Biomedical and Biopharmaceutical Research
Jornal de Investigação Biomédica e Biofarmacêutica

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I Simpósio Nacional de Nanociência e Nanotecnologia Biomédica

13 MAIO 2011
Auditório Agostinho da Silva

10h00 – 11h00 - Sessão 1:
A nanotecnologia aplicada à terapêutica

12h00 – 13h10 - Sessão 2:
Caracterização de nanossistemas

15h30 – 16h30 - Sessão 3:
Nanoquímica e nanofísica

17h10 – 18h15 - Mesa Redonda:
O mundo Nano para além do determinismo tecnológico

Inbn
Laboratório de Nanociência e de Nanotecnologia Biomédica

Informações:
António Costa
Fac. Ciências e Tecnologias da Saúde
Universidade Lusófona
Campo Grande, 376
1749.024 Lisboa, Portugal
Tel: +351 217515550

Associação Lusófona para o Desenvolvimento do Ensino e Investigação em Ciências da Saúde
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Biomedical and Biopharmaceutical Research
Jornal de Investigação Biomédica e Biofarmacêutica

Biomedical and Biopharmaceutical Research
Jornal de Investigação Biomédica e Biofarmacêutica

Suplemento / *Suplement*

I Simpósio Nacional de Nanociência e Nanotecnologia Biomédica

Lisboa, 13 de Maio de 2011
Universidade Lusófona de Humanidades e Tecnologias
Auditório Agostinho da Silva

Comunicações Orais

Sessão 1: A Nanotecnologia aplicada à Terapêutica.
CO.01 Estratégias antitumorais e a importância de pensar "Nano" - João Nuno Moreira (FFUC/CNC)

CO.02 Abordagem nanotecnológica no desenvolvimento de uma vacina veterinária - António Almeida (FFUL/iMed.UL)

CO.03 Nanopartículas auto-estruturadas para entrega de genes com vista ao tratamento de neuropatias do sistema nervoso periférico - Ana Paula Pêgo (INEB)

C0.04 Conferência magistral: Avanços da Nanomedicina na Terapêutica, Maria Jose Alonso (Universidade de Santiago de Compostela).

Sessão 2: Caracterização de nanossistemas
CO.05 Preparação e caracterização de nanopartículas biodegradáveis inteligentes para a libertação controlada de substâncias activas usadas no tratamento do cancro - Jorge Coelho (FCTUC)

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CO.07 Nanossistemas na imagiologia molecular: desenvolvimento e aplicações - Isabel Prata (IBILI/FMUC)

C0.08 Conferência magistral: Biossensores e sistemas nano com tecnologia fotónica, Teresa Neves-Petersen (Universidade de Aalborg).

Sessão 3: Nanoquímica e Nanofísica.
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CO.14 Nanopartículas: análise e percepção de risco - José Palma de Oliveira (FPUL)

Sessão 1: A Nanotecnologia aplicada à Terapêutica

Moderadora

Sérgio Simões

Currículo Resumido

Sérgio Simões has a degree in Pharmaceutical Sciences, a Master degree in Engineering Sciences and a Ph.D. in Pharmaceutics. He has a position as assistant professor at Faculty of Pharmacy of the University of Coimbra – Portugal. He is a co-founder, Member of the Board and Vice-president of Bluepharma, Indústria Farmacêutica, S.A.. He is also Executive Chairman of Luzitin, S.A.. His research activity has been focused on the development of targeted nanocarriers for drug and nucleic acid delivery aiming at their application for gene therapy. He has published more than 100 SCI journal papers, 7 book chapters, inventor in 5 patents and he is the author/co-author of 250 presentations in international conferences and workshops. Over the last years, he has conducted several research projects in the field of Biotechnology and gained a significant expertise on the development, industrial transfer and manufacturing of pharmaceutical dosage forms for the European and US market.

Academic degrees
Habilitation in Pharmaceutics, University of Coimbra (2005)
Ph.D. in Pharmacy, University of Coimbra (1999)
M.Sc. in Engineering Sciences, University of Coimbra (1994)
Degree in Pharmaceutical Sciences, University of Coimbra (1991)

Professional activity
Assistant Professor of Pharmaceutical Biotechnology/Technology at the Faculty of Pharmacy - University of Coimbra (since 1999 -)
Member of the board of Bluepharma, Indústria Farmacêutica, S. A. (since 2001 -)
Head of R&D department at Bluepharma, Indústria Farmacêutica, SA (since 2001 -)
Research member at the Center for Neurosciences and Cell Biology (since 1995 -)

Relevant scientific activity
Author/co-author of 7 book chapters
Author/co-author of 102 research papers in peer reviewed journals
Author/co-author of 250 presentations in international conferences and workshops
Supervisor/co-supervisor of 16 Ph.D. Thesis and 3 post-doc projects in the pharmaceutical technology/biotechnology field
Principal investigator/participant of several research project funded by the EU, National research agency, NATO, ADI and QREN

CO.01 Estratégias antitumorais e a importância de pensar "Nano»

Interventor

João Nuno Moreira

Currículo resumido
Licenciado em Ciências Farmacêuticas (FFUC), Mestre em Biologia Celular (FCTUC) e Doutorado em Farmácia, na especialidade de Tecnologia Farmacêutica (FFUC/Universidade de Alberta, Canadá). Actualmente é Professor Auxiliar da Faculdade de Farmácia da Universidade de Coimbra e Investigador Principal do Centro de Neurociências e Biologia Celular (CNC) da mesma Universidade.
A sua actividade científica centra-se no desenvolvimento de nanossistemas de base lipídica para a entrega de fármacos ou ácidos nucleicos na terapêutica antitumoral. No âmbito das actividades de investigação científica que desenvolve é autor de vários trabalhos científicos publicados em revistas internacionais com arbitragem científica, um pedido de registo de patente nos Estados Unidos da América, dois capítulos de livros e de várias comunicações orais ou sob a forma de painel em encontros científicos nacionais e estrangeiros. Participa na orientação/co-orientação de teses de Doutoramento, de Mestrado e de Licenciatura. Tem experiência como investigador responsável na coordenação de projectos de investigação financiados pela FCT e Indústria Farmacêutica. É membro activo da plataforma de colaboração MIT-Portugal, tendo sido palestrante convidado em cursos avançados no MIT. Tem participado na organização dos cursos avançados "Principles and Practices in Drug Development" e de "Oncobiologia". Foi co-promotor do projecto "Smart Medicines", o qual foi premiado com o prémio InovCapital para a melhor ideia de negócio, no âmbito do concurso Arrisca Coimbra 2009 e com o 2º lugar no concurso "Idea to Product I2P 2010", organizado pela COTEC Portugal – Associação Empresarial para a Inovação. Desde 28 de Junho de 2010, é membro da Comissão de Avaliação de Medicamentos do INFARMED, na qualidade de membro proposto pela Ordem dos Farmacêuticos.

Resumo da Comunicação
Como é sobejamente reconhecido, a utilização de quimioterapia convencional é llimitada por graves efeitos secundários decorrente da sua acção ao nível de células sãs e da sua inerente falta de selectividade para células neoplásicas. Nesta comunicação serão discutidas as novas oportunidades proporcionadas pela nanotecnologia na resolução deste problema. Será dado especial ênfase às nanotecnologias de base lipídica direccionadas por ligandos, fruto do trabalho que há mais de dez anos tem vindo a ser desenvolvido no Centro de Neurociências e Biologia Celular e na Faculdade de Farmácia da Universidade de Coimbra.

CO.02 Abordagem nanotecnológica no desenvolvimento de uma vacina veterinária

Interventor

António Almeida

Currículo resumido

Professor Catedrático de Tecnologia Farmacêutica da Faculdade de Farmácia da Universidade de Lisboa. É Licenciado em Ciências Farmacêuticas (Farmácia Industrial) pela mesma Faculdade (1987) e Doutorado (Ph.D., Tecnologia Farmacêutica) pela University of Aston, Birmingham, UK (1993). Fez a Agregação em Ciências Farmacêuticas (Tecnologia Farmacêutica), na Faculdade de Farmácia, Universidade de Lisboa (2005). Foi Presidente da Assembleia de Representantes (1998/02) e Vice- -Presidente do Conselho Científico da Faculdade de Farmácia, Universidade de Lisboa (2003/08). É desde 1996, perito da Comissão de Avaliação de Medicamentos do INFARMED. Ao longo da sua carreira científica realizou trabalho de investigação no Instituto Gulbenkian de Ciência, Oeiras (1987-1988), na University of Aston, Birmingham - UK (1993), no Chemical and Biological Defence Establishment, Ministry of Defence, Porton Down, Reino Unido (1992/1993). Foi Investigador Convidado na Freie Universität Berlin, Institut für Pharmazie, Berlim, Alemanha (1995/1996). É actualmente investigador do grupo NanoDDS do iMed.UL (Research Institute for Medicines and Pharmaceutical Sciences), Faculdade de Farmácia, Universidade de Lisboa, onde é responsável por uma equipa de investigação na área da formulação de medicamentos utilizando sistemas de micro e nanopartículas poliméricas e lipídicas. É autor e co-autor de várias publicações em livros e revistas científicas internacionais, assim como de algumas patentes. Tem exercido regularmente, desde 1997, as funções de avaliador científico de várias revistas científicas internacionais, sendo membro do corpo editorial do Journal of Biomedical Nanotechnology, do Journal of Microencapsulation e do Journal of Drug Delivery Science and Technology.

Resumo da Comunicação

A gurma é uma doença contagiosa do tracto respiratório superior dos equídeos. Caracteriza-se por inflamação das mucosas nasofaríngea e oral, descarga nasal purulenta e formação de abcessos nos nódulos linfáticos adjacentes após ingestão ou inalação do agente etiológico Streptoccocus equi equi. Ligada à parede celular do S. equi, a proteína M (SeM) é uma molécula com propriedades antigénicas, capaz de conferir protecção contra a infecção causada pela estirpe patogénica. A formulação de uma vacina destinada a ser administrada por via nasal de modo a induzir, simultaneamente, a produção e secreção local de anticorpos que evitem a colonização do epitélio respiratório pelo S. equi e a produção de anticorpos específicos circulantes que previnam a sua propagação a outros tecidos, pode revelar-se extremamente útil pois as vacinas actualmente existentes ou carecem da eficácia necessária ou apresentam efeitos secundários que limitam a sua aplicação generalizada. Desde a década de 1990, temos vindo a estudar a integridade e actividade in vivo dos antigénios totais de S. equi micro- ou nanoencapsulados em diversos sistemas transportadores poliméricos (PLGA, PCL, quitosano) e lipídicos (SLN e lipossomas) para vacinação por via nasal, tentando estabelecer uma comparação entre os resultados obtidos com os diversos sistemas. Esta comparação tem em conta a influência dos parâmetros de formulação sobre a integridade dos antigénios, os estudos de libertação in vitro e a caracterização do tipo de resposta imunitária produzida em modelo animal apropriado.

CO.03 Nanopartículas auto-estruturadas para entrega de genes com vista ao tratamento de neuropatias do sistema nervoso periférico

Interventor

Ana Paula Pêgo

Currículo resumido

Doutorou-se em Química de Polímeros e Biomateriais em Ciências de Engenharia pela Universidade de Twente (Países Baixos) em Novembro de 2002. É Investigadora Auxiliar do INEB desde Maio de 2004, desenvolvendo a sua actividade no grupo NEWTherapies. É Professora Auxiliar convidada da Faculdade de Engenharia da Universidade do Porto (FEUP). Ana Paula Pêgo tem uma sólida experiência em química de polímeros e na concepção de novos biomateriais para aplicação em Neurociências. Neste momento coordena a equipa – Biomaterials for Neurosciences - pertencente ao grupo NEWTherapies. O seu trabalho de investigação é focado nas seguintes áreas: (i) desenvolvimento de enxertos híbridos (células + matrizes) e nanopartículas desenhadas à escala molecular para entrega de agentes terapêuticos (ADN, ARNi) para promover a regeneração nervosa e (ii) a criação de plataformas de teste de agentes terapêuticos para a promoção da regeneração de tecidos. Outras palavras-chave que caracterizam o trabalho em curso são: medicina regenerativa, nanomedicina, interacção célula-material, células estaminais neurais e ética.

Resumo da Comunicação

Uma grande parte das patologias associadas ao sistema nervoso periférico não encontra ainda hoje uma resposta clínica eficaz. A modulação da expressão genética a nível de populações celulares específicas do sistema nervoso pode abrir novos caminhos na criação de novas estratégias terapêuticas. A terapia genética tem como princípio o uso de ADN/ARN como uma pro-droga, levando, por exemplo, à expressão in-situ de uma proteína terapêutica ou à regulação da expressão proteica a nível de uma determinada população celular. Almejando o desenvolvimento de novas estratégias com vista o tratamento de neuropatias do sistema nervoso periférico, o nosso grupo tem vindo a desenvolver sistemas não-virais auto estruturados, optimizados para a transfecção de neurónios periféricos. Nesta apresentação iremos abordar o trabalho realizado nesta área tendo por base vectores (nanopartículas) à base de quitosano, um polímero de origem natural, funcionalizados com um fragmento de uma neurotoxina. O conjunto de modificações químicas que foram realizadas com o objectivo de aumentar a estabilidade e a especificidade das nanopartículas constituídas por quitosano e ADN serão discutidos. Os resultados apresentados irão demonstrar a capacidade destes complexos para transfectar especificamente células do sistema nervoso periférico.

