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CBiOS Science Sessions - 2016 -

New methods to explore efficacy and safety of natural origin products

Novos Métodos de Exploração de Eficácia e Segurança de Produtos de Origem Natural

Stefânia Duz Delsin

Faculdade de Ciências Farmacêuticas de Ribeirão Preto, Universidade de São Paulo, Av. do Café, s/n, Monte Alegre, Ribeirão Preto - SP, Brasil
CBiOS - Research center for Health Sciences & Technologies, U. Lusófona, Campo Grande 376,
1749-024 Lisboa, Portugal

Abstract

Recently, probiotics have gained prominent role in dermatology due to their potential in the improvement of atopic eczema, atopic dermatitis, skin rejuvenation properties as well as the innate immunity of the skin. In this study, the microorganism CIDCA and its derivatives were evaluated in relation to their inhibitory capacity of proteinases and antioxidant capacity. For this, micromethods were developed, and first, preliminar results were obtained, with the objective of evaluating such properties as antioxidant, antoi-acetylcholinesterase, anti-tyrosinase, anti-elastase and anti-collagenase. This way, CIDCA and its derivatives showed to have a great potencial as actives to be explored by the pharmaceutical industry, and may present safety and efficacy for being used in topical formulations and orally administered since this study has shown that CIDCA-PFL can inhibit proteinases like acetylcholinesterase (86,84%) and CIDCA-S can inhibit elastase (65,10%) wich can be involved indirectly or directly with the degradation of the extracellular matrix (ECM) or with
dermatological disorders, and so, contributing with premature aging skin. In addition, it was found a promising activity against acetylcholinesterase, with an IC50 of 15,74 µg/mL. Finally, in relation to antioxidant, anti-tyrosinase and anti-collagenase capacity, although lower values were obtained, CIDCA-S still revealed 19,51% in relation to antioxidant capacity, RM inhibited 30,94% in relation to tyrosinase and FK inhibited 43,49% in relation to collagenase enzyme.

Lecturer's resumé

Stefânia Duz Delsin, Pharmacy-Biochemistry student at Faculty of Pharmaceutical Sciences of Ribeirão Preto, University of Sao Paulo. Her main interests in research field are natural products applied to cosmetic area, exploring potential biological activities as antioxidant and metalloproteinase inhibition activities of the extracellular matrix. Moreover, she has interest in the R&D area of cosmetic formulations and in clinical studies applied in humans.

Effectiveness of Hypopressive Exercises in women with pelvic floor dysfunctions

Eficácia dos Exercícios Hypopressivos em mulheres com disfunção do assoalho pélvico

Beatriz Navarro Brazález

Faculty of Medicine and Health Sciences, Physiotherapy Department, University of Alcala

Abstract

Pelvic floor dysfunctions include urinary and anal incontinence, pelvic organ prolapse or sexual disorders among others. Pelvic floor dysfunctions affect nearly 25% of women, prevalence that reaches the 50% in parous women. Pelvic floor physical therapy focused on the training of pelvic floor muscles is included in the first-line conservative treatment to tackle pelvic floor dysfunctions. New exercises in the treatment of pelvic floor muscles, such as Hypopressive exercises, are emerging and to assess their efficacy, reliable measuring tools are essential. Therefore, three are the main aims of this study:
1. Assess the reliability and the correlation of common assessment instruments of female pelvic floor muscles.
2. Describe the pelvic floor muscle action during a Hypopressive exercise.
3. Evaluate the effectiveness of Hypopressive exercises versus common pelvic floor muscles physical therapy in women diagnosed with pelvic floor dysfunctions.
To achieve the objectives, three phases are being developed. First, an intra and inter-rater reliability study and a descriptive cross-sectional correlation study of four pelvic floor muscles measuring instruments. Second, a descriptive cross-sectional study of the electromyography action of pelvic floor and abdominal muscles during a Hypopressive exercise comparing to a voluntary pelvic floor muscle contraction. Third, a single-blind randomized clinical trial in ninety women with pelvic floor dysfunctions.
Preliminary results indicate that vaginal perineometry, vaginal dynamometry and surface electromyography were all found to be reliable tools in the assessment of pelvic floor muscles strength in women with pelvic floor dysfunctions. The digital palpation is an intrarater reliable method, however, it should not be used to compare results between different examiners. For longitudinal studies, perineometry and dynamometry are more suitable strength assessment instruments.

Lecturer's resumé

Beatriz Navarro Brazález é graduada em fisioterapia pela Universidade de Alcalá no ano 2010, Mestre em Investigação nos Cuidados de Saúde pela Universidade Complutense de Madrid em 2012, e estudante de doutoramento na Universidade de Alcalá. Trabalha como fisioterapeuta geral na assistência privada, como membro investigador no Grupo de Fisioterapia nos Processos de Saúde da Mulher da Universidade de Alcalá, e como professora colaboradora de grado e pós-grado de Fisioterapia. As suas áreas de investigação envolvem ensaios clínicos na prevenção das lesões vasculares e neuromusculares em mulheres após cirurgia do cancro da mama, na prevenção de desordens do assoalho pélvico após a gravidez e no tratamento dessas doenças. Também participa em estudos de validação de questionários e ferramentas de medição, tendo já várias publicações em revistas e congressos nacionais e internacionais.

