg/dL. Then, the rats were randomized in two groups: one for the test formulation and the other for the administration of a solution containing blank nanoparticles and free insulin, both groups with 6 rats. After one hour fasting with free access to water, the first group was administered *per os* 1 mL of an aqueous solution containing the insulin nanoparticles (50 IU/Kg); under the same conditions, it was administered 1 mL of the solution containing the blank nanoparticles and free insulin (equivalent dose) to the second group. The glucose measurements were taken at time zero and after administration, with intervals of 0.5 h for the first 2 h and thereafter at intervals of 1 h.
Results: Nanoparticles size range was from 820 and 1045 nm. Zeta potential was comprehended between +24.23 and +28.74 mV, with an EE of 36%. 5 of 6 rats of the test group lowered their blood glucose to less than 40 mg/dL after 5 h and 4 of 6 rats of the second group remained at hyperglycemic status. *In vivo* trial was suspended after 5 h.
Conclusions: Despite the need of further studies, this study showed that insulin was encapsulated into PLGA nanoparticles and after orally administered insulin maintains its bioactivity.
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P027. Evaluation of topical anti-inflammatory activity of *Cymbopogon citratus* on *in vivo* model

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Introduction: *Cymbopogon citratus*, commonly known as lemongrass, is one of the most common herbs used in popular medicine in the treatment of inflammation and inflammatory disorders. Our previous studies of the essential oil-free infusion obtained from *C. citratus* have revealed a great anti-inflammatory activity. Phytochemical studies showed the presence of tannins, phenolic acids (caffeic and p-coumaric acid derivatives) and flavonoids (O- and C-glycosyl derivatives of apigenin and luteolin) [1].
Objectives: Taking into account that *C. citratus* oral extract shows acute anti-inflammatory and antioxidant properties, the aim of this work was to evaluate *in vivo* the topical anti-inflammatory activity of *C. citratus* leaves essential oil-free infusion topical formulation (CcI), a tannin-rich fraction topical formulation (CcT) and a flavonoid-rich fraction topical formulation (CcF).
Methods: *C. citratus* leaves essential oil-free infusion, tannin-rich fraction and flavonoid-rich fraction were prepared as previously descrived [1]. CcI, CcT and CcF were formulated in a water/oil emulsion cream in a concentration of 0.3%. Male Wistar rats weighing 160-250g, n=6-8/group, with free access to water and fasted for 24 h prior to experiments were used. The evaluation of the *in vivo* anti-inflammatory activity of CcI, CcT and CcF was performed by the carrageenan-induced rat paw edema model using the non-steroidal anti-inflammatory drug diclofenac as a positive control [2].
Results: The results obtained for edema reduction from the acute inflammatory model were 47% for CcI, 47% for CcT, 43% for CcF and 82% for topical diclofenac sodium. It means that topical CcI, CcT and CcF applied before carrageenan administration significantly prevent edema formation when compared with the negative control group. These results also suggest that the possible compounds responsible for the observed effects are the poliphenolic compounds, specifically tannins and flavonoids.
Conclusions: These results showed that *C. citratus* topical formulation possesses anti-inflammatory activity, confirming that *C. citratus* is able to reduce inflammation and helps to justify this popular use as an anti-inflammatory agent.
References: [1] J. Med. Food (2010), **13**: 1-10. [2] Proc. Soc. Exp. Biol. Med. (1962) **111**: 544-547.
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P028. Evaluation of chronic anti-inflammatory of *Cymbopogon citratus* on *in vivo* model

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Introduction: *Cymbopogon citratus*, commonly known as lemongrass, is one of the most common herbs used in popular medicine in the treatment of inflammation and inflammatory disorders. Previous phytochemical studies of the essential oil-free infusion obtained from *C. citratus* have revealed the presence of tannins, phenolic acids (caffeic and *p*-coumaric acid derivatives) and flavonoids (*O*- and *C*-glycosyl derivatives of apigenin and luteolin) [1].
Objectives: Taking into account that *C. citratus* extract shows acute anti-inflammatory and antioxidant properties, the aim of this work was to evaluate *in vivo* the chronic anti-inflammatory activity of *C. citratus* leaves essential oil-free infusion.
Methods: The cotton pellet induced granuloma was used to assess the effect of oral *C. citratus* infusion in the expression of pro- and anti-inflammatory cytokines. Granuloma was induced by subcutaneous implant of a sterile cotton pellet (10mg) in the dorsal area of anaesthetized animals [2]. Male Wistar rats weighing 160-250g, n=6/group, with free access to water and food were used. Test groups received orally *C. citratus* infusion (68.24 mg/kg), for 5 consecutive days, twice a day, according to the traditional medicine. Indomethacin was used as reference drug (25 mg/kg p.o.). Cytokines were quantified recording to an ELISArray kit.
Results: Cytokines are one of the most important groups of chemical inflammatory mediators during an inflammatory episode, acting as anti- and pro-inflammatory agents. The results obtained for this chronic inflammation model show that the oral treatment with *C. citratus* reduced INF-α, IL-2, IL-4, IL-6, IL-10, IL-12, IL-13, TNFα, GM-CSF and RAMPES levels and induced the IL-1α and IL-1β production, being these results supported by *in vitro* studies. It suggests that the anti-inflammatory activity observed for *C. citratus* leaves essential oil-free infusion is related to the cytokines regulation during an inflammatory episode.
Conclusions: This work contributes to the scientific validation of the traditional uses of *Cymbopogon citratus* and the discovery of the mechanism underling its activity.
References: [1] J. Med. Food (2010) **13**: 1-10. [2] J. Ethnopharmacol. (2003) **88**: 195-198.
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P029. Inexistence of nanoproduct regulamentation (GMP): what alternatives we have?