CO.04 Conferência Magistral – Avanços da Nanomedicina na Terapêutica

Interventor

Maria José Alonso

Currículo resumido

María José Alonso is Full Professor of Biopharmaceutics and Pharmaceutical Technology at the University of Santiago de Compostela. She has worked as a post-doctoral research fellow at the University of Paris-Sud - France (1986-87) and as a visiting professor at the University of Angers, France (1989), and at the Massachusetts Institute of Technology (1991-92). Maria José was a pioneer of the drug delivery and nanotechnologies field in Spain (since 1987). She has been a member of several scientific and advisory boards of the most relevant international associations in the drug delivery and nanomedine field (CRS, FIP, APGI, EUFEPS…) and she was the founder of the Spanish-Portuguese Chapter of the Controlled Release Society (1995). She has also organized several international conferences and workshops. María José has been responsible of more than 30 research projects and contracts, 7 of which have been financed by prestigious institutions, i.e. the WHO, the "Bill ad Melinda Gates Foundation" and the EC. She has adviced 20 Ph.D. students and 15 post-docs. She is the author of more than 180 international scientific articles and book chapters (H factor 45), 14 patents and more than 200 presentations at international symposia, for many of which (30) she was an invited speaker. She is actually a member of 10 Editorial Boards of prestigious journals in the field of drug delivery and nanomedicine. In 2010, Maria José was elected a member of the Galician Academy of Pharmacy and The Royal National Academy of Pharmacy.

Resumo da Comunicação

Despite the recent introduction of the nanomedicine term in the scientific arena, the origin of nanomedecine and, in particular that of nano-drug-delivery, dates from the early 60's. It is thanks to these original ideas, that we have a currently significant number of nanopharmaceuticals into the market. However, the full potential of nanomedicine is still to come, and this will be feasible because over the last decades we have initiated a non-return pawthway, which is the adoption of transdisciplinary and translational approaches. Our group, being committed with the translation of ideas from the university through novel pharmaceutical technology, has designed novel nanostructured materials intended to transport drugs and antigens across biological barriers and deliver them to the target tissue. A large amount of this research has been defined to have a special impact in developing world nations. During my presentation, I would like to focus on new therapies consisting of multifunctional nanocarriers containing small drugs and antigens. This work is leading to the development of novel therapies for cancer and diabetes, and new nanovaccines as single-dose and needle-free strategies for improving the immunization coverage of women and children

Sessão 2: Caracterização de nanossistemas

Moderador

Bruno Sarmento

Currículo resumido

Bruno Sarmento, Pharm. D., Ph.D., is an Assistant Professor of the Pharmaceutical Sciences Department of the Health Sciences Institute and a Research Scientist in the Pharmaceutical Technology Department of the Faculty of Pharmacy of Porto University. He conducted his studies at several national and international universities. His field of research is focused on delivery of biotechnological drugs by means of colloidal targets, development of new drug delivery systems using polymeric and lipid nanoparticles and therapeutic polymers and controlling delivery of drugs across biological barriers, namely gastrointestinal tract, ocular mucosa and BBB. Most of the research projects he is involved are related with nanomedicine drug delivery, focusing on drug targeting for cancer, metabolic and infection diseases, controlled release for regenerative medicine or chronic pathologies as well as the study of drug absorption using cellular and animal models. He is a member of a wide range of national and international scientific societies. He maintains collaborating studies with the Faculty of Pharmacy of the University of Copenhagen, the Faculty of Pharmacy of the University of Helsinki, the Faculty of Pharmacy of the University of Pavia, the Faculty of Pharmacy of the University of Barcelona and the Federal University of Bahia, between others. Author of various scientific papers (more than 40), book chapters (more than 8) and international scientific conference presentations (more than 50). Bruno Sarmento is frequently asked to lecture in conferences and universities both national and international. He is also a peer reviewer of international scientific journals and research project evaluator of funding agencies.

CO.05 - Preparação e caracterização de nanopartículas biodegradáveis inteligentes para a libertação controlada de substâncias activas usadas no tratamento do cancro

Interventor

Jorge Coelho

Currículo resumido

Jorge Fernando Jordão Coelho é Professor Auxiliar do Departamento de Engenharia Química da Faculdade de Ciências e Tecnologia de Universidade de Coimbra. Desenvolve investigação na área de materiais poliméricos, nomeadamente: síntese de polímeros por métodos convencionais (via radicalar e reacção gradual) e avançados (polimerização radicalar viva); dendrímeros; química supramolecular (preparação de nanopartículas a partir de estratégias de "self-assembly"); e caracterização mecânica, térmica e estrutural de polímeros. É autor de 32 artigos em revistas internacionais com revisão e vários capítulos de livros.

Formação académica

Esta comunicação abordará várias estratégias de preparação de nanopartículas biodegradáveis utilizando polímeros que respondem a estímulos externos. Será efectuado um pequeno sumário dos avanços mais recentes nesta área de investigação, tocando aspectos como: o potencial deste campo de investigação, os desafios em aberto e perspectivas futuras.

Como exemplo serão apresentados dois projectos de investigação que se encontram em decurso e que utilizam abordagens distintas para a libertação do mesmo tipo de compostos.

CO.06 - Experimentação animal

Interventor

Isabel V. de Figueiredo S. Pereira

Currículo resumido
Professora Auxiliar e Investigadora do Centro de Estudos Farmacêuticos (CEF) na Faculdade de Farmácia. Autora e/ou co-autora em mais de 150 comunicações orais e em forma de poster em reuniões científicas nacionais e internacionais. Integrou a comissão de trabalho que elaborou o Plano de Estudos da Licenciatura em Ciências Bioanalíticas da Faculdade de Farmácia da Universidade de Coimbra. Integrou a Comissão de Acompanhamento do Processo de Bolonha na Faculdade de Farmácia. Integrou o grupo de trabalho responsável pela Implementação do Processo de Bolonha na Faculdade de Farmácia. Foi membro do Grupo Coordenador dos Cursos de Farmacoterapia, organizados pelas Faculdades de Farmácia de Coimbra, Porto e Lisboa, Ordem dos Farmacêuticos e, responsável pela co-organização e leccionação de cursos de formação contínua. Colabora com o departamento de formação da Escola de Pós-Graduação em Saúde e Gestão, da Associação Nacional das Farmácias. Colabora com a Cofanor na realização dos Curso de Pós-graduação. Integra o Conselho Científico do Instituto de Ciências Nucleares Aplicadas à Saúde. Possui creditação concedida pela Direcção Geral de Veterinária que determina ser considerada pessoa competente para utilizar animais de laboratório. Integra a bolsa de peritos de Avaliação de Medicamentos Veterinários. É coordenadora do Estágio curricular do Mestrado Integrado em Ciências Farmacêuticas. Prémios: 2º Prémio em 2002 (Liga Portuguesa Contra a Epilepsia). Patentes: 1.

Formação académica
O modelo animal, nos dias de hoje, é utilizado em todos os campos da investigação biomédica. A relação entre o Homem e os animais de outras espécies ganhou contornos mais definidos, o recurso a outras espécies tem regras e uma ética estabelecida, a transposição dos resultados do animal para a espécie humana tem critérios claros e objectivos e o Homem tomou consciência de que faz parte de um conjunto interligado, em que os elos se entrelaçam e a sua sobrevivência depende da sobrevivência de todos. É crucial distinguir entre o direito dos animais e o bem-estar animal. A comunidade científica defende o bem-estar animal, o que significa garantir a saúde e o cuidado adequado dos animais de experimentação, conforme a Declaração Universal dos Direitos dos Animais, proclamada pela UNESCO em Bruxelas, em 27 de Janeiro de 1978. A realização de um protocolo experimental com recurso a modelos animais é uma tarefa complexa. É virtualmente impossível fornecer regras específicas para a escolha do modelo animal ideal, uma vez que as muitas considerações que devem ser feitas antes que um protocolo possa ser realizado, podem condicionar essa mesma escolha, no entanto, esta deve ser criteriosa e é sem dúvida o ponto fulcral do todo o desenho experimental. A escolha de um modelo inadequado implicará um comprometimento quer na análise quer na interpretação dos resultados obtidos. Cada investigador deve ter objectivos concretos, conhecer a espécie animal utilizada, ter consciência do seu trabalho e das suas conclusões, sabendo que as informações por si obtidas poderão vir a ser utilizadas por outros, podendo beneficiar ou prejudicar centenas de milhares de pessoas. O investigador deve ser rigoroso em cada passo do seu trabalho.
Muitos exemplos da grande contribuição que os modelos animais deram à ciência encontram-se espelhados nos diferentes Prémio Nobel da Medicina e da Fisiologia. De salientar, estudos nos sistemas circulatório e digestivo, na diabetes, na descoberta de vacinas, no desenvolvimento de técnicas de transplantes de órgãos e mesmo na descoberta de novos fármacos que proporcionam o aparecimento de novos tratamentos.
Na verdade, o recurso a modelos animais é, em muitos casos, uma obrigação.

CO.07 - Nanossistemas na imagiologia molecular: desenvolvimento e aplicações

Interventor

Isabel Prata

Currículo resumido

Maria Isabel Prata é Licenciada em Química e Doutorada em Bioquímica, ramo de Bioinorgânica, pela Universidade de Coimbra. Tem como principais interesses de investigação a imagiologia médica e a obtenção de novos traçadores para imagiologia médica, nomeadamente, de agentes de contraste para Ressonância Magnética e de radiofármacos para PET (tomografia de emissão de positrões) e SPECT (tomografia por emissão de fotão simples). Desenvolve no IBILI, Faculdade de Medicina da Universidade de Coimbra, projectos de aplicação de nanopartículas em diagnóstico.

Formação académica

Imaging in disease conditions is fundamental for diagnostic and prognostic. Imaging agents are molecular constructs presenting both efficient sensor/reporter groups (fluorescence, MRI, SPECT, PET, etc.) and targeting moieties to determine organ/cell localization. Nanoscale objects, dendrimers, transition and noble metal and semiconductor nanoparticles (NPs), lipossomes, micelles, etc., with dimensions under 100 nm, close to that of biological macromolecules e.g. proteins, have emerged in recent years as invaluable platforms for the study of biomolecular processes, for imaging and pharmacologic intervention – targeting and controlled drug-delivery. Traditionally, a major limitation in the use of nanoparticles in vivo is their premature elimination from the circulatory system by the mononuclear phagocyte system (MPS), which can rapidly remove them from the bloodstream and prevent the nanoparticles from reaching their target. The disposal of particles through the MPS follows a complex pathway beginning with the adsorption of serum proteins (opsonins) onto the surface of the nanoparticle, followed by macrophage recognition, phagocytosis, and subsequent sequestration in the liver, spleen and bone marrow. The elucidation of design parameters, for example, molecular weight, core shell architecture, and so forth, of functionalized nanoparticles that can avoid opsonin adsorption and thereby slow down their elimination by the MPS is a critical first step in the development of targeted nanoparticle systems.

CO.08 - Conferência Magistral: Biossensores e sistemas nano com tecnologia fotónica

Interventor

Maria Teresa Neves-Petersen

Currículo resumido

Education

*1*994 - Graduation in Biochemical Engineering (5 year) - Technical University of Lisbon, IST

1994 - Master - School of Biological and Medical Sciences, Cell and Molecular Biology, University of St. Andrews, Scotland

2000 - Ph.D. in "The Role of Electrostatic Interactions and Light in Protein Structure and Function", Aalborg University, Denmark, in collaboration with University of Maryland, USA

2003 - PostDoc – Biophysical studies of biomolecules at interfaces and the effect of UV light on proteins (studies done at Aalborg University, Denmark; the University of Maryland, USA and Lund Laser Center, Lund University, Sweden)

Resumo da Comunicação

Light assisted molecular immobilisation (LAMI) is a novel technology that results in spatially localised covalent coupling of a large variety of protein molecules and other biomolecules onto thiol reactive surfaces, e.g. thiolated glass/quartz, gold or silicon [1-4]. The reaction mechanism behind the reported new technology involves light-induced breakage of disulphide bridges in proteins upon UV illumination of nearby aromatic amino acids resulting in the formation of reactive molecules which will bind thiol reactive surfaces [5]. This new technology allows for dense packing of different bio-molecules on a surface (enzymes, antibody Fab fragments, cancer markers such as prostate specific antigen PSA) allowing the creation of multi-potent functionalised active new materials [2, 3, 6-9]. We have recently shown that the new photonic technology combined with the Fourier-transforming properties of lenses as well as with a simple millimeter scale feature size spatial mask allows for molecular immobilisation with diffraction limited resolution, achieving ~700 nm resolved patterns of immobilised biomolecules [8, 9]. LAMI technology is ideal to couple drugs and other bio-molecules to nanoparticles which can be used as carriers into cells for therapeutic purposes. We hereby show that this technique has the outstanding potential for nanomedicine allowing the creation of sensitive nanoprobes aimed at binding therapeutically interesting molecules. These nanoprobes have biological and medical applications such as bioseparation, biosensing and drug delivery. Thiol derivatised silica nanoparticles have been prepared. In order to explore the interesting magnetic properties of magnetite, superparamagnetic Fe3O4/SiO2 Core-shell nanoparticles have also been prepared and functionalised with thiol groups. We have immobilised prostate specific antigen (cancer marker), human serum albumin, bovine serum albumin and insulin onto thiol derivatised nanoparticles using LAMI technology. All nanoparticles and the protein-nanoparticle bioconjugates were characterised with Dynamic Light Scattering, Scanning Electron Microscopy, Energy Dispersive X-ray spectroscopy, UV-Vis absorption and Fluorescence spectroscopy and Fluorescence microscopy. We are currently following the interaction between insulin immobilised onto superparamagnetic nanoparticle carriers with insulin receptor protein present in muscle cells and monitoring the induced cellular metabolic changes. Since magnetite based nanoparticles are magnetic in nature, we potentially can guide the molecular carrier to a particular cellular location by means of magnetic fields, being this way able of triggering an ON-OFF cellular response (work in progress).