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Indoor air quality in baby rooms: a study about VOC levels

Qualidade do Ar Interior em Quartos de Bebés – Estudo das Concentrações de Compostos Orgânicos Voláteis (COV)

Raquel Rodrigues dos Santos[1], Ana Sofia Fernandes[2] e Liliana Mendes[2]

[1]Administração Regional de Saúde de Lisboa e Vale do Tejo (ARSLVT)
[2]CBiOS - Research centerfor Health Sciences & Technologies, U. Lusófona, Campo Grande 376,
1749-024 Lisboa, Portugal

Abstract

A importância da qualidade do ar interior na saúde das crianças é claramente reconhecida, em especial no contributo para o aparecimento de doença respiratória. Os COV poderão ser protagonistas de relevo nesta problemática. Com o objetivo de contribuir para o aumento do conhecimento científico, que neste domínio favoreça a saúde ambiental da criança, iniciou-se um estudo transversal, através de colheitas de amostras de ar e aplicação de um questionário. Procuram-se associações entre os níveis de COV no interior do quarto de bebés, e as características ambientais do quarto e eventuais episódios de pieira/sibilância. Uma vez que ainda se encontra a decorrer, apuraram-se as principais oportunidades e constrangimentos associadas às várias etapas da sua execução. Apesar de diversas dificuldades sentidas, em especial com a disponibilidade dos constituintes da amostra, acredita-se que estão reunidas as condições para que se possa concluir o estudo e apurar resultados.

Lecturer's resumé

Nascida a 27 de fevereiro de 1974, Licenciada em Saúde Ambiental, Mestre em Saúde Pública e Doutoranda em Ciências da Saúde. Com formação pós-graduada em áreas como Políticas de Saúde, Administração e Gestão em Saúde, Epidemiologia Espacial, Alta Direção em Administração Pública e em Gestão Pública. Iniciou carreira profissional em 1997, como TDT na área de Saúde Ambiental na ARSLVT, alternada e paralelamente tem sido Docente do Ensino Superior Público e Privado, Consultora e Formadora e tem desenvolvido diversas funções de Administração e Gestão, nomeadamente, Diretora do Centro de Saúde de lombo-lombo do Governo Provincial de Cabinda. Em 2011 foi mãe e talvez por esse facto, a partir de então, se tenha dedicado ao estudo da Saúde Ambiental das Crianças. É Vice-presidente da Sociedade Portuguesa de Saúde Ambiental e Membro do Corpo editorial da Revista Iberoamericana de Salud Ambiental.

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A medicinal chemistry approach for the development of novel anti-tumor agents

Desenvolvimento de novos agentes anti-tumorais utilizando química medicinal

Maria M. M. Santos

Instituto de Investigação do Medicamento (iMed.ULisboa), Faculdade de Farmácia da Universidade de Lisboa, Lisboa, Portugal

Abstract

One of the most appealing targets for developing anticancer treatments is the p53 transcription factor. This protein is involved in tumor suppression by triggering cell death, cell cycle arrest and senescence, and it is inactivated in all types of cancers either by mutation or inhibition by endogenous negative regulators (e.g. MDM2 and MDMX). Several strategies are being developed to activate p53, and in particular targeting p53-MDM2 interaction has emerged as a promising approach to treat cancers that retain wild type p53 function.

In the last years our group has been interested in the development of novel chemotypes that activate the tumor suppressor p53 by inhibition of the p53-MDM2 interaction. In particular, we have developed several spirooxindole and tryptophanol derivatives which were shown to activate the p53 pathway. Structure-activity relationship studies led to the discovery of potent inhibitors which are being optimized towards clinical candidates. Here, we will present our most recent results in the development of novel small molecules with in vitro antitumor activity.

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- Eur. J. Med. Chem. 2014, 22, 266-272.
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Lecturer's resumé

M Santos graduated in Applied Chemistry (1999) and did a PhD in Organic Synthesis with A. M. Lobo (2004, New Univ of Lisbon). She was a post-doc at Amat-Bosch's research group (Barcelona Univ, Spain) working in asymmetric synthesis of biological active compounds (2004-2006). Since 2006, she has been working in the area of medicinal chemistry at the Faculty of Pharmacy (university of Lisbon). Currently, she is an FCT investigator at the Faculty of Pharmacy. In 2015, she received the "Portuguese award for the Best Young Organic Chemist 2015" from the Organic Chemistry Division of the Portuguese Chemical Society (SPQ) and sponsored by BIAL.

Research in Santos's group combines organic synthesis and medicinal chemistry with applications for the synthesis of bioactive molecules. The ultimate goal of her basic research is the development of novel chemical families that act on important therapeutic targets, such as caspases, p53, and NMDA receptors.