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Introduction: Nanomedicine is defined as the application of nanotechnology in medicine, which has allowed the achieve a lot advances in healthcare by acting in the areas of therapy, diagnostic/ imaging and regenerative medicine, exploring new physical and chemical properties of materials at the nanoscale [1-4]. This scale is compatible with the functional units in living organisms where the nanoproducts provide innovative properties [2, 3].
GMP for medicines for human use are rules and guidelines developed by organizations and institutions whether public or private, national and international regulatory authorities, in cooperation with the pharmaceutical industry, to achieve levels of quality, safety and efficacy throughout the process manufacture of medicinal products [1]. Despite all the efforts, which have been made by this area, the development and evolution of GMP for medicines for human use, there is no explicit regulatory harmonization and consensus for the nanoproducts [1].
Objectives: This study intends to sensitize participants in GMP for medicines for human use, for the absence of specific GMP for these nanoproducts.
Methods: Current guidelines of GMP for medicinal products for human use of EC / EEA, ICH, PIC / S and WHO were consulted.
Results: The existing regulation for the Manufacture of Biological Active Substances and Medicinal Products for Human Use are those that can be closely reconciled to the nanoproducts. There are more than 40 nanoproducts in the market but no specific GMP guidelines have been established for them.
Conclusions: It is extremely important and necessary the involvement of the various sectors; it is intended that all participants reach to an harmonization and consensus throughout the process inherent to the manufacture of nanoproducts in order to ensure quality, safety and efficacy. All these requirements will provide more confidence to the patient, more quality to the market and therefore to the pharmaceutical sector and health. Nanoproducts involve materials and biological processes that may exhibit inherent variability. Thus, the extent and nature are naturally variable.
References: [1] Gouveia, Bruno. Boas Práticas de Fabrico de Medicamentos para Uso Humano, ULHT, *Master thesis* (2013). [2] Nanomedicine. Nanotechnology for Health (2006). [3] Vision Paper and Basis for a Strategic Research Agenda for NanoMedicine (2005). [4] Contribution of Nanomedicine to Horizon 2020. White Paper to the Horizon 2020 Framework Programme for Research and Innovation (2013).

P030. Novel nanoparticulate platform for delivery of tumor associated antigens

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Introduction: Breast cancer is one of the leading cancer-related death cause among women worldwide. The conventional forms of treatment do not specifically target the tumour. Therefore, there is an urgent and critical need of new therapeutic strategies for breast cancer patients. Therapeutic cancer vaccines are novel immunotherapeutic approaches used to overcome host immunosupression induced by tumor cells.
Objectives: The main goal of this study is to develop a polymeric nanoplatform based on antigen-loaded poly(lactide-co-glycolide) acid (PLGA)-based nanoparticles (NPs). To deliver the antigen to dendritic cells (DCs) and improve T cell efficiency within tumor.
Methods: PEGylated-PLGA-based NPs (PLGA-PEG) were formulated by double-emulsion solvent evaporation technique using ovalbumin (OVA) as a model antigen. Other polymers were used in order to best attain the most efficient parameters for cancer immunotherapeutic treatment. NPs size and surface charge were determined by Dynamic light scattering and Laser Doppler Velocimetry, respectively. Antigen structural integrity was confirmed through SDS-PAGE gel electrophoresis. To confirm NPs safety, DCs were incubated with increasing concentrations of PLGA-PEG-based NPs (100-1000 μg/mL) and their viability was tested using MTT and Alamar Blue assays.
Results: PLGA-PEG NPs presented an average size of 131 nm, with a polydispersity index (PdI) of 0.16 and a zeta potencial (ZP) of –4.78 mV. Encapsulation efficiency (EE) and loading capacity (LC) were 60 % and 30 μg/mg, respectively. Increase in mean diameter of PLGA-PEG NPs was observed when chitosan (CS) (PG_CS) was used (167 nm, PdI 0.167), ZP values were close to neutrality (-1.66 mV), which is desired in the design of a therapeutic cancer vaccine to overcome the premature capture of NPs by macrophages. EE and LC of PG_CS were 57.5 % and 29 μg/mg, respectively.
PG_CS NPs modified by Pluronic F127 (PG_CS_PL) presented higher mean diameter values (180 nm with a PdI 0.18), and ZP (ZP -1.78 mV), but lower EE and LC (32 % and 16 μg/mg).
NPs safety was quantified using MTT and Alamar Blue® assays. DCs were incubated 24 h with increasing concentrations (100-1000 μg/mL) of three different batches of PLGA-PEG-based NPs: PG_CS, PG_CS_PL and PG_PL. Formulated NPs did not decrease cell viability.
Conclusions: Having in consideration the results herein described, it is possible to state that the developed PLGA-PEG-based NP constitutes a promising platform for the delivery of antigens to DCs, key players in tumor immunology.
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