[1] Protein Sci. (2002) 11: 588-600. [2] Protein Sci. (2006) 15: 343-51. [3] Proteomics (2007) 7: 3491–3499. [4] Angew. Chem. Int. Ed. (2008) 47: 9618-9647. [5] Biophysical J. (2009) 97: 211-226. [6] Biosensors and Bioelectronics (2006) 21: 1553-1559. [7] Protein Science (2010) in print. [8] J. Nanoscience and Nanotechnology (2009) 9: 4333–4337. [9] Proteomics (2009) 9: 1–4.

Sessão 3: Nanoquímica e Nanofísica

Moderador

Maria do Céu Costa

Currículo resumido

Licenciou-se em Farmácia, fez Doutoramento e Provas de Agregação na Universidade de Lisboa, especializou-se com Mestrado em Química Orgânica Tecnológica pela Universidade Nova de Lisboa, e, mais recentemente, fez duas Pós-Graduações em Gestão Pública (FORGEP e CAGEP) no Instituto Superior de Gestão de Lisboa. Foi Coordenadora do 1º Curso de Formação Pós-graduada em Fitoterapia e Medicamentos Fitoterápicos em Portugal na Universidade Lusófona. É sócia fundadora da Sociedade Portuguesa de Fitoquímica e Fitoterapia, tendo sido eleita sua Vice-Presidente em 7 de Maio de 2010. Foi perita da EMEA de 1999 a 2008, na área da Qualidade, e exerceu em Comissão de Serviço o cargo de Directora da Direcção da Avaliação Técnico-Científica de Medicamentos no INFARMED, I.P., de 2005 a 2008. É actualmente membro de Comissão Técnica Especializada do Conselho Científico da ASAE e perita da Rede Europeia de Riscos Emergentes, da Agência Europeia de Segurança Alimentar (EFSA). Realizou investigação nas Universidades de Cambridge (UK) e de Konstanz (DE), no Imperial College (London, UK), Auckland University (New Zeland) e Faculdade de Farmácia da Universidade de Sydney (Austrália). Paralelamente à carreira de Investigação tem tido uma carreira docente universitária, tendo leccionado desde 1979, Química Orgânica, Farmacêutica e de Produtos Naturais, Bioquímica, Análise Estrutural, Farmácia Natural e Bromatologia. Ainda como aluna foi Monitora, e depois Assistente na Faculdade e Farmácia de Lisboa, Professora Convidada na Universidade da Madeira, no Instituto Politécnico de Lisboa, e é actualmente Responsável pelas Unidades Curriculares de Análise de Alimentos e Investigação Aplicada em Análises Clínicas e Saúde Pública da Escola Ribeiro Sanches, do Grupo Lusófona. Tem mais de 100 publicações incluindo várias patentes, nacionais, europeias e mundiais.ced cellular metabolic changes. Since magnetite based nanoparticles are

C0.09 - A Nanoquímica nas Ciências da Saúde

Interventor

Marise Almeida

Currículo resumido

Licenciada em Química pela Faculdade de Ciências da Universidade de Lisboa e Doutorada em Química, pela mesma Universidade. É docente de Bioquímica e Química na Faculdade de Medicina Dentária da Universidade de Lisboa (FMDUL) e de Química Analítica e Métodos Instrumentais de Análise na Escola Superior de Saúde Ribeiro Sanches (ERISA). É membro da Unidade de Investigação em Ciências Orais e Biomédicas da FMDUL. Nos últimos anos tem desenvolvido investigação na área da biodegradação de materiais dentários. Tem participado em Projectos de Investigação nesta área, quer como investigadora principal quer como participante. Tem cerca de 60 comunicações Orais e em Poster em conferências internacionais da especialidade. Publicou cerca de 25 artigos científicos em revistas internacionais da especialidade com "peer-review". É membro da rede europeia COST " Biological adhesives: from biology to biomimetics".

Resumo da comunicação

A Nanotecnologia é actualmente reconhecida como uma área de trabalho transversal a vários sectores da actividade humana com especial relevo para a Saúde. Neste domínio, são já várias as contribuições existentes, onde se destacam a nanoencapsulação de fármacos, o uso de nanopartículas na área da imagiologia médica e implementação de novas ferramentas de diagnóstico, o desenvolvimento de metodologias, quer para a criação de nanodispositivos quer para a produção de materiais para implantes.

Na área da Medicina dentária, as nanotecnologias e a nanoquímica em particular, assumem um papel relevante na obtenção e desenvolvimento, por exemplo, de novos materiais nanoestruturados usados em tratamentos dentários, tais como os nanocompósitos, de materiais com propriedades adesivas acrescidas ou ainda, na obtenção de superfícies quimicamente transformadas, de forma a aumentar a osteointegração de implantes dentários.

Ainda na Medicina Dentária, desenham-se actualmente outras áreas de intervenção e contribuição da nanoquímica, nomeadamente, na área da Endodontia, com o desenvolvimento de materiais com propriedades antimicrobianas. Estas aplicações criaram recentemente o termo nanodentisteria como uma área da Medicina Dentária, onde a aplicação das nanotecnologias e da nanoquímica é usada de forma a se obter intervenções e procedimentos mais eficazes e duradouros no restabelecimento da saúde oral.

C0.10 - Uma contribuição para imagem fotoacústica e perfis de profundidade fotoacústicos em tempo real

Interventor

Carlos Serpa

Currículo resumido

Actualmente é Investigador Auxiliar no Grupo de Fotoquímica da Universidade de Coimbra. Após completar o seu Doutoramento em 2004, realizou um Pós-doutoramento no Caltech (EUA), sob a orientação de Harry B. Gray. Os seus interesses de pesquisa abrangem uma vasta gama de investigação básica e aplicada no domínio da interacção entre a Luz e a Matéria. Interesses de investigação actuais estão focados na utilização de ondas de pressão fotoacústicas para permeação activa, na Química de Materiais para aplicações nos domínios da Energia e da Saúde e no folding de proteínas e péptidos. Carlos Serpa é co-autor de 27 artigos em revistas internacionais com avaliação, de mais de 70 conferências e um pedido de patente. Foi distinguido com o Prémio BES Inovação 2008, a mais prestigiada distinção portuguesa em inovação tecnológica, pelo seu trabalho em novos métodos de permeação cutânea activa. Carlos Serpa é actualmente Investigador Principal de projectos de I&D focados na entrega trans-cutânea activa de medicamentos e em materiais nanoestruturados para aplicações em Energias Renováveis. Participa em actividades docentes de graduação e pós-graduação no Departamento de Química da Universidade de Coimbra. A recente frequência do Curso de Empreendedorismo de Base Tecnológica, promovido pela Universidade de Coimbra, revela o seu interesse acerca de I&D industrial e em transferência de tecnologia.

Resumo da comunicação

A Tomografia Fotoacústica é uma técnica muito promissora para imagiologia médica, combinando o contraste de técnicas ópticas com a profundidade de penetração e resolução espacial de técnicas acústicas. Com a actual tecnologia laser, a Tomografia Fotoacústica tem o potencial de detectar estruturas internas até 5 a 7 cm da fonte de luz. A resolução espacial de detecção acústica, utilizando transdutores de ultra-som comerciais, depende da frequência das ondas ultra-sónicas e está actualmente próxima de 50 μm de tamanho. A Tomografia Fotoacústica é um método não invasivo e não destrutivo para imagem quando emprega radiação infravermelha inofensiva na janela fototerapêutica (700-900 nm), já que os ultra-sons empregues para detectar estruturas internas são inócuos.

Muito poucos agentes de contraste combinam uma intensa absortividade no infravermelho próximo com fotoestabilidade e biocompatibilidade. Neste trabalho apresenta-se a prova de princípio de que um agente de contraste recentemente desenvolvido oferece essa combinação e mostram-se algumas imagens elementares obtidas. Adicionalmente, demonstra-se que o agente de contraste tende a acumular-se em tumores. Mostram-se ainda experiências preliminares do estudo da cinética de difusão do agente de contraste em profundidade através da pele, contribuindo para acentuar o seu potencial de utilização como agente de contraste em Tomografia Fotoacústica. O mapeamento da difusão em tempo real do agente de contraste através da pele foi obtido após irradiação da sua absorção no infra-vermelho próximo, onde a pele é transparente.

C0.11 - Síntese de óxidos/hidróxidos de ferro nano-estruturados por química sol-gel

Interventor

Luisa Durães

Currículo resumido

Actualmente é Professora Auxiliar no Departamento de Engenharia Química da Faculdade de Ciências e Tecnologia da Universidade de Coimbra, onde tem desempenhado trabalho de docência e de investigação desde 1995. Obteve a sua Licenciatura em Engenharia Química pela mesma faculdade em 1993. Desde então, alcançou com distinção os graus de Mestre e de Doutora, ambos em Engenharia Química, em 1999 e 2008, respectivamente, tendo-se especializado em combustão e detonação de materiais energéticos e, mais recentemente, na síntese de materiais nanoestruturados por tecnologia sol-gel. Já orientou com sucesso 10 trabalhos de Mestrado e neste momento orienta 2 trabalhos de Doutoramento. Tirando partido da sua experiência na participação de 6 projectos internacionais até 2008, contribuiu activamente depois do seu Doutoramento no desenvolvimento de 4 projectos na área da nanotecnologia, todos com financiamento aprovado. Além disso, já publicou 14 artigos em revistas/livros com arbitragem e cerca de 40 artigos em actas de conferências. Também efectuou a revisão de vários artigos científicos e pertence ao corpo editorial da revista Engineering (Scientific Research Publishing). Fez ainda parte da comissão que elaborou a proposta de criação do Doutoramento em Engenharia Química (Bolonha) da Universidade de Coimbra e tem estado ligada a este curso desde 2009 fazendo parte da sua Comissão de Coordenação.

Resumo da comunicação

Os óxidos e oxihidróxidos de ferro encontram-se em grande abundância na natureza (solos, águas, organismos vivos) e são um bom exemplo de versatilidade química, neste caso do ferro. Existem em muitas fases sólidas diferentes, amorfas e cristalinas, com formas e tamanhos de partícula diversos, apresentando vários estados de oxidação do metal e comportamentos magnéticos únicos. De facto, a química do ferro muda significativamente com as condições ambientais (temperatura, pH, presença de água e ligantes, etc.), o que explica a relativa facilidade de interconversão de fases e constitui a chave para a síntese em laboratório de produtos à medida da aplicação. Os óxidos de ferro, quando em tamanho nanométrico, podem ter aplicação na área biomédica, nomeadamente, como agentes de contraste para imagem por ressonância magnética e como ferrofluidos biocompatíveis. Neste trabalho sintetizaram-se óxidos/hidróxidos de ferro nano-estruturados por tecnologia sol-gel, partindo do sistema ternário Fe(NO3)3·9H2O/etanol/óxido de propileno. A secagem dos géis por secagem evaporativa ou por extracção por fluidos supercríticos permitiu produzir xerogéis e aerogéis, respectivamente, os quais foram caracterizados física, química e estruturalmente, para avaliar o efeito do método de secagem nas suas propriedades. Concluiu-se que os materiais obtidos são compostos por agregados de cristalites, sendo o tamanho destas de ~ 1 nm (estimativa de Scherrer) no caso dos xerogéis e de 10-30 nm (TEM) nos aerogéis. Possuem elevada porosidade e área de superfície específica, designadamente, 50 % e 150 m2·g-1 nos xerogéis e 90 % e 400 m2·g-1 nos aerogéis, mas os segundos destacam-se nestes parâmetros. Com base nos resultados de FTIR, XRD e espectroscopia de Mössbaeur, concluiu-se que a ferrihidrite de 2 linhas (Fe5O7(OH)·4H2O) é a fase constituinte mais provável dos xerogéis e aerogéis. Devido ao pequeno tamanho das suas cristalites, a ferrihidrite apresenta-se com comportamento superparamagnético, quer nos xerogéis quer nos aerogéis. Na literatura encontra-se bem documentada a conversão da ferrihidrite para α-Fe2O3 ou Fe3O4 por simples desidratação ou por controlo de temperatura e pH, o que torna o material sintetizado muito promissor para o uso como precursor de óxidos de ferro para aplicações biomédicas.

Mesa Redonda – O mundo Nano para além do determinismo tecnológico

Moderador

Luís Monteiro Rodrigues

Currículo resumido
Licenciatura em Ciências Farmacêuticas na Faculdade de Farmácia da Universidade de Lisboa (1977-1981)
Provas de Aptidão Pedagógica e Capacidade Científica (equivalente ao Grau de Mestre) na área de Fisiologia - Aprovado com Muito Bom com Distinção (1986-1988)
Faculdades de Medicina de Badajoz (Universidade de Estremadura-Espanha) e de Ciências Médicas da Universidade Nova de Lisboa (formação pré-clínica completa em Medicina) (1986-1992)
Doutoramento em Fisiologia pela Universidade de Lisboa - Aprovado com Muito Bom com Distinção e Louvor (1989-1992)

Actividade profissional
Docência: Universidade de Lisboa em Ciências Farmacêuticas (1983-), Medicina (1986-1992), Análises Clínicas (1996-), Engenharia Biomédica e Biofísica (2006-) e na Universidade Lusófona em Ciências Farmacêuticas (2000-) e em Ciências Dermatocosméticas (2006-). É Coordenador de diversas unidades curriculares em áreas da sua especialidade.
Investigação: Coordenador Sénior em projectos diversos de Investigação desde 1992. É director do Centro de Investigação em Ciências e Tecnologias da Saúde (CBIOS) da Universidade Lusófona e coordenador da UDE (Unidade de Dermatologia Experimental - CBIOS). É editor executivo da Revista Lusófona de Ciências e Tecnologias da Saúde (2004-). Desde 1990 publicou cerca de 350 trabalhos de natureza científica, correspondentes a diversos trabalhos desenvolvidos nas áreas supracitadas. Desde 1986, publicou cerca de 40 trabalhos de natureza essencialmente pedagógica. Desde 1990, apresentou os resultados dos seus trabalhos em cerca de 300 comunicações prestadas à comunidade científica nacional e internacional.