More information can be found at: http://www.ff.ul.pt/~mariasantos/

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Isolation, modelling and phytosome forms of antibacterial and anti-proliferative compounds from Plectranthus spp

Isolamento, modelação e formas fitossomais de componentes antibacterianos e antiproliferativos de Plectranthus spp

Diogo Matias

CBIOS - Research Center for Health Sciences & Technologies,
U. Lusófona, Campo Grande 376,
1749-024 Lisboa, Portugal

Abstract

Natural products were a sustainable source of chemical diversity. Namely, diterpenes obtained from the Plectranthus genus possess several pharmacologic applications. This work intended the antibacterial and cytotoxicity screening of extracts from three plants of the genus Plectranthus obtained by the combination of different solvents and extractions methodologies. The active components were then incorporated into phytosomes in order to improve their delivery and stability.
Some of the P. madagascariensis extracts showed relevant antibacterial (Staphylococcus spp.), antioxidant and cytotoxic (MDA-MB-231) activities. Those extracts were rich in polyphenols as rosmarinic acid and diterpenes as 7α,6β- dihydroxyroyleanone and coleon U. The most antibacterial extract was selected for its incorporation into phytosome. This extract presented potent activity against skin pathogens as S. aureus (including methicillin-resistant strains) and S. epidermidis. The obtained phytosomes were spherical and amorphous with average size of 191.3±75.3 nm. After encapsulation with chitosan an improvement of the surface charge (+21±12 mV) and size dispersion (0.2) with average particle size of 1082±363 nm and encapsulation efficiency of 57.7±6.0%. Those particles showed a sustained release over 10h and maintenance of the antibacterial activity verified in the extract.
This study demonstrated the potential of the genus Plectranthus as source of lead anticancer and antibacterial agents. The developed micro-encapsulated extract of P. madagascariensis corresponds to a topical antibacterial candidate.

Lecturer's resumé

Integrated master in Pharmaceutical Sciences (FFUL) and PhD student in Health Sciencies (ULHT/UAH) in partial time. Have developed its professional background in the pharmacy and in academic research passing for the iMed. UL and IMM/FMUL. His main research interests were at the identification of natural compounds with anticancer and antibacterial activity and their delivery in nanoparticle formulations. He is the author of some articles and book chapters in his areas of interest. Have also functions as adjunct pharmacist into a local pharmacy and is member of the OF, SPFito and SPQ.

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Universidade Lusófona, Campo Grande 376, 1749-024 Lisboa, Portugal

Intellectual Property – Patenting

Propriedade Intelectual – Patenteamento

Rui Gomes

J. Pereira da Cruz, Rua Vitor Cordon 14, 1249-103 Lisboa, Portugal

Abstract

Intellectual Property (IP) has been existing for centuries, but in the last few decades a huge increase in its use and, consequently, its relevance, has occurred. This large exploitation of IP Rights is present in very different areas: artistic creations such as music compositions, live performances, technological innovation or client-company relationship. All these and many others are defined in the several types of IP: Industrial Property – which relates to patents, trademarks or designs –, and Copyright and Related Rights – related to artistic creations or performances.
The relevance of IP finds its basis in the benefits it provides, such as the grant of a monopoly for a specific time period and territory, being a special kind of property, an intangible asset which may be exploited by itself. It not only affects major business players but also SMEs and non-profit entities, such as public universities, research institutions or NGOs.
In this seminar IP will be put in context, and a special focus will be given to technological innovation, how and why should technological innovation be thought in the context of IP, and how can the tools related to IP be put into practice, while benefiting the development of the activity of any entity – non or for profit.
Basic and very practical concepts – mostly related to patents but also broadly applicable in IP – will briefly be presented, enabling the audience to put them into practice in work situations.

Lecturer's resumé

Consultant in Industrial Property specialized in patents since 2014, with the qualification of Portuguese Industrial Property Attorney and in process of obtaining the qualification of European Patent Attorney.
PhD in Technological Physics since 2013 before the University of Coimbra and with professional experience as R&D Engineer in the Industrial Instrumentation area (2008-2011), having also worked as a Patent Examiner in the Portuguese Institute of Industrial Property (2011-2014).
Experienced in training in the Intellectual Property area, both for dissemination and for specific training purposes.
For more information, consult https://pt.linkedin.com/in/rngomes.

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Biomarkers in wastewater

Biomarcadores em águas residuais

Álvaro Lopes

Faculty of Veterinary Medicine – ULHT, Campo Grande, 376, 1749-024 Lisboa

Abstract

As questões ambientais estão na ordem do dia e a grande maioria dos cidadãos tem a consciência dos múltiplos potenciais de risco ecológico que podem advir por uma utilização não racional dos recursos e pelos desperdícios e produtos gerados pelas sociedades de amplo consumo. A maioria dos estados membros da União Europeia, a exemplo do que se faz em outras partes do mundo, têm programas de monitorização permanente das situações de risco ambiental. Do vasto conjunto de substâncias químicas colocadas no ambiente surgiu há alguns anos atrás (anos 90) a preocupação relativamente ao "destino" e "risco" da grande quantidade de produtos farmacêuticos que são tomados (e eliminados) diariamente, e que são massivamente colocados nas redes de saneamento, tratados em ETAR com graus de eficácia variável e posteriormente relançados para os sistemas hídricos e ambiente com consequências ecológicas difíceis de prever em toda a sua plenitude. Dados estes factos, a monitorização de resíduos farmacêuticos em águas residuais, rios e lagos tem vindo a ser razoavelmente implementada por todos os estados membros da UE, Portugal incluído, embora ainda de uma forma limitada e pontual. Uma das consequências positivas destas preocupações tem-se manifestado num aumento progressivo da valoração de deteção de outros possíveis biomarcadores que as águas residuais possam conter e que venham a permitir estudos epidemiológicos de melhor qualidade. O autor focará nesta sua apresentação as potencialidades e limitações da análise de águas residuais, nomeadamente para monitorização de consumos de substâncias psicoativas, o âmbito e atividades dos Programas SCORE e COST da Comissão Europeia e uma breve descrição dos estudos que já foram efetuados em Portugal (pelo autor) e em diversos países da União Europeia assim como as perspetivas futuras desta abordagem analítica.