Outras funções e cargos
É Consultor de empresas nas áreas da sua especialidade. Além disso, é membro do Grupo de Métodos Instrumentais de Análise de Produtos Cosméticos da União Europeia (1992-1993), membro da Comissão Científica de Cosmetologia na União Europeia (actual SCCNFP) (1993-1998), membro Fundador (1995) e Secretário do EEMCO Group, membro Fundador (1995) da EBRA e Membro do Grupo de Trabalho sobre Produtos Cosméticos do INFARMED (1996-2001). Desde 2000, é Presidente da Direcção da ALIES. É sócio Fundador e Presidente da Direcção da SPCC.

Prémios e distinções (últimos 5 anos)
"Young Investigator´s Award", 11th Congress European Burn Association, Lisboa, Portugal. Atribuído pela EBA & SPCPR em 2005.
Prémio melhor Poster do II Congresso Nacional de Ciências Dermatocosméticas. Atribuído pela SPCC em 2009.
Prémio Melhor Trabalho Científico Original do XXVII Congresso Nacional de Cosmetologia (S. Paulo, Brasil). Atribuído pela ABC em 2010.

CO.12 –Patenteamento e Propriedade Intelectual aplicado à Nanotecnologia

Interventor

Alberto Canelas

Currículo resumido

Licenciado em Engenharia Química em 1971 pelo Instituto Superior Técnico - Universidade Técnica de Lisboa. É Agente Oficial de Propriedade Industrial, junto do Instituto Nacional da Propriedade Industrial e mandatário Europeu de Patentes, junto do Instituto Europeu de Patentes. Além disso, é mandatário acreditado junto do Instituto para a Harmonização do Mercado Interno. É membro das associações A.I.P.P.I., F.I.C.P.I., A.C.P.I., E.C.T.A., E.P.I., UNION, A.M.E.P.. Pertence ao grupo de trabalho de Biotecnologia criado no âmbito da F.I.C.P.I. (Fédération Internationale des Conseils en Propriété Industrielle). Faz igualmente parte da Comissão de Biotecnologia criada no âmbito da UNION (Union of European Practitioners in Intellectual Property) e também da Comissão de Patentes da mesma organização. É ainda membro do Comité de Biotecnologia do E.P.I. (Institute of the Professional Representatives before the European Patent Office). Trabalha na firma J. PEREIRA DA CRUZ, S. A. (consultores e mandatários em Propriedade Industrial), de Lisboa, no seu Departamento de Patentes, desde 1983. Tem desenvolvido actividade essencialmente na área das patentes da Indústria Química e Farmacêutica, nomeadamente, no estudo, elaboração e apresentação de pedidos de patente. Tem igualmente dado pareceres técnicos e participado como perito em processos de contencioso envolvendo patentes nessa área. Para além disso, tem participado como orador em diversos Seminários e Conferências dentro da sua área de especialidade.

Resumo da Comunicação

A Nanotecnologia e as diferentes organizações:
- União Europeia
- OCDE
- Instituto Europeu de Patentes (EPO)
- Organização Mundial da Propriedade Intelectual (OMPI)
- Instituto de Patentes e Marcas dos Estados Unidos (USPTO)
- Instituto Nacional da Propriedade Industrial (INPI)

A nanotecnologia e as Patentes Europeias
- A natureza interdiciplinar da nanotecnologia e a necessidade de classificar adequadamente os documentos de patente:
- Nanobiotecnologia
- Nanotecnoogia para o processamento, armazenamento e transmissão de informação
- Nanotecnoogia para os materiais e ciência de superfície
- Nanotecnolgia para a interacção, detecção ou accionamento
- Nanoóptica
- Nanomagnetismo
- Tratamento dos pedidos de patente
- Desafios e oportunidades no campo da nanotecnologia

A nanotecnologia e as patentes em Portugal
- Posição e iniciativas do INPI
- Exemplo de patentes no campo da biotecnologia em Portugal

CO.13 –Patenteamento e Propriedade Intelectual aplicado à Nanotecnologia

Interventor

Rogério Gaspar

Currículo resumido

Rogério Gaspar is currently Full Professor in the Pharmaceutics Department, Faculty of Pharmacy at the University of Lisbon. Early in his career, both at the University of Coimbra and whilst undertaking his Ph.D. studies at the Université Catholique de Louvain in Brussels, he developed an interest in advanced drug delivery systems. He has continued to work in this area, and has more than 20 years experience in the design and evaluation of nanoparticles and liposomes for drug (e.g. Leishmaniasis and cancer) and gene (cytosolic) delivery.

More recently, these interests have broadened to other aspects of the nanomedicine field including the design of vectors for MRI imaging and also understanding the cellular mechanisms that govern the action of nanomedicines.

Throughout his career, Rogerio Gaspar has been called upon to support the development of Portuguese Regulatory strategy and his participation on numerous national committees led to invitations to join EMEA as an advisory expert. He also has been a participant on several EMEA Working Groups developing both European Regulatory Strategy and overseeing International Harmonization. He was a member of the Working Group on Human Medicines of the European Council (European Union) during the Portuguese Presidency, being the Chairman of this group when political agreement was reached on the new European Clinical Trials Directive (2000).

These aspects of his career give Rogerio a unique perspective of both nanomedicines research and development and the Regulatory process.

CO.14 –Nanopartículas: análise e percepção de risco

Interventor

José Palma de Oliveira

Currículo resumido

José Manuel Palma nasceu em Setúbal e continua a viver nessa cidade e é Professor de Psicologia Ambiental e Percepção de Risco na F. Psicologia da Universidade de Lisboa. Foi Presidente da Society for Risk Analysis – Europe em 2000/2001 (sociedade científica) e activista da Quercus tendo sido Presidente da Direcção Nacional (para além de Vice-presidente e Presidente da Assembleia Geral) durante parte dos anos 90. Foi membro da Direcção da European Federation of Transport and Enviroment desde 1997 até 2010, para além de perito do ECOSOC e junto dos grupos de trabalho científicos que prepararam as directivas sobre ruído (EU). Foi membro efectivo do Conselho Nacional da Água e Comissão de Acompanhamento do Empreendimento de Fins Múltiplos de Alqueva (entre 97 e Janeiro de 01). Foi presidente do Conselho de Administração da Fundação para a Protecção das Salinas do Samouco entre 2001 e 2008. É consultor (gestor de risco) na Ambimed (tratamento de resíduos hospitalares perigosos), na Secil (co-incineração de combustíveis alternativos RIB e Perigosos), e Presidente dos Parques de Ecologia Industrial (grupo SGR /Transucatas). Dos seus trabalhos na área de análise, gestão e percepção de risco destacam-se: I. Linkov and J. M. Palma-Oliveira (Eds.) (2001). Assessment and management of environmental risks: Methods and applications in eastern european and developing countries. Dortrech: Kluwier; I. Druzhinina and J. M. Palma-Oliveira (2004) Radioactive contamination of wild mushrooms: a cross-cultural risk perception study. J. Environmental Radioactivity 74: 83-90; A. Mata, D. Antunes, R. Gaspar de Carvalho, C. Rodrigues and J. M. Palma-Oliveira (2006) Risk Communication: How to communicate and to be understood successfully. In C. Guedes Soares and E. Zio (Eds.) Safety and reliability for managing risk, 1321-1327. London: Taylor and Francis; J. M. Palma-Oliveira, R. G. de Carvalho, S. Luís and M. Vieira (2009). Knowing much while knowing nothing: perceptions and misperceptions about nanomaterials. In Igor Linkov and Jeffery Steevens (Eds.) Nanomaterials: risks and benefits (437-462). Netherlands: Springer. Tem actualmente vários artigos em fase de submissão sobre percepção de risco das nanotecnologias.

Resumo da Comunicação

Será discutido a expansão do trabalho em nano e nas suas aplicações e o desafio que isso coloca na definição de metodologias de análise e gestão de risco. Serão analisadas brevemente algumas estratégias de análise de risco e feita a sua comparação metodológica. Será defendido que a principal razão para essa decalage entre a análise de risco e a aplicação dos nanomateriais é a especificidade do olhar da sociedade sobre as nano. Durante os últimos anos tem aumentado exponencialmente o trabalho sobre percepção de risco das nanopartículas. Apesar do conhecimento da população sobre esses assuntos ser muito limitado, o padrão de resultados é bastante consistente em várias partes do mundo. Assim, para além de uma atitude geral positiva da parte dos europeus e da capacidade para criar um "mapa mental" relativamente detalhado das nano, os resultados apontam para uma diferenciação bastante acentuada entre a percepção de risco e atitude em relação à nanotecnologia em geral e das suas aplicações concretas. Para além de uma revisão global sobre esta linha de percepção de risco, serão apresentados um conjunto de trabalhos realizados pela nossa equipa que pretendem entender quais as diferenças entre as percepções de risco das diferentes aplicações e quais são os predictores que se podem considerar gerais (i.e., que se aplicam a todas os objectos de aplicação – da alimentação à medicina) e os que são muito mais sensíveis a elementos contextuais. Esta visão pormenorizada da percepção de risco das nano será fundamental para ajudar a definir uma estratégia de gestão de risco.

Comunicações em Posters

Lista de Resumos Submetidos

P.01 DEVELOPMENT AND CHARACTERIZATION OF MODIFIED-PULLULAN-BASED NANOPARTICLES FOR PROTEIN DELIVERY – M. Dionísio, A. M. R. Costa and A. Grenha.

P.02 HYDROCORTISONE-LOADED POLY(ε-CAPROLACTONE) NANOPARTICLES FOR TARGETED DELIVERY TO THE SKIN SURFACE: OPTIMIZATION OF THE SOLVENT DISPLACEMENT METHOD – C. Silva, C. Rosado and C. P. Reis.

P.03 PREFORMULATION STUDY: SELECTION OF A METHOD FOR PRODUCTION OF PLGA NANOPARTICLES FOR LATER ENCAPSULATION OF AZELAIC ACID – N. Martinho, C. Rosado, C. Silva and C. P. Reis.

P.04 ENCAPSULATION OF ROSMARINIC ACID INTO CHITOSAN NANOPARTICLES – S. Baptista da Silva, M. Amorim, A. Oliveira, O. Ramos, M. Pintado, D. Ferreira and B. Sarmento.

P.05 LIPOSOMAL IMATINIB – MITOXANTRONE COMBINATION: FORMULATION DEVELOPMENT AND THERAPEUTIC EVALUATION IN AN ANIMAL MODEL OF PROSTATE CANCER – A. C. Pinto, J. N. Moreira and S. Simões.

P.06 IMPROVED SHELF-LIFE OF CATIONIC SOLID LIPID NANOPARTICLES – S. Doktorovova, A. M. Silva, C. M. Lopes, P. Martins-Lopes and E. B. Souto.

P.07 EFFECT OF TYPE OF POLOXAMER AND PRODUCTION PARAMETERS IN THE ENCAPSULATION OF INSULIN INTO POLYMERIC MICELLES – F. Andrade, F. Antunes, M. Videira, D. Ferreira and B. Sarmento.

P.08 IMPROVING INSULIN DELIVERY BY MIXED NANOPARTICLES FROM PLGA AND AMPHIPHILIC CYCLODEXTRINS – A. F. Soares, A. Gèz, C. Ribuot, P. Faure, L. Choisnard, R. A. Carvalho, J. G. Jones, D. Wouessidjewe and F. J. Veiga.

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P.021 EVALUATION OF ANALGESIC ACTIVITY OF AGRIMONIA EUPATORIA L. INFUSION ON IN VIVO MODELS – T. Santos, J. Ferreira, G. Costa, V. Francisco, M. M. Caramona, M. M. Castel-Branco, M. T. Batista and I. V. Figueiredo.

P.01 Development and characterization of modified-pullulan-based nanoparticles for protein delivery

Marita Dionísio, Ana M. Rosa da Costa and Ana Grenha*IBB - Institute for Biotechnology and Bioengineering/CBME - Centre for Molecular and Structural Biomedicine, University of Algarve, Portugal

Introduction: Due to their interesting properties, natural polysaccharides have received increased attention in the field of drug delivery systems design. In particular, these materials seem to be very promising for the preparation of nanocarriers. Nanoparticles have been claiming great ability to associate biopharmaceuticals and to increase cellular uptake [1]. Their preparation through a mild polyelectrolyte complexation is considered very advantageous, avoiding aggressive preparation conditions such as organic solvents and high shear forces. Pullulan is a non-ionic exopolysaccharide of fungal origin which usefulness has been explored for various biomedical applications, including drug and gene delivery, as well as tissue engineering [2].

Objectives: Neutral polymers like pullulan do not allow a direct application of the referred polyelectrolyte complexation method, due to the lack of charges on the molecule structure. Therefore, the aim of this work was to prepare pullulan derivatives which could be used to prepare pullulan-based nanoparticles by complexation with other natural polymers with reported successful applications in drug delivery, such as chitosan and carrageenan.