Lecturer's resumé

Álvaro Lopes é licenciado em Farmácia e Doutorado em Toxicologia pela Universidade de Lisboa. Durante três décadas trabalhou na área das Ciências Forenses (Toxicologia) paralelamente com alguma atividade académica universitária e de investigação que hoje em dia constituem a sua principal dedicação. É um dos dois representantes nacionais do designado Management Committee do programaCOST ES1307 – Sewage Biomarker Analysis for Community Health Assessment. É docente da ULHT-FMV

A Contribution for a Better Comprehension of Donkey Dentistry: the Importance of Dental Care

Uma contribuição para uma melhor compreensão da medicina estomatológico-dentária aplica a asininos: a importância dos cuidados dentários

João Brandão Rodrigues

Faculty of Veterinary Medicine – ULHT, Campo Grande, 376, 1749-024 Lisboa
CBiOS - Research center for Health Sciences & Technologies, U. Lusófona, Campo Grande 376, 1749-024 Lisboa, Portugal

Abstract

Dentistry is the branch of medicine that is involved in the study, diagnosis, prevention and treatment of pathological conditions of the oral cavity, the maxillofacial area and the adjacent and associated anatomical structures, as well as their impact on general health and welfare.

Equid dentistry should be assumed as something prophylactic, through the early diagnosis and treatment of oral and dental disorders in a conservative way, avoiding its development into potentially clinical significant pathology and irreversible damage occurs. Owners should be aware that clinical signs do not always manifest on early stages, with a high number of animals suffering from asymptomatic dental and oral disorders, especially donkeys that are stoic by nature.

It is intended during the presentation to approach and explain the most significant results found in a prospective cross-sectional study in 800 donkeys, divided in 7 age groups (ranging 0-34 years), mainly focused on those clinical aspects that will allow to better understand the principles of donkey dentistry, as well as the most significant differences between horses and donkeys. It is very important to mention that 95.5% of all donkeys included in this study were examined for the very first time and never received any dental treatment. Thus, the results presented in this study allow a more complete comprehension of how dental disorders evolve with age, as well as to identify those disorders affecting specific age groups.

The 74% of donkeys suffer from incisors disorders: craniofacial abnormalities (49.25%), abnormalities in the occlusal surface (21.63%), fractures (17%), periodontal disease (16.13%) and diastemata (14.38%) were the main disorders recorded. Regarding cheek teeth (CT), the 82.75% of donkeys were diagnosed with disorders: CT enamel overgrowths (73.13%), focal overgrowths (37.25%), periodontal disease (23.50%) and diastemata (19.88%) were the main disorders recorded. Some uncommon pathological conditions were diagnosed, such as the presence of supernumerary teeth, salivary duct lithiasis and development of macroscopic focal gingival hyperplasia.

The results obtained during this study highlighted the importance of regular dental care in donkeys, but also draws attention to the importance of similar studies in other equids populations, in order to better understand the prevalence of oral and dental disorders, improving their health and welfare.

Lecturer's resumé

João Brandão Rodrigues – DVM, PhD

Licenciado em Medicina Veterinária pela Universidade de Trás-os-Montes e Alto Douro, em 2007.

Dedicou-se desde então aos equídeos de trabalho, principalmente na área da Medicina Estomatológico-dentária, com várias participações como orador em congressos nacionais e internacionais, assim como várias publicações científicas sobre o tema.

Especialista em "Odontología y Cirúgia Maxilofacial Veterinarias", pela Universidade Complutense de Madrid, desde 2011.

Participa regularmente em projectos internacionais directamente relacionados com bem-estar e a saúde dos equídeos de trabalho, participando como instrutor na sua área de especialização e como consultor externo para as questões de saúde e bem-estar de asininos.

Doutorado em Ciências Veterinárias pela Universidade de Trás-os-Montes e Alto Douro desde 2013, tendo dedicado a sua investigação à patologia estomatológico-dentária que afecta as populações de raças asininas ameaçadas (Burro Mirandês e Asno Zamorano-leonês).

Actualmente é docente na Faculdade de Medicina Veterinária da Universidade Lusófona de Humanidades e Tecnologias (FMV-ULHT), na área da clínica de equídeos.

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Characterization of Lusitano's Pure Blood Pressure Centers using two pressure plates

Caracterização de Centros de Pressão do Puro Sangue Lusitano com recurso a duas placas de pressão

Pequito M.[1]; Gomes-Costa M.[2]; Prazeres J.[1]; Bragança M.[1]; Roupa I.[2]; Fonseca R.G.[1]; Abrantes J.[1,2]

[1]Faculdade de Medicina Veterinária, Universidade Lusófona de Humanidades e Tecnologias
[2]MovLab, Universidade Lusófona de Humanidades e Tecnologias

Abstract

Caracterizar a distribuição simultânea dos centros de pressão (CdP) dos 4 apoios do cavalo e a relação espacial com outros CdP fornece informação sobre a estabilidade postural e desempenho biomecânico.
Este estudo caracteriza, em estação, os CoP palmares, plantares, ortogonais, diagonais e total.
Oito Puros Sangue Lusitano saudáveis, corretamente aparados, permaneceram em estação, com a cabeça em posição neutra e com os anteriores sobre uma placa de pressão Footscan® e os posteriores sobre outra idêntica. Os dados foram adquiridos com o software Footscan® Balance, sincronizados a uma frequência de 50 Hz. Registaram-se os dados de cinco ensaios de 8 segundos, em três períodos diferentes, intervalados de 15 minutos. As coordenadas dos CoP palmares, plantares e ortogonais foram medidos e dos diagonais e global foram calculados. A origem do referencial cartesiano foi comum para ambas as placas. A relação dos deslocamentos do CdP entre cada ensaio e cada período, assim como entre os posteriores e os anteriores foram analizados através do teste de Kruskall-Wallis. Relativamente à localização espacial, as médias das coordenadas do CdP dos cascos anteriores e posteriores formam um polígono próximo do rectângulo. Os CdP ortogonais esquerdo e direito estão alinhados com o eixo das ordenadas (x) dos CdP palmares e plantares, estando, no entanto, oCdP ortogonal esquerdo ligeiramente desviado caudalmente no eixo das abcissas (y). Os CdP ortogonais anterior e posterior estão alinhados com o eixo y dos CdP palmares e plantares, estando o CdP ortogonal anterior ligeiramente desviado para a direita no eixo x. Ambos os CdP diagonais, assim como o global, estão espacialmente próximos, aproximadamente no centro do polígono formado pelos CdP dos 4 cascos. Relativamente ao comportamento dos CdP dos 4 apoios, verificou-se que a média dos mesmos para cada membro apresenta um baixo desvio padrão. Também o deslocamento total de cada CdP, durante a totalidade dos 8 segundos de cada ensaio, é baixo, sendo o menor de 21,5 mm e o maior de 76,7 mm. Observou-se que o deslocamento total dos posteriores é maior do que o dos anteriores. Finalmente, não se encontraram diferenças significativas nos deslocamentos entre cada ensaio, nem entre cada período de experiência.
Evidenciou-se que o cavalo em estação tem o CoP total próximo do centro do polígono formado pelos quatro cascos. Também se observa que o cavalo apresenta uma grande estabilidade postural, deslocando mais os membros posteriores que os anteriores. Finalmente a análise dos CdP é um teste com boa repetibilidade.
Este estudo foi financiando pela Fundação para a Ciência e a Tecnologia (FCT) através do projecto PTDC/CVT/113480/2009 - Biomecânica equina: Análise cinemática e dinamométrica em locomoção equina normal e na comparação do efeito de diferentes conformações e tratamentos ortopédicos.

Lecturer's resumé

Licenciado em Medicina Veterinária, na U.T.L., em 2001
Internato, opção Equinos, na U. de Liège, Bélgica, em 2002
Mestrado em Medicina de Equinos na U. de Liège, Bélgica , em 2004
Doutoramento em Medicina de Equinos, na U. de Liège, Bélgica , em 2015
Clínico no Hospital Veterinário Universitário da U. de Liège, Bélgica, entre 2002 e 2004
Frequência de residência alternada para o E.C.E.I.M., na U. de Liège, Bélgica, entre 2005 e 2008
Clínico privado, em Chantilly, França, entre 2004 e 2008 e, a tempo parcial, em 2014
Docente na F.M.V. – U.L.H.T. desde setembro de 2008

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Application of photoplethysmography to monitor heart rate in dogs

Aplicação da fotopletismografia para monitorização da frequência cardíaca em cães

Rui Assunção[1], Henrique Silva[2,3], João Requicha[1], Luis Lobo[1,4], Luis Monteiro Rodrigues[2,3]

[1]Universidade Lusófona, Faculty Vet Medicine, Campo Grande 376, 1749-024, Lisboa, Portugal
[2]Universidade Lusófona, School of Health Sc & Technologies, CBiOS (Research Center for Biosciences and Health Technologies), Campo Grande 376, 1749-024, Lisboa, Portugal
[3]Universidade de Lisboa, Faculty of Pharmacy, Pharmacol. Sc Depart, Lisboa, Portugal
[4]Hospital Veterinário do Porto, Travessa Silva Porto 174, 4250-475 Porto, Portugal

Abstract

Photoplethysmography (PPG) is a non-invasive optical measurement technique which detects volume changes in the microvasculature. PPG application is widespread in the clinical practice, and its technology is already used, for example on pulse oximetry, and digital blood pressure. The purpose of this pilot study was to apply PPG to monitor the heart rate of dogs during anaesthesia and to characterize the major components of the PPG signal.

This study was carried out at Hospital Veterinário do Porto in 4 dogs of both sexes (1 male and 3 females), from 7 months old to 6 years old and from different breeds. PPG signal was recorded in the tail during 3 to 5 minutes before and after general anaesthesia with a reflection PPG sensor coupled to the Bitalino system (Plux, Portugal). The animals were anaesthetized with an intramuscular injection of methadone (0.35 mg/Kg) plus dexmedetomidine (5 µg/Kg). Heart rate was obtained from the PPG signal using a Matlab based algorithm. Furthermore, the PPG signal was decomposed into its main frequencies with the Wavelet transform.

The pulse wave analysis showed the mean heart rate decreased from 119.57 bpm before anaesthesia to 75.06 bpm after anaesthesia. On a wavelet-based periodogram several bands of activity were found. The band relating to the heart activity ranged between 1.53 Hz and 2.86 Hz before anaesthesia to 0.97 Hz and 1.81 Hz after anaesthesia, also supporting the reduction in heart rate.

These results revealed that PPG is useful in the clinical setting to monitor the effect of general anaesthesia on the cardiac activity of dogs.