Methods: Simple chemical alterations were performed in pullulan, introducing in the polymer sulphate, carboxylate and amine groups, which resulted in different charged derivatives. These were characterised by Fourier-transform infrared spectroscopy (FTIR), nuclear magnetic resonance (NMR) and gel permeation chromatography (GPC). Nanoparticles based on modified-pullulan were prepared by polyelectrolyte complexation and their mean size and zeta potential were characterised in freshly prepared samples by photon correlation spectroscopy and laser Doppler anemometry, using a Zetasizer® Nano Series ZS (Malvern Instruments, UK).

Results and discussion: FTIR, NMR and GPC confirmed the derivatives structure, which enabled the preparation of nanocarriers with sizes of 240 – 400 nm and strong positive zeta potential up to +50 mV. The determination of in vitro cytotoxicity of developed derivatives and nanoparticles is currently being assessed in Caco-2 cells, using the MTT assay. The next step of protein association will give a further insight on the ability of these vehicles as protein carriers.

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P02 - Hydrocortisone-loaded poly(ε-caprolactone) nanoparticles for targeted delivery to skin surface: optimization of the solvent displacement method

Catarina Silva[1,2],*, Catarina Rosado[2] and Catarina Pinto Reis[1]
[1]CBIOS - Laboratory of Nanoscience and Biomedical Nanotechnology, Universidade Lusófona de Humanidades e Tecnologias, Lisboa, Portugal
[2]CBIOS - Experimental Dermatology Unit, Universidade Lusófona de Humanidades e Tecnologias, Lisboa, Portugal

Introduction: Nanoparticles (NP) are drug carriers which allow the increasing of the drug bioavailability and targeted delivery . Several researchers have shown the potential of the poly-ε-caprolactone (PCL) NP as a biodegradable and bioerodible polymer that provides a promising solution for longer-term drug delivery systems . Hydrocortisone (HC) is a mild corticosteroid well suited for skin absorption [6].

Objectives: This study assessed the influence of manufacturing conditions on the PCL-NP main physical parameters such as particle size (PS), zeta potential (ZP), polydispersity index (PI) and on the amount of drug encapsulated (namely as encapsulation efficiency, EE).

Methods: PCL-NP were prepared by solvent displacement procedure . Parameters such as stirring speed, type and concentration of surfactant were considered. The HC content in the supernatant was measured by spectrophotometry at 242 nm and the EE of HC into PCL-NP was then calculated.

Results: Measurement analysis of the PS indicated a significant decrease of the mean particle diameter in the presence of a surfactant agent ($p<0.05$). Span80® produced the smallest NP with a mean diameter of 201.5 ± 23. However, no influence on the PS and on ZP was demonstrated with the increase of the surfactant concentration from 2% to 4% and the stirring rate (range from 100 to 800 rpm). The addition of a surfactant increased the mean value of the ZP, but decreased the PI value. In the presence of the drug, mean particle size slightly increased after loading (231.6 ± 15). In terms of the ZP, the particles were negatively charged (-5.09 mV) and the PI value was 0.033. EE was around 59%.

Conclusions: PCL-NP may offer a potential system for targeted delivery to the skin surface, especially for highly lipophilic molecules such as HC. The optimization of the PCL-NP procedure allowed the achievement of small size particles, with a homogeneous distribution and an average negatively charged surface after loading which may be crucial for in vivo interaction with the cell membrane. Further studies will focus on skin permeation assays and assess the drug release from the particles.

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P03 Preformulation study: selection of a method for production of PLGA nanoparticles for later encapsulation of azelaic acid

Nuno Martinho[1,2],*, Catarina Rosado[2], Catarina Silva[1,2] and Catarina Pinto Reis[1]
1CBIOS - Laboratory of Nanoscience and Biomedical Nanotechnology, Universidade Lusófona de Humanidades e Tecnologias, Lisboa, Portugal
2CBIOS - Experimental Dermatology Unit, Universidade Lusófona de Humanidades e Tecnologias, Lisboa, Portugal

Introduction: Controlled drug delivery systems making use of polymeric nanoparticles to tissue-specific targeting have become a common strategy [1]. PLGA is a suitable synthetic biocompatible polymer, widely used due to its good mechanical properties, low toxicity and predicable pharmacokinetics that offer a range of possibilities for drug delivery [2; 5-6].

Objectives: This study aims to select a suitable method based on solvent-diffusion-evaporation to produce stable nanoparticles in the range of 200-300 nm by comparing the modified-spontaneous emulsification solvent diffusion method (m-SESD) with classical solvent displacement (SD). The size and zeta potential were determined as the key parameters for the evaluation and selection of the method.

Methods: Nanoparticles were produced by m-SESD [3] and SD methods [4]. Parameters such as the type of organic solvents (acetone, ethanol or dichloromethane), acetone/ethanol (A/E) ratio with m-SESD method, and different coating polymers (Poloxamer or PVA) in order to modulate the zeta potential and stabilize the resulting nanoparticles were evaluated. The size and zeta potential were measured using light scattering and electrophoretic light scattering, respectively.

Results: Both m-SESD and SD allowed the production of nanoparticles in the 200-300 nm range. However, statistical differences were noticed ($p<0.05$) by which m-SESD produced smaller particles (198.90 ± 30.58 nm versus 226.70 ± 3.68 nm) while zeta potential was not different between the two methods. On the other hand, when dichloromethane was used resulted in particle aggregation while zeta potential remained the same. The use of Poloxamer proved to be more effective than PVA in terms of smaller particles and zeta potential with a 3-9 fold negativity value. The correct ratio of A/E for the m-SESD was accessed to be a mixture of 8:2.

Conclusion: PLGA nanoparticles were produced by both methods without the use of chlorinated or other ICH 2 solvents. The use of a correct ratio of A/E led to uniform and smaller particles. For this reason, m-SESD method by the use of Poloxamer will be further evaluated for encapsulation of azelaic acid.

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P04 Encapsulation of rosmarinic acid into chitosan nanoparticles

S. B. Silva[1,*], M. Amorim[2], A. Oliveira2, O. Ramos[2,3], M. Pintado[2], D. Ferreira[1] and B. Sarmento[1,4]
1 -Department of Pharmaceutical Sciences, Faculty of Pharmacy, University of Porto, Portugal
2 - Biotechnology School, Portuguese Catholic University - Porto, Portugal
3 - Institute of Chemical and Biological Technology, New University of Lisbon, Oeiras, Portugal
4 - CICS – Heath Sciences Research Center, Instituto Superior de Ciências da Saúde - Norte, Gandra, Portugal

Introduction: The use of nanotechnology in medical sciences is a innovation that promises a new age of health. Among the different approaches explored so far, chitosan exhibits favourable and unique biological properties, such as biocompatibility, biodegradability, non-antigenic, non-toxicity and mucoadhesiveness . On the other hand, natural extracts have been incorporated in chitosan films, or macro/microparticles, becoming more effective as antimicrobial or improving the antioxidant protection. The combination of antioxidants and nanoparticles is seen as the key to success in the topical administration of therapeutic antioxidants . Phenolic compound like rosmarinic acid are potent natural antioxidant with a multitude of biological activities like anti-inflammatory, antimutagen, antibacterial and antiviral as well as high potential radical scavenging activity .

Objectives: In this study, chitosan nanoparticles with rosmarinic acid were prepared and characterized in order to ensure their best size, efficient encapsulation and to test the antioxidant activity performance.

Methods: Chitosan nanoparticles were prepared by ionic gelation and the encapsulation of rosmarinic acid was tested in different theoretical loadings (5%, 10%, 15%, 20%, 30%, 40% and 50%) fairly to the initial concentration of chitosan, to guarantee the best ratio between chitosan and the acid. Spectrophotometric measurements as well as flurometric techniques were carried out to determine the antioxidant capacity of the nanoparticles with rosmarinic acid such as ABTS and ORAC method and the encapsulation efficiency was measured by a validated HPLC method. Size and zeta potential of the nanoparticles were measured using zeta potential analyzer utilizing phase analysis light scattering - Zeta Pals.

Discussion and conclusions: The data showed that the best results considering the evaluation of nanoparticles size, zeta potential, encapsulation efficiency and antioxidant activity were the 40% of rosmarinic acid loading. Pharmaceutically, the systems are expected to increase the capacity of maintaining the antioxidant activity during the preparation process, to optimize the release of the compound from the nanoparticles. A good control of the physicochemical properties and stability of the nanoparticles to improve the bioavailability and therapeutic efficacy is expected, without compromising the safety performance of the drug.

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P05 - Liposomal imatinib – mitoxantrone combination: formulation development and therapeutic evaluation in an animal model of prostate cancer

Ana Catarina Pinto[1,3], João Nuno Moreira[1,2] and Sérgio Simões[1,2,3,*]
1- Laboratory of Pharmaceutical Technology, Faculty of Pharmacy, University of Coimbra, Portugal
2- Center for Neurosciences and Cell Biology, University of Coimbra, Portugal
3- Bluepharma, Indústria Farmacêutica S.A., S. Martinho do Bispo, Coimbra, Portugal

Introduction: Mitoxantrone plus prednisone is a palliative treatment for hormone-refractory prostate cancer (HRPC) but without survival benefit. Imatinib has shown activity against HRPC but only in the preclinical setting. Our previous in vitro cytotoxicity screening study established that their free combination act additively to inhibit proliferation of PC-3 cells.

Objectives: We aim to develop a liposomal imatinib-mitoxantrone (LIM) formulation with improved in vivo therapeutic activity.
Methods: Imatinib and mitoxantrone were simultaneously co-loaded into DSPC:Chol liposomes by means of a (NH4)2SO4-generated proton gradient method. The optimized formulation was characterized in terms of mean size diameter, loading parameters and drug retention in human serum. In vivo antitumor activity of developed LIM formulation was evaluated in a nude mice bearing subcutaneous PC-3 xenograft model.

Results: LIM formulation exhibited maximal encapsulation efficiency (> 95%) and enhanced drug retention for both drugs. Additionally, this LIM formulation, administered at a low mitoxantrone dose (0.5 mg/kg), showed a tumor inhibition activity (TGI = 66.7% and 4.0-fold tumor volume increase) slightly superior to that of liposomal mitoxantrone (LM) at 2 mg/kg (TGI = 53.0% and 4.2-fold volume increase). Therefore, therapeutic activity of mitoxantrone was significantly improved by co-loading with imatinib since a four times lower dose was needed to achieve an equivalent growth inhibition effect.

Conclusions: The loading parameters and drug retention properties of our LIM formulation, combined with its in vivo antitumor activity, make this formulation an excellent strategy to improve the therapeutic index of mitoxantrone and a promising candidate for clinical development in prostate cancer therapy.

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P06 - Improved shelf-life of cationic solid lipid nanoparticles

S. Doktorovova[1,*], A. M. Silva[2], C. M. Lopes[1,3], P. Martins-Lopes[1] and E. B. Souto[1,3]
[1]Insititute of Biotechnology and Bioengineering, Centre of Genomics and Biotechnology, University of Trás-os-Montes and Alto Douro (IBB/CGB-UTAD), Vila-Real, Portugal
[2]Centre for the Research and Technology of Agro-Environmental and Biological Sciences, University of Trás-os-Montes and Alto Douro (CITAB-UTAD), Vila-Real, Portugal
[3]Faculty of Health Sciences, Fernando Pessoa University, Porto, Portugal

Introduction: Cationic solid lipid nanoparticles (cSLN) have already been proposed as an alternative over cationic liposomes for non-viral gene delivery, bringing important advantages e.g. well-acceptable materials of pharmaceutical use, site-specific delivery, low cost and single-step production process [1]. Shelf-life stability of standard cationic liposomal reagents is usually reported for a period of about 6 months.

Objectives: The aim of this work was to develop cSLN with improved physicochemical stability for gene delivery.
Materials and methods: Imwitor 900 P (IMW) and Lutrol F68 were provided by Sasol GmbH, Germany, Compritol 888 ATO (COM) was provided by Gattefosse SA, France. Cetyl trimethylammonium Bromide (CTAB) was obtained from Sigma, Portugal. cSLN were produced by high shear homogenization method [2] and stored in glass vials at room temperature. Mean particle size (z-ave), size distribution (PdI) and zeta potential (ZP) were analysed by Zetasizer Nano ZS (Malvern Instruments), and solid state was confirmed by differential scanning calorimetry (DSC 821e, Mettler).

Results: One day after production, IMW-based cSLN showed z-ave = 159 ± 6 nm, PdI = 0.34 and ZP = 54.8 mV. These cSLN were stable for max. of 21 days being unsatisfactory for the present purpose. COM-based cSLN had z-ave = 180 ± 15nm, PdI = 0.27 and ZP = 56.2 mV on the day of production and after 340 days at room temperature the recorded values were z-ave = 173 ± 8 nm, PdI = 0.71 and ZP = 45.5 mV (n = 3). These results anticipate improved physicochemical stability than those published elsewhere (180 days) [3]. The higher PdI obtained after 340 days indicates presence of aggregates with size above 1 µm. High ZP of 51.4 mV (IMW) and 50.3 mV (COM) was maintained after purifying cSLN by centrifugation, indicating that most of CTAB might still be adsorbed on particles surface. Solid state of cSLN was confirmed by DSC.

Conclusions: Cationic SLN based on Compritol showed better physicochemical stability over Imwitor cSLN, similar to those of negatively charged SLN. The parameters reported here foresee cSLN as promising laboratory platform for in vitro/in vivo gene delivery.

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P07 - Effect of type of poloxamer and production parameters in the encapsulation of insulin into polymeric micelles

F. Andrade[1,*], F. Antunes[1], M. Videira[2], D. Ferreira[1] and B. Sarmento[1,3]
[1]Department of Pharmaceutical Technology, Faculty of Pharmacy, University of Porto, Portugal
[2]iMed.UL – Research Institute for Medicines and Pharmaceutical Sciences, Faculty of Pharmacy, University of Lisbon, Portugal
[3]Department of Pharmaceutical Sciences, Instituto Superior de Ciências da Saúde – Norte, Portugal

Introduction: Therapeutic proteins have emerged as useful and promising drugs in the treatment of various diseases, however, and despite their high therapeutic potential, its administration in the active conformation through non-invasive routes, has been shown to be an enormous challenge for the pharmaceutical industry
Nano-based drug delivery systems, including micelles, could overcome some problems such low mucosal permeability and instability by protect the proteins from degradation, and enhance their transepithelial transport. Poloxamers are nonionic triblock copolymers composed by a central block of relatively hydrophobic polyoxypropylene surrounded on both sides by the blocks of relatively hydrophilic polyoxyethylene conforming to the general formula HO(C2H4O)a (C3H6O)b (C2H4O)a. They present the capacity to self-assembly into micelles at concentrations above the critical micelle concentration Actually are available different types of poloxamers presenting different characteristics.

Objectives: To assess the effect of type of poloxamer and type of agitation during production in the characteristics of insulin-loaded micelles.

Methods: Insulin-loaded micelles (n = 3) were prepared using the hydration of polymeric film technique. The polymers (30 mg) were dissolved in 3 mL of chloroform that was removed under vacuum and a polymeric film was obtained. Insulin aqueous solution (1 mg) was added to this film to get 10 mg/mL polymer concentration, followed by extensive vortexing or sonication. Nonincorporated insulin was separated by filtration of micelle suspension through a 0.2 µm filter and quantified using the HPLC method developed and validated previously]. Particle size was measured by dynamic light scattering (DLS) at 25 °C with a detection angle of 90° and zeta potential by phase analysis light scattering (PALS) (n = 3). Association efficiency (AE%) as the amount of insulin associated with the micelles was also calculated.

Results: In terms of size, micelles present a mean diameter between 250 and 420 nm and the produced with higher molecular weight poloxamer are slightly bigger (188<407<338). Sonication appears to produce smaller micelles with lower polydispersity when compared to vortexing. The zeta potential of micelles is close to zero and the AE is about 20% for all micelles (Table 1).

Table 1

Type of poloxamer	188		338		407	
Agitation	vortexing	sonication	vortexing	sonication	vortexing	sonication
Mean diameter	241,07 ± 186,50	257,63 ± 177,83	416,60 ± 63,99	278,80 ± 145,27	305,27 ± 91,26	254,73 ± 142,68
Polydispersity	0,23 ± 0,37	0,03 ± 0,05	0,25 ± 0,21	0,07 ± 0,12	0,19 ± 0,25	0,10 ± 0,05
Zeta potential	3,43 ± 2,86	1,46 ± 0,73	-0,98 ± 5,63	1,06 ± 4,11	3,68 ± 7,06	-1,00 ± 7,95
AE%	22,04 ± 2,72	17,76 ± 4,07	23,65 ± 0,48	21,12 ± 2,16	26,69 ± 4,47	18,93 ± 2,11

Discussion and conclusion: Sonication appears to produce particles with lower mean diameters and less polydispersity, with the exception of micelles produced with poloxamer 188. It also appears that the increasing in the size of micelles is proportional to the increasing of the molecular weight of poloxamer used. In all cases, micelles have a charge close to neutrality, which suggest the presence of polyoxyethylene on their surface . The association efficiency was similar between the different types of poloxamer, although the micelles produced by sonication appear to produce micelles with slightly lower association efficiency. This could be due to the lower mean diameter of particles obtained by sonication. Although, the appearance of the slightly differences between the different micelles, they are not significant (p>0.05). Further studies are needed with higher number of batches to analyze the effect of other parameters like polymer:insulin ratio, agitation time or the solution used during the film hydration.

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P08 - Improving insulin delivery by mixed nanoparticles from PLGA and amphiphilic cyclodextrins

A. F. Soares[1, 2,*], A. Gèze[3], C. Ribuot[3,] P. Faure[3], L. Choisnard[3], R. A. Carvalho[1], J. G. Jones[1] D. Wouessidjewe[2] and F. J. Veiga[2]
[1] Department of Life Sciences, University of Coimbra, Portugal
[2] Faculty of Pharmacy, University of Coimbra, Portugal
[3] Université Joseph Fourier, Grenoble, France

Introduction: Nanoparticle (NP) delivery systems are useful in optimizing the administration of labile peptide drugs, notably insulin [1]. NPs can be prepared using mild conditions by nanoprecipitation [2] from biocompatible and biodegradable Poly(D,L-lactide-co-glycolide) (PLGA). Cyclodextrins (CD) and their derivatives are widely used in drug delivery to improve drugs' solubility, bioavailability and stability [3]. Amphiphilic CD derivatives can also be used to prepare NPs by nanoprecipitation [4].

Objectives: Prepare biologically-relevant NPs from biocompatible materials for insulin delivery.

Methods: Amphiphilic CD grafted C10 alkyl chains (CDC10) by enzyme catalysis [5] and PLGA were dissolved in acetone and poured over a polysorbate 80 solution under magnetic stirring at 25 ºC, solvents were partially removed by evaporation and NPs recovered as a colloidal suspension. Insulin was added as a commercial preparation to the aqueous phase during NP preparation. Particle size was determined by quasi-elastic light scattering and morphology addressed by transmission electron microscopy, insulin association was determined indirectly by HPLC quantification of insulin in the supernatant after centrifugation of NP suspensions. Biological action was measured as the hypoglycemic effect after subcutaneous (sc) injection to fasted rats.

Results: Particles in the nano-size range (117-123 nm) with ~90% insulin association were obtained from PLGA or a PLGA-CDC10 (5:1) blend. NPs did not elicit hypoglycemic response; both insulin(ins)-NP formulations were able to promote a sustained hypoglycemic effect from 0.5 to 2 h by opposition the 2h-peak observed for a free insulin standard. Moreover, a prolonged effect was detected at 6 h for ins-PLGA-CDC10. Areas above the curve in the glycemia profiles from 0 to 8 h after sc injection were: 199 (insulin), 171 (ins-PLGA) and 282 (ins-PLGA-CDC10, $p<0.05$ versus insulin and ins-PLGA).

Conclusions: An original nanosystem for insulin delivery was developed. Hypoglycemic effect of sc insulin was enhanced and prolonged by including a novel CDC10 derivative in ins-PLGA NPs making this mixed system a promising candidate for improving insulin therapy.

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P09 - Evaluation of absorption enhancer effect of dextran sulfate/chitosan nanoparticles as oral insulin delivery system involving triple co-culture in vitro cell models

F. Antunes[1], F. Andrade[1], D. Ferreira[1] and B. Sarmento[1,2,*]
[1]Department of Pharmaceutical Technology, Faculty of Pharmacy, University of Porto, Portugal
[2]Health Sciences Research Center (CICS), Dep. Pharm. Sci., I. S. C. S. N., Gandra, Portugal

Introduction: Several modifications and improvements of Caco-2 cells need to be investigated to generate a more predictable cell model, namely developing co-culture cell models that mimic as close as possible the physiological constitution of the intestine. Nanoparticles are under consideration for oral delivery of proteins like insulin by stabilizing, by ensuring biological activity during transit through the gastrointestinal tract and by facilitating absorption and delivery to the target site [1].

Objectives: To evaluate the absorption enhancer effect of dextran sulfate/chitosan nanoparticles on two in vitro triple co-culture cellular models based on Caco-2 cells to partially reproduce the characteristics of intestinal enterocytes, mucus-secreting cells and M-cells in order to design a model that more accurately mimics the small intestinal epithelial layer.

Methods: Inverted and normal oriented in vitro models of triple co-culture Caco-2/HT29/Raji B were assessed according to Kérneis et al. [2] and des Rieux et al. [3]. Transepithelial Electrical Resistance (TEER) values of the cell membranes were monitored. Monocultures of Caco-2 cells were used as controls. Insulin transport was monitored by HPLC.

Results and discussion: Insulin absorption was higher when HT29 cells were introduced on the co-culture than Caco-2 cells as single epithelial cells. Insulin absorption from insulin-loaded-dextran sulfate/chitosan nanoparticles was higher on normal oriented cell cultures. TEER values of normally oriented inserts were slightly lower than those of inverted inserts (Figures 1 and 2).

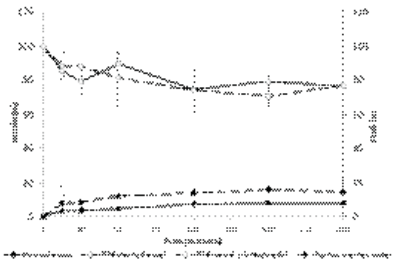


Figure 1. Cumulative insulin transport and TEER measurements from insulin-loaded-dextran sulfate/chitosan nanoparticles across normal oriented and inverted Caco-2/Raji B co-culture models.

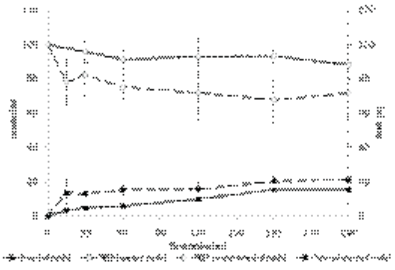
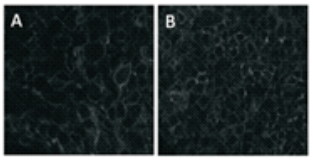


Figure 2. Cumulative insulin transport and TEER measurements from insulin-loaded-dextran sulfate/chitosan nanoparticles across normal oriented and inverted triple Caco-2/HT29/Raji B co-culture models.



The integrity of the cell cultures was also observed by fluorescence microscopy (Figure 3).
Figure 3. Fluorescence microscopy image of Caco-2/HT29/Raji B cells (A) and Caco-2/Raji B cells (B). Caco-2 and HT29 co-culture labeled with Alexa Fluor WGA (magnification 20x).

Conclusions: Results demonstrate that Caco-2/HT29/Raji B triple co-culture normal oriented may be reliable to obtain a more physiological, functional and reproducible in vitro model of the intestinal barrier to study protein absorption. Also, dextran sulfate/chitosan nanoparticles enhance the insulin absorption through the cellular models used, compared to insulin solution.

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P10 - Modification of a galactomannan-based polysaccharide and application in drug delivery

Luís Braz[1,2,*], Ana Grenha[3], Domingos Ferreira[4], Ana M. Rosa da Costa[1,5] and Bruno Sarmento[4,6]
[1]CIQA – Centre for Research in Chemistry of Algarve, University of Algarve, Portugal
[2]School of Health, University of Algarve, Portugal
[3]CBME - Centre for Molecular and Structural Biomedicine, IBB – Institute for Biotechnology and Bioengineering, University of Algarve, Portugal
[4]Dep. Pharmaceutical Technology, Faculty of Pharmacy, University of Porto, Portugal
[5]Dep. Chemistry and Pharmacy, Faculty of Science and Technology, University of Algarve, Portugal
[6]CICS – Health Sciences Research Center, Instituto Superior de Ciências da Saúde-Norte, Portugal

Introduction and objectives: Polymeric nanoparticles have been presented as promising tools to successfully meet the challenge of delivering biopharmaceuticals by oral route. Natural polymers are frequently used in drug delivery systems, since they easily comply with the mandatory requisites of biocompatibility, biodegradability and non-toxicity. The objective of this work is to design a new nanometric drug delivery system, completely based on Locust Bean Gun (LBG), a galactomannan-based polysaccharide abundant in the Portuguese region of Algarve. Nanoparticles are to be obtained by a simple ionic interaction method but, as LBG is devoid of charge, synthesis of positively and negatively charged LBG derivatives were carried out in order to produce pure LBG-based nanoparticles.

Methods: Two charged LBG derivatives, one cationic and one anionic, were synthesized by methods already used to modify other polymers, which were adapted for our purposes. Amination was performed by alkylation with glycidyltrimethylammonium chloride [1] and the sulfate derivative was obtained using SO3.DMF as sulfating agent [2]. Chemical modifications were confirmed by FTIR spectroscopy. Cytotoxicity of these derivatives was tested by the MTT cell viability assay. Nanoparticles were obtained by ionic complexation between the sulfate and the amine derivative, and characterized by photon correlation spectroscopy (size) and laser Doppler anemometry (zeta potential).

Results and discussion: LBG sulphate functionalization was confirmed by FTIR spectroscopy, through the appearance of a S=O asymmetric stretching band at 1255 cm-1. Evidence for formation of the amino functionalized derivative comes from the broadening of the band at 1088 cm-1 and the new bands at 1480 and 914 cm-1.
The MTT cell viability assay revealed the absence of overt cytotoxicity of derivatives up to 0.5 mg/mL, after 3 h incubation with Caco-2 cells.
Nanoparticles were obtained with different mass ratios and their sizes varied between 200 and 450 nm, further evidencing negative charges around -30 mV.

Conclusions: Developed LBG-based nanoparticles display adequate physicochemical properties for drug delivery purposes.