Lecturer's resumé

Rui Miguel Vitorino Assunção was born in 1992 in Vila Franca de Xira, Portugal. He is Master student at the Faculty of Veterinary Medicine of University Lusófona. During the last years, Rui attended to conferences in Veterinary Medicine field and had internships in Veterinary Clinic of Laranjeiras (Lisbon, 2013) and Faculty of Veterinary Medicine of Vienna (2014). The traineeship was at the Veterinary Hospital of Porto during 6 months in 2015-2016 in the area of Small Animals Internal Medicine and Surgery. Rui had also a participation in LIAC 2015 as co-author of a poster.

Looking into the oscillatory properties of the laser Doppler flowmetry signal in human microcirculation

Compreender melhor as propriedades oscilatórias da fluxometria por laser Doppler na microcirculação humana

Henrique Silva[1,2], Hugo Ferreira[3], Marie-Ange Renault[4], Alain-Pierre Gadeau[4], Julia Buján[5], LM Rodrigues[1,2]

[1]CBIOS, Universidade Lusofona, Lisboa, Portugal.
[2]Pharmacol Sc, Universidade de Lisboa, School of Pharmacy, Lisboa, Portugal.
[3]Institute of Biophysics and Biomedical Engineering, Universidade de Lisboa, School of Sciences, Lisboa, Portugal.
[4]Adaptation cardiovasculaire à l'ischémie, U1034, Université de Bordeaux & INSERM, U1034, Pessac, France.
[5]Dept Medicine and Medical Specialities, CIBER-BBN Alcalá de Henares, Madrid, Spain

Abstract

A fluxometria de laser Doppler (FLD) é a técnica mais utilizada para a quantificação não-invasiva da microcirculação cutânea. São frequentemente utilizados testes de provocação com o intuito de alterar as condições basais de perfusão e induzir respostas reflexas, das quais se retiram parâmetros que permitem a distinção entre estadios fisiológicos e fisiopatológicos. O objetivo deste estudo foi o de utilizar a provocação de inalação de oxigénio a 100% em modelos humano e animal com o intuito de caraterizar a sua resposta vascular in vivo, registada com FLD. No modelo humano foram utilizados 65 voluntários saudáveis divididos em dois grupos com base na idade (grupo 1: 18-30 anos; grupo 2: 45-65 anos), nos quais se fez um registo unilateral com FLD. No modelo animal foram utilizados 16 murganhos C57BL/6 saudáveis (sexo masculino, 8-27 semanas de idade) anestesiados com uma mistura de ketamina-xilazina, nos quais se fez um registo bilateral de FLD. Em ambos os modelos a provocação consistiu num registo basal de 10 minutos, seguida da respiração normobárica de oxigénio de 10 minutos e de um período de recuperação de 10 minutos. O sinal de FLD foi analisado com a transformada de wavelet (TW), a detrended fluctuation analysis (DFA) e a análise de entropia à multiescala (MSE). A inalação de oxigénio criou hiperóxia que, por seu lado, induziu diferentes respostas vasculares, consistentes entre os dois modelos. No modelo humano 70% dos voluntários respondeu com vasoconstrição e os restantes com vasodilatação, enquanto que no modelo animal foram encontrados três perfis de resposta: vasoconstrição bilateral, vasodilatação bilateral e resposta mista. A análise com TW, DFA e MSE permitiu distinguir os diferentes tipos de resposta vascular. Este estudo revelou pela primeira vez a heterogeneidade da resposta vascular à inalação de oxigénio num modelo humano e animal, contribuindo para o conhecimento atual sobre os mecanismos de regulação da microcirculação.

Lecturer's resumé

Mestre em Ciências Farmacêuticas em 2011 pela Universidade de Lisboa (Faculdade de Farmácia). Atualmente é assistente na Universidade Lusófona e assistente convidado na Universidade de Lisboa como membro do staff de Fisiologia. É aluno do programa de doutoramento em Ciências da Saúde na Universidade de Alcalá-Universidade Lusófona. O seu projeto de doutoramento centra-se na caracterização da microcirculação periférica através da análise das propriedades oscilatórias do sinal de fluxometria por laser Doppler. É autor e co-autor de várias publicações científicas em revistas internacionais e apresentou os seus resultados em diversos congressos nacionais e internacionais.

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Proceedings | *Resumos*

III Symposium of Nanoscience and Biomedical Nanotechnology
III Simpósio Nacional de Nanociência e Nanotecnologia Biomédica

April | Abril 15/04/2016
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Programa

Open Session | *Sessão de abertura*

Reitor da Universidade Lusófona de Humanidades e Tecnologias, Mário Moutinho
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1st Session |*Sessão 1*
Nanomedicine | *Nanomedicina*

Chairman | Moderador - **Catarina Pinto Reis**
Speakers | *Prelectores*
Jorg Kreuter
Sandra Simões
Catarina Silva

1st Session (continuation) | *Sessão 1 (continuação)*
Nanomedicine | *Nanomedicina*

Chairman **|** Moderador - **Luís P. Fonseca**
Speakers | *Prelectores*
Elias Fattal
Pedro Viana Baptista
Diogo Matias

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

Chairman | Moderador - **Hugo Alexandre Ferreira**
Speakers | *Prelectores*
Sónia Fraga
Lino Ferreira
Filipe Pereira