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P11 Design, cell uptake and transport in a simulated vaginal fluid of polymeric nanoparticles intended for microbicide development

José das Neves[1,*], Bruno Sarmento[1,2], Rebecca L. Carrier[3], Mansoor M. Amiji[4] and M. Fernanda Bahia[1]
[1]Dep. of Pharmaceutical Technology, Faculty of Pharmacy, University of Porto, Portugal
[2]Dep. of Pharmaceutical Sciences, Instituto Superior de Ciências da Saúde-Norte, Portugal
[3]Dep. of Chemical Engineering, Northeastern University, Boston, MA, USA
[4] Dep. of Pharmaceutical Sciences, School of Pharmacy, Northeastern University, Boston, MA, USA

Introduction: Recent clinical trial results indicate that topically-applied microbicide formulations may be helpful tools in preventing the sexual transmission of HIV/AIDS. Nanotechnology-based delivery systems can be utilized to enhance delivery efficiency and residence of the antiretroviral drug at the mucosal-epithelial cell interface.
Objectives: To assess the feasibility of engineered polymeric nanoparticles as novel delivery systems for microbicide antiretroviral drugs.

Methods: Poly(ε-caprolactone) (PCL) nanoparticles were developed in order to deliver the NNRTI dapivirine. Different surface modifiers were used to obtain nanoparticles. These were characterized for their size, shape, surface charge, morphology and drug release. The retention and transport of formulated nanoparticles in a simulated vaginal fluid (SVF) were assessed by multiple-particle tracking (MPT) studies. Cellular uptake was examined in different epithelial and phagocytic cells.

Results: PCL nanoparticles had mean particle diameter of 180-200 nm and zeta potential varying from - 53 mV to +45 mV according to the surface modifiers used. Drug release studies in SVF showed fast release of around 50% within the first 15 min, followed by sustained release for 4-8 hours. MPT experiments indicated that nanoparticles predominantly moved through sub-diffusive transport within the SVF. Small differences were observed among different surface-modified nanoparticles. Also, there was a large fraction of the nanoparticles immobilized within the SVF. Rapid cellular uptake of rhodamine-123-loaded nanoparticles was observed in different cell types, thus indicating that nanoparticles may provide an interesting platform for intracellular delivery and accumulation of microbicide drugs in cells relevant for mucosal HIV transmission.

Conclusions: These results seem to support the development of polymeric nanoparticles

P12 - Ibuprofen nanoparticles for oral delivery: characterization and in vivo toxicity evaluation

C. Fernandes[1],*, S. Candeias [2], I. Figueiredo[3], R. Garcia[3], A. S. Cabrita[4] and C. Reis[1]
[1]CBIOS – Laboratory of Nanoscience and Biomedical Nanotechnology (LNBN), Universidade Lusófona de Humanidades e Tecnologias, Lisboa, Portugal
[2]CBIOS – Nutrition and Analitical Methods Unit (UNMA), Universidade Lusófona de Humanidades e Tecnologias, Lisboa, Portugal
[3]Pharmacology Laboratory, Faculty of Pharmacy, Universidade de Coimbra, Portugal
[4]Experimental Pathology Laboratory, Medicine Faculty, Universidade de Coimbra, Portugal

Introduction: Ibuprofen is a non-steroidal anti-inflammatory drug (NSAID) widely used as analgesic and antipyretic. However, therapeutic effects are usually accompanied by undesirable effects, in particular for NSAIDs, the gastric toxicity.

Objectives: Since nanotechnology has created new perspectives in pharmacology, it is intended, using this area, to reduce the gastric toxicity of NSAIDs, using ibuprofen as a model drug.

Methods: Ibuprofen nanoparticles were produced by emulsification-diffusion method [1], using polylactic acid (PLA) as polymer, because it is biodegradable, biocompatible, and shows excellent results in the field of nanotechnology [2]. Encapsulation efficiency of ibuprofen was determined by spectrophotometry [3]. Size and zeta potential were determined by photon correlation spectroscopy (PCS) and by electrophoretic mobility, respectively. Particle morphology was assessed by SEM. In vivo test was conducted with Wistar rats (test group (n = 7), control groups with phosphate buffer pH 7.4 (n = 2), free drug (n = 3) and water (n = 1)). The total daily dose of ibuprofen was 12 mg/kg (3 i.d.) for 10 days. Thereafter, the animals were anesthetized, sacrificed and random fragments were collected from various organs and tissues. All fragments were observed microscopically. Blood samples were collected and analyzed by HPLC [4].

Results: The encapsulation efficiency was 84 ± 5%; the particle size was 281.1 ± 66.7 nm and zeta potential was -4.3 mV. The analysis by SEM showed particles with spherical shape and not agglomerated. In respect to the analysis of histological results, there was a reduction of gastric toxicity when compared with control groups. HPLC analysis confirmed the absorption of the drug. Although, the concentrations were lower in the test group when compared to the free drug.

Discussion and conclusion: The study was successful in terms of the development and characterization of the formulation. Moreover, there was a reduction in gastric lesions caused by ibuprofen. However, serum levels differed between the test and control with ibuprofen probably due to the presence of polymer network, which may have delayed the drug release [5]. Thus, further studies are needed to optimize the formulation such as using other polymers and/or other methods of encapsulation.

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P13 - Development of indomethacin nanocapsules for oral delivery and evaluation of its gastrointestinal toxicity

Cláudia Pires[1,*], Isabel Vitória[2], A. S. Cabrita[3], Rita Garcia[2] and Catarina Pinto Reis[1]
[1]CBIOS – Lab. Nanoscience and Biomedical Nanotechnology (LNBN), ULHT, Lisbon, Portugal
[2]Lab. Pharmacology and Pharmaceutical Care, Faculty of Pharmacy, University of Coimbra, Portugal
[3]Lab. Experimental Pathology, Faculty of Medicine, University of Coimbra, Portugal

Introduction: Indomethacin is a nonsteroidal anti-inflammatory drug widely used in therapy. However, after oral administration, there are often side effects such as abdominal discomfort and gastric ulceration [1]. Herein, and like previously described [2], nanotechnology can be considered as a viable, non-toxic and safe alternative to deliver the free drug.

Objectives: To develop polymeric nanocapsules of indomethacin and to reduce its adverse effects on the gastric mucosa.

Methods: The method used in the production of nanocapsules was the emulsification/solvent evaporation of solvent [2]. The polymer used was PLGA. We studied several process parameters and formulation based on a reference method [3]. The selection of the most adequate formulation was based on the smaller size and greater stability obtained. These parameters were analyzed by spectroscopy photo correlation and by measuring the zeta potential by electrophoretic mobility. The encapsulation efficiency was determined by spectrophotometry at 318 nm. In vivo assay was performed with Wistar rats (test group (n = 7) and control groups with the free drug (n = 3), vehicle (n = 2) and water (n = 1)). The treatment lasted for 5 days (dose of indomethacin administered 10 mg/kg). After 5 days of treatment, we performed the animal necropsy and randomly collected some fragments of tissues for histological evaluation.

Results: The smallest size (167.2 ± 94.7 nm with polydispersity index of 0.488) was obtained using a stirring rate of 1200 rpm and half the concentration of emulsifier agent and double the concentration of drug. This formulation had a stable zeta potential (-33.19 mV) and an encapsulation efficiency of 55%. Additionally, there were no cases of ulceration in the stomach of the test group. In contrast, gastrointestinal toxicity was observed in the group of animals dosed with the free drug.

Discussion and conclusions: This technique produced small nanocapsules with a unimodal size distribution and very stable. This study also demonstrated that encapsulation of indomethacin reduces its toxicity on the gastric mucosa. Therefore, it can be applied to other drugs as well as other applications in the next future.

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P14 - In vitro transdermal delivery of drug loaded alginate microspheres

Maguie Cangueiro[1,*], Patrícia Carvalho[2], Catarina Rosado[2] and Catarina P. Reis[1]
[1]CBIOS - Laboratory of Nanoscience and Biomedical Nanotechnology, Universidade Lusófona de Humanidades e Tecnologias, Lisboa, Portugal
[2]CBIOS - Experimental Dermatology Unit, Universidade Lusófona de Humanidades e Tecnologias, Lisboa, Portugal

Introduction: Encapsulation technique has emerged a few years ago as a promising technology for new drug delivery systems [1]. The production of alginate microspheres via internal gelation offers a method of encapsulation for a variety of biologically active agents [2, 3]. In this study, alginate microspheres were prepared by emulsification/internal gelation technique.

Objectives: The purpose of this study was the controlled release of caffeine (hydrophilic drug) through a encapsulation process into alginate and its subsequent comparison with the permeation of a lipophilic drug, prednisolone, using the same process and to compare those results with traditional dosage forms of caffeine for transdermal delivery.

Methods: Preparation of alginate particles: A 2% (w/v) sodium alginate solution was prepared with the drug at 1% (w/v) and CaCO3 at 5% (w/v). The solution was dispersed at 400 rpm into 50 mL of paraffin oil containing 1.5 mL of Span 80. 15 min later, 20 mL of paraffin oil containing 300 µL of acetic acid were added to the w/o emulsion. Microparticles were recovered using acetate buffer at pH 4.5 and centrifugation cycles [4]. A gel and cream containing caffeine at 1% were also prepared and the viscosity was measured. Morphological and particles size analysis: Morphology was determined by optical microscopy. Size distribution of microparticles was determined by laser diffractometry [5]. Encapsulation efficiency (EE): The EE was determined from the difference between the total free drug and the initial amount of the drug. In vitro release study: The in vitro skin permeation studies were performed on Franz diffusion cells using silastic as permeation membrane.

Results: An encapsulation efficiency of 51% was obtained for the caffeine and 34% for the prednisolone acetate. Particles were spherical with a mean diameter of 45 µm. In vitro permeation results of the O/W cream (8.46 µg/cm2.h-1) and gel (7.61 µg/cm2.h-1) indicated a high release of caffeine. In contrast, alginate microspheres demonstrated the lowest caffeine flux (0.179 µg/cm2.h-1) probably due to a controlled release. For prednisolone, the permeation flux was similar (0.165 µg/cm2.h-1).

Discussion and conclusions: This study confirmed that this technique successfully produces large quantities of small and spherical particles. The highest value of encapsulation efficiency was obtained with caffeine which result is consistent with alginate hydrophilic nature. Additionally, similar permeation rates were obtained for different encapsulants using the same process of particle production. However, when compared to traditional dosage forms, slower permeation flux of caffeine was obtained with microparticles. Thus, this technique can be successfully applied to controlled release of hydrophilic and lipohilic drugs and it can be considered as an important tool for controlled transdermal delivery.

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P15 - Poly(ε-caprolactone) nanoparticles produced by solvent displacement method: the influence of different formulation and process parameters on physical characteristics

Catarina Silva[1,2,*], Catarina Rosado[2], Nuno Martinho[1,2] and Catarina Pinto Reis[1]
[1]CBIOS - Laboratory of Nanoscience and Biomedical Nanotechnology, Universidade Lusófona de Humanidades e Tecnologias, Lisboa, Portugal
[2]CBIOS - Experimental Dermatology Unit, Universidade Lusófona de Humanidades e Tecnologias, Lisboa, Portugal

Introduction: Poly-ε-caprolactone (PCL) is a semi-crystalline hydrophobic polymer with long-term biodegradable and bioerodible characteristics, which makes it very suitable for controlled drug delivery . The drug release rate of PCL nanoparticles (NP) depends on the type of formulation, method of preparation and on the polymer content . There are several methods to produce PCL-NP [4]. One of them is the solvent displacement method which is based on spontaneous emulsification of an organic internal phase, which contains the dissolved polymer, into an aqueous external phase, in the presence of a stabilizer agent .

Objectives: This study will compare different formulation and process parameters of the solvent displacement method for PCL-NP preparation and assess its influence on the NP physical parameters.

Methods: PCL-NP were prepared according two different techniques of the solvent displacement method. First, 125 mg of PCL dissolved in 25 mL of acetone were added to an aqueous phase consisting of 50 mL of distilled water containing 2% (w/v of the aqueous phase) of Span 80®. The emulsion was stirred at 800 rpm using a mixer equipped with a marine impeller . On the other hand, 625 mg of PCL dissolved in 100 mL of acetone were gently poured into 200 mL of distilled water containing 625 mg of Pluronic® F-68 under moderate magnetic stirring (400 rpm). Both resulting suspensions of NP were centrifuged at 12600 X g during 1 hour .
The mean size, polydispersity index, zeta potential and stability of the NP formulations were then measured.

Results: Smaller particles were achieved with Span 80® technique (167.1 ± 48 versus 210.9 ± 43; n = 3). Additionally, this technique also showed a higher homogeneous NP distribution, indicated by a smaller polydispersity index (0.068 versus 0.114) and higher stability according zeta potential measurement.

Conclusions: Depending of the selected formulation and process parameters, smaller and more stable particles can be produced using the same NP production method. These parameters will influence the drug rate release and, finally, its future therapeutic application.

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P16 - Development and optimization process of PLGA nanoparticles obtained by m-SESD method

Nuno Martinho[1,2,*], Catarina Rosado[2] and Catarina Pinto Reis[1]
[1]CBIOS - Laboratory of Nanoscience and Biomedical Nanotechnology, Universidade Lusófona de Humanidades e Tecnologias, Lisboa, Portugal
[2]CBIOS - Experimental Dermatology Unit, Universidade Lusófona de Humanidades e Tecnologias, Lisboa, Portugal

Introduction: The modified-spontaneous emulsification solvent diffusion method (m-SESD) is a method that provides simplicity, low cost, low energy outlay, versatility for lipo-and hydrophilic drugs, high encapsulation efficiency and makes use of non-toxic solvents [1-5]. Nanoparticles have several advantages in topical delivery and PLGA microparticles have already been successful delivered to the pilosebaceous unit, making PLGA an ideal polymer to test [3].