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

Chairman | Moderador - **José Paulo Farinha**
Speakers | *Prelectores*
João Pedro Conde
João Rodrigues
Beatriz Nobre

Open Session

From Left to Right / Da esquerda para a direita:
Maria Dias
Mário Moutinho
Luís Monteiro Rodrigues
Isabel Vitória Figueiredo

1st Session | Sessão 1
Nanomedicine | *Nanomedicina*

Chairman / *Moderador*

Catarina Pinto Reis

Resumé / *Currículo Resumido*
Catarina Reis is currently Assistant Professor of Pharmaceutical Technology I and II in the School of Sciences and Health Technologies at Universidade Lusófona de Humanidades e Tecnologias (Lisbon, Portugal). She attended the University of Coimbra and graduated in Pharmaceutical Sciences in 2003. Her doctoral studies were undertaken at the same university with important international collaborations. She is author or co-author of several articles, book chapters and inventor of patents.

C.01 - Across the blood-brain barrier: Nanoparticles
Passagem através da barreira hematoencefálica: Nanopartículas

Speaker / *Prelector*

Jorg Kreuter

Resumé / *Currículo Resumido*
Prof. Dr. Jörg Kreuter studied pharmacy at the Philipps-University Marburg, Germany and got his Ph.D. in 1974 as well as his Habilitation (Science Doctor) in 1982 at the Eidgenössische Technische Hochschule (ETH) in Zürich, Switzerland. In 1977, he was working as a postdoc at the University of Kansas in Lawrence and in 1979 at the University of Michigan in Ann Arbor. In 1983, he was a visiting professor at the University of Wisconsin in Madison and in 1984 became a professor at the Goethe-University Frankfurt, Germany. He was dean twice, 1988-1989 and 1997-1998. He is one of the pioneers in nanoparticles and has over 300 publications.

Abstract / *Resumo da Comunicação*
The blood-brain barrier (BBB) represents an insurmountable obstacle for the delivery of most drugs to the central nervous system (CNS). One of the possibilities to overcome this barrier is drug delivery to the brain using nanoparticles. Drugs that have been transported into the brain and led to a pharmacological effect after intravenous injection using this carrier include the hexapeptide dalargin, the dipeptide kyotorphin, loperamide, tubocurarine, doxorubicin, and the NMDA receptor antagonists MRZ 2/576 and MRZ 2/596. To achieve a significant transport across the blood-brain barrier the coating of the nanoparticles with polysorbate 80 (Tween® 80) or poloxamer 188 was a key factor.
Experiments with the extremely aggressive glioblastoma 101/8 transplanted intracranially showed a long-term survival for 6 months of up to 40% of the rats after intravenous injection of the polysorbate 80-coated nanoparticle preparation. The surviving animals showed a total remission by histological investigation. Untreated controls died within 10 - 20 days, doxorubicin controls and uncoated doxorubicin nanoparticle groups died between 10 – 50 days. The cardiac, and testicular toxicity of doxorubicin was very significantly reduced by binding the drug to poly(butyl cyanoacrylate) and even more considerably by binding to human serum albumin nanoparticles. Similar PLGA nanoparticles presently are in Clinical Phase I.
The mechanism of the drug transport across the blood-brain barrier with the nanoparticles appears to be transcytosis across the BBB. After injection of the nanoparticles, apolipoproteins E or A-I adsorb on the particles surface mediating the interaction with the respective receptors on the endothelial cells.

1st Session | Sessão 1
Nanomedicine | *Nanomedicina*

C.02 - Nanovesicles for dermal and transdermal delivery
Nanovesículas para administração dérmica e transdérmica

Speaker / *Prelector*

Sandra Simões

Resumé / *Currículo Resumido*
Sandra Simões is a researcher at the Faculty of Pharmacy, University of Lisboa. Her research work has been focused on design, development and biological evaluation of drug delivery systems for topical application, dermal and transdermal delivery, with special application on topical delivery of antioxidants and on chemoprevention of skin photocarcinogenesis.

Abstract / *Resumo da Comunicação*
Nanocarriers have been successfully used to enhance the clinical efficiency of several drugs. More recent approaches in modulating into and through-the-skin delivery led to the development of specialised nanoparticulated systems. Ultradeformable vesicles (UDV) have recently become a promising tool for the development of improved and innovative dermal and transdermal therapies. The stratum corneum is the main penetration barrier of the skin. This barrier efficiently precludes the transdermal delivery of most drugs with molecular weight greater than 500 Da and/or with inadequate oil–water partition coefficient. Different UDV have been tested for the incorporation of actives of distinct properties and examined for controlled release, retention and skin permeation enhancement evaluated by in vitro, ex-vivo and in vivo assays. Drug entrapment in UDV can facilitate localized drug skin deposition and overcome the skin barriers for an efficient drug delivery. Additionally, nanovesicles protect sensitive drugs from degradation, increasing the formulation stability. High hydrophilicity of such nanovesicle surface and extreme vesicle shape adaptability allow non-invasive transport of both large and small drugs from the skin surface into the body (systemic delivery), the peripheral tissues (region-specific delivery) and the skin (dermal delivery).