Objectives: This study aimed to evaluate different parameters in the production of 200-300 nm nanoparticles by m-SESD to further encapsulate azelaic acid.
Methods: Nanoparticles were produced with modifications of the original m-SESD [4]. Parameters such as the presence or absence of a stabilizer, the type of the stabilizer (Poloxamer or sodium laurylsulfate - SLS), speed rate of dropping the oil phase into the aqueous phase, extra addition of water after nanoparticles formation, presence of an encapsulated model drug (ibuprofen - IBU), enhancement viscosity of the oil phase (by the use of PEG400) and freeze drying effects were investigated. The size and zeta potential were determined as the key parameters for the evaluation of the parameters to be use.

Results: m-SESD was a stable process which produced nanoparticles with a size range from 200 to 300 nm but only in the presence of a stabilizer. No statistical differences in the mean size were noticed between different speed rate of dropping, the presence of IBU, enhancement of viscosity or extra addition of volume of water. However, the type of stabilizer and the enhancement of viscosity were important for the stability of the nanoparticles. SLS was the most successful stabilizer which led to a zeta potential around -65 mV, followed by PEG400 with -20 mV. The freeze-drying process increased particles size but also improved nanoparticles stability (based on the higher negative zeta potential) except for the use of PEG400.

Conclusions: m-SESD will be further modified to achieve the characteristics required to further exploit in the encapsulation of azelaic acid for topical delivery. This study confirms that the use of stabilizer is an important parameter to the range of nanoparticles intended and the use of SLS will be further pursed due to its high particle stability.

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P17 - Development of azelaic acid-loaded PLGA nanoparticles by m-SESD method for topical treatment of acne

Nuno Martinho[1,2,*], Catarina Rosado[2] and Catarina Pinto Reis[1]
[1]CBIOS - Laboratory of Nanoscience and Biomedical Nanotechnology, Universidade Lusófona de Humanidades e Tecnologias, Lisboa, Portugal
[2]CBIOS - Experimental Dermatology Unit, Universidade Lusófona de Humanidades e Tecnologias, Lisboa, Portugal

Introduction: Nanoparticles (NP) have several advantages as controlled and targeted drug delivery system due to its small size and good stability [1]. As a result, for skin disorders like acne, a targeted drug delivery directly to the pilosebaceous unit (PU) is an important issue and should be a promising pathway to be pursued [2; 4-5]. Azelaic acid (AzA) is an inhibitor of 5α-redutase and DHT with anti-keratinizing properties [3]. In this study, AzA will be encapsulated into PLGA-NP since it is considered an effective and suitable drug to be delivered to PU for acne treatment [2, 5].

Objectives: This study aims to develop AzA-loaded PLGA-NP by m-SESD method and evaluate the in vitro permeation performance by diffusion studies.
Methods: The AzA-loaded PLGA-NP were produced by m-SESD method [1] with two different surfactants (Poloxamer – POL – and sodium laurylsulfate – SLS) at different concentrations (0.1 to 1%) and at different stirring speed rates (400 to 700 rpm). Size and zeta potential were measured by using light scattering and electrophoretic light scattering, respectively. The non-encapsulated drug in the supernatant was quantified and encapsulation efficiency (EE) was determined by spectrophotometry at 210 nm. In vitro permeation studies were performed using Franz cells. A commercial gel and cream containing AzA were used as controls.

Results: Nanoparticles in the range of 200-500 nm were produced according to the conditions used. For the same conditions (stabilizer concentration and stirring rate), SLS produced smaller and more stable particles (9 fold increase in the zeta potential negativity value). The stabilizer type and concentration as well as stirring rate strongly influenced EE. However, for different conditions accessed, EE was higher when POL was used as a stabilizer (≈70% versus ≈60%). In vitro permeation tests revealed different mean fluxes for free and encapsulated AzA.

Conclusions: Nanoparticles in the range of 200-300 nm with high EE and stability were successfully produced. Stirring rate, type of surfactant and its concentration were considered as the major factors. In vitro studies showed different permeation behaviors but further studies are intended to evaluate drug permeation through human skin.

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P18 - Functional hydrogel nanocomposites for biomedical applications

A. M. Salgueiro, L. Ferreira, J. Moreira, B. J. Goodfellow, A. M. Gil, T. Trindade and A. L. Daniel-da-Silva*
CICECO and Department of Chemistry, University of Aveiro, Portugal

Introduction: A new generation of nanostructured materials with interesting functionalities for biomedical applications is emerging based on recent attempts to find new methods of early-diagnosis of diseases and more effective therapies. In this context, bionanocomposites composed of a biopolymer matrix and inorganic nanofillers appear as attractive materials because they bring together the intrinsic functionalities of inorganic nanoparticles (NPs) and the biointerfaces (biocompatibility and low toxicity) offered by polymers of natural origin [1].

Objectives: This work aims to develop novel biopolymer based nanocomposites with functionalities (thermal, optical, magnetic) useful for bioapplications, including medical imaging and drug delivery.

Methods: Hydrogel nanocomposites comprising a thermosensitive polymer matrix and dispersed nanophases with magnetic and/or optical properties (iron oxide and gold NPs) were prepared following in situ and ex situ preparative routes [2]. Nanogel particles of selected formulations of composites were prepared using nano-reactors (microemulsions) in order to adjust the final particles' size distribution [3]. Bulk and nanosized composites were obtained and extensively characterized by various techniques that allowed to acquire new insights regarding their morphological (SEM, TEM), optical (UV-Vis-NIR spectroscopy), thermal (DSC), rheological and magnetic properties. The nanocomposites were evaluated regarding their behavior as carriers for the release of a model drug in simulated physiological conditions.

Results: The nanocomposites described here form superparamagnetic thermoreversible gels with optical absorption in the Visible-NIR spectral window due to previously selected inorganic NPs used in their preparation. The use of microemulsions afforded hydrogel composite NPs with narrow size distribution and average size <100 nm (essential in the development of nanocarriers with prolonged blood circulation times). The composite NPs were thermosensitive in the vicinity of 37ºC and were successfully conjugated to an antibody. The release rate of a model drug was found to be dependent on the set up temperature, here investigated as control parameter.

Conclusions: Perspectives of applications of these nanocomposites on medical diagnosis and drug delivery are discussed. In particular, the resulting composites are of potential interest as contrast enhancers in magnetic resonance imaging and as nanocarriers for remotely triggered drug delivery.

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P19 - Modelling of the heating of liposomes by laser radiation

H. Vilhena[1] and J. Coelho[1,2,*]
[1]Laboratório de Óptica, Lasers e Sistemas, Departamento de Física, FCUL
[2]Instituto de Biofísica e Engenharia Biomédica, Departamento de Física, FCUL

Introduction: Liposomes have an ever growing role in nanomedicine as drug delivery systems. These relatively impermeable spherical vesicles are composed by a lipid membrane, similar to the membranes in biological cells, around an aqueous core. Due to their ability to encapsulate a large variety of molecules in their inner core they have become an important tool not only in the field of drug delivery but also in genetic therapy and food and nutrition fields. Given their small size, the manipulation of this kind of structures is sometimes performed by optical trapping (one or more laser beams focused on a given sample, interacting by means of optically induced forces).

Objectives: The purpose of this work was to create a numerical simulator for the resulting effects of heating a liposome by laser irradiation.

Methods: The laser beam was defined as having an irradiance Gaussian distribution in a 2D axisymmetrical geometry. The inner region of the liposome and the surrounding medium were defined as being composed of water and the software Comsol Multiphysics was used in solving the heat diffusion equation by the finite elements method.

Results: As an example, a liposome with a diameter of 2000 nm, was irradiated for 1 s by two laser beams in opposing directions and focused in the center of the vesicle. Both beams had a wavelength of 1064 nm and total emitted power of 200 mW. It was obtained a temperature rise of about 15ºC. This variation is in accordance with experimental results obtained in similar conditions and is representative of the difficulties encountered in the manipulation by optical traps of this kind of structures. These difficulties arise from the fact that the temperature values that are reached are close to the typical phase transition temperatures of lipid membranes resulting in a variation of their permeability. On the other hand, altering the membrane permeability can be further explored in the localized release of drugs by optical rather than chemical methods.

Conclusions: The present model could become an important tool in the development of innovative methods for drug delivery and tumor hyperthermia. The performed tests demonstrate that the simulation at smaller scales is limited only by the available computational capacity.

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P20 - Evaluation of analgesic activity of Uncaria tomentosa (Willd.) DC fraction on in vivo models

J. Ferreira[1,3,*], T. Santos[1,3], V. Francisco[2,3], G. Costa[2,3], M. M. Caramona[1,3], I. V. Figueiredo[1,3], M. T. Batista[2,3] and M. M. Castel-Branco[1,3]

[1]Laboratório de Farmacologia e Cuidados Farmacêuticos, Faculdade de Farmácia, Universidade de Coimbra, Portugal

[2]Laboratório de Farmacognosia, Faculdade de Farmácia, Universidade de Coimbra, Portugal

[3]Centro de Estudos Farmacêuticos, Universidade de Coimbra, Portugal

Introduction: Uncaria tomentosa (willd.) DC. (Rubiaceae), commonly known as cat´s claw, is a vine that grows in the Amazon rainforest and is widely used in traditional Peruvian medicine. Bark decoctions are indicated by the Peruvian Indians to the treatment of several ailments such as viral infections, cancer, gastric illnesses (gastric ulcers), arthritis and other inflammatory disorders [1, 2]. Previous in vivo studies confirm the anti-inflammatory and analgesic properties of Uncaria tomentosa bark decoctions [3, 4]. In a previous study, it was proved that a Proanthocyanidins (PA) free decoction does not present anti-oxidant properties, unlike the brute decoction, confirming that this activity is based in the large capacity of these phenols to radical scavenging and suggesting that this activity may be related to the anti-inflammatory and analgesic properties of Uncaria tomentosa [5].

Objectives: The main purpose of this study was to evaluate the analgesic activities of a Proanthocyanidins rich fraction of Uncaria tomentosa (U.t.) decoction in animal models of pain and to relate this activity to these compounds.

Material and methods: U.t. bark decoction was prepared according to the traditional Peruvian medicine, as previously described [5]. The different PA fractions were obtained by gel chromatography on a Sephadex LH-20 column according to their behavior under UV observation and the presence of these compounds was confirmed by HPLC analyses. The test groups received fractions at the doses of 4.38 mg/kg (D1) and 8.76 mg/kg (D2) p.o., according to the traditional Peruvian medicine. The hot-plate test [4, 6] was used to evaluate in vivo central analgesic activity, employing morphine as reference drug (10 mg/kg) and the writhing test (diclofenac 10 mg/kg as reference drug) [4, 6] was used to evaluate in vivo peripheral analgesic activity. The analgesic potency of the fraction was expressed as percentage of reduction of latency response and number of writhing responses, respectively. The studies were performed in male mice weighing 25-30 g, fasted and with free access to water at least 18 h prior to the experiments.

Results and discussion: The results obtained for the peripheral analgesic model were 0% for D1, 51.57% for D2 and 75.23% for positive control group, indicating that oral treatment with U.t. PA rich fraction prevents peripheral pain in double dosage. Although, for the central pain model, results show that this fraction does not present analgesic activity. All together, these results suggest that the analgesic activity of U.t. is related to the nociception mechanism of pain, likely associated with the inflammatory process, suggesting that PA prevent the development of inflammatory response.

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P21 - Evaluation of analgesic activity of Agrimonia eupatoria L. infusion on in vivo models

T. Santos[1, 3,*,] J. Ferreira[1, 3], G. Costa2, 3, V. Francisco[2, 3], M. M. Caramona[1, 3], M. M. Castel-Branco[1, 3,] M. T. Batista[2,3] and I. V. Figueiredo[1,3]
[1]Laboratório de Farmacologia e Cuidados Farmacêuticos, Faculdade de Farmácia, Universidade de Coimbra, Portugal
[2]Laboratório de Farmacognosia, Faculdade de Farmácia, Universidade de Coimbra, Portugal
[3]Centro de Estudos Farmacêuticos, Universidade de Coimbra, Portugal

Introduction: Agrimonia eupatoria L. is a plant found on clay soils, namely in Portugal. In popular medicine, Agrimonia eupatoria L. is used for the treatment of several disorders including inflammation [1]. In 2006, a hydro-alcoholic extract of Agrimonia was chemically characterized. This extract presented a prevalence of several ? avonoids [2] that have anti-oxidative activity by reducing free radicals, namely ROS [2]. It has been demonstrated that the ethyl-acetate fraction of Agrimonia eupatoria L. aqueous extract shows anti-oxidant activity and lower toxicity levels.

Objectives: Based on the referred findings, the aim of this work was to evaluate the analgesic activity of Agrimonia in animal models of acute pain.

Methods: The ethyl acetate fraction of the aqueous extract was obtained from the infusion of the aerial parts of the plant, according to the usages in traditional medicine, followed by the treatment with n-hexane to remove the fat-soluble compounds. The resulting solution was repeatedly extracted with ethyl acetate. The in vivo analgesic experiments were performed in mice. The hot-plate test (morphine as reference drug) [3] and the writhing test (diclofenac as reference drug) [3] were used to evaluate, respectively, the central and peripheral analgesic activities. In both models test groups were used with two concentrations of the fraction.

Results and discussion: The results for the central analgesia model indicate no activity of the ethyl acetate fraction of Agrimonia. However, for peripheral analgesia, a percentage of inhibition of 46.9% was observed, indicating a very significant difference between the negative control group and the higher dose test group, although this value does not overlap the effect produced by the reference drug used. These results and the presence of anti-oxidative compounds in the extract indicate that the analgesic activity of Agrimonia is the indirect consequence of the ability of these compounds to reduce inflammation, namely its biochemical mediators that are also involved.

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