C.03 - Ligand-functionalized nanoparticles for treatment of melanoma *in situ*
Nanopartículas funcionalizadas com ligandos para o tratamento in situ do melanoma

Poster selected for oral communication / *Poster selecionado para comunicação oral*

Catarina Silva

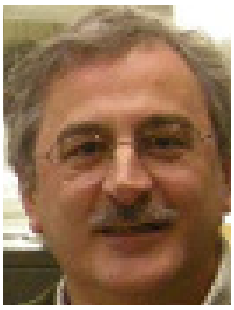
Resumé / *Currículo Resumido*
Catarina Oliveira Silva has a Pharm.D. since 2012 granted by Universidade Lusófona (ULHT). Her master thesis was a result of collaboration between University of Santiago de Compostela in Spain and ULHT on the development and characterization of nanocapsules as transdermal drug delivery systems. Since 2013 she is dedicated to a PhD Program in Biomedical Sciences, organized in cooperation of the University of Alcalá Henares in Spain and ULHT, directed by Dr. Jesús Molpeceres, Dr. Catarina Pinto Reis and Dr. Patrícia Rijo. She also worked as a researcher for Fundação para a Ciência e Tecnologia (FCT) with the project "A new approach to phototherapy tumor targeting: focusing the light through diffusion" (Ref: PTDC/888 BMD/0611/2012), in partnership with Aalborg University in Denmark and University of Minho in Portugal. Catarina has also collaborated as a teaching assistant at ULHT and has published 14 papers, including 3 book chapters, 5 oral communications and several posters presentations in national and international congresses. Her main interests include nanomedicine, melanoma, drug delivery and phototherapy.

Abstract / *Resumo da Comunicação*
Introduction and objectives: Hybrid nanoparticles (NP) made of polymers or natural substances like modified polyesters, lipids, polysaccharides and proteins, hold multiple functionalities and a promising role in localized cancer therapy. When applied to more superficial cancers such as melanoma in situ (i.e., before formation of metastases), NP may improve anti-tumor therapies, preventing cancer evolution to metastatic stages.
Objectives: Aiming the application of nanosystems as targeted anti-tumor platforms, two strategies have been developed: 1) ligand functionalized gold NP for photothermal therapy and 2) ligand functionalized polymeric NP encapsulating a cytotoxic drug.
Materials and Methods: Firstly, hybrid NP, coated with hyaluronic and oleic acids (HAOA) and functionalized with α-melanocyte stimulating hormone (α-MSH), were prepared by a modified solvent displacement method [1]. Parvifloron D, a natural diterpene with marked cytotoxicity, was encapsulated into NP. NPs were characterized in terms of size, PI, zeta potential, pH, morphology and long-term stability. Encapsulation efficiency (EE, %) was determined for loaded NP. The presence of HAOA and α-MSH on the surface of the NP was studied by TEM, FTIR, NMR, X-PS, DSC, CD and CLSM. Internalization of NP and in vitro cytotoxicity on melanoma and non-melanoma cell lines were assessed and in vivo assays were conducted on SCID mice. Secondly, HAOA-coated gold NPs, conjugated with Epidermal Growth Factor (EGF) and with a plasmon absorption band at the near-infrared (NIR) range (i.e., 650-900 nm), were prepared [2]. NPs were characterized in terms of size, PI, zeta potential, pH and morphology. The presence of coating and EGF on the NP's surface was studied by TEM, fluorescence spectroscopy, CD and CLSM. Internalization of NP into human lung carcinoma cells and in vitro cytotoxicity assays were conducted, as well as in vivo assays on SCID mice.
Results and Discussion: Hybrid α-MSH-conjugated HAOA-coated NP showed a mean size of 300 nm (PI: 0.2), while EGF-conjugated HAOA-coated gold NP had 100 nm (PI: 0.2). Parvifloron D-loaded NP showed EE = 87% and a sustained release profile. Both HAOA-coated NP showed a negative zeta potential (-10 mV). Both HAOA coating and ligand attachment was confirmed by the described techniques. Both NP were able to internalize the cells overexpressing specific cancer receptors and reduce melanoma cells viability on in vitro cytotoxicity assays. In vivo experiments showed that both photothermal and chemotherapy were able to inhibit tumor growth, by necrosis.
Conclusions: Developed nanosystems appear to be promising platforms for melanoma treatment, presenting the desired structure and a robust performance for targeting and local anti-tumor therapies.
References: [1] Oliveira Silva, C. et al. Internat. J. Pharm. 493(1-2), 271-284, 2015. [2] Oliveira Silva, C. et al. PLoSOne 10(12), e0144454, 2015.

1st Session (cont.) | Sessão 1(cont.)
Nanomedicine | *Nanomedicina*

Chairman / *Moderador*

Luís P. Fonseca

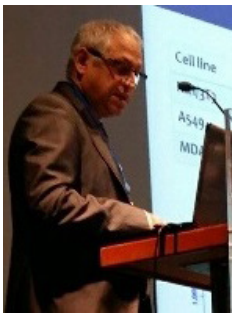


Resumé / *Currículo Resumido*
Luís P. Fonseca is Associate Professor at the Department of Bioengineering of Instituto Superior Técnico (I.S.T.), University of Lisbon, and senior researcher at the BioEngineering Research Group (BERG), based in the Institute for Bioengineering and Bioscience (iBB) at I.S.T.
After graduating in Chemical Engineering in 1983, he got an MSc in Biochemical Engineering in 1989, a PhD in Biotechnology in 1995, and later his DSc in Biotechnology 2006, all at I.S.T.
Luis P. Fonseca stayed one year (1998-1999) as a Post-Doctoral Fellow at The School of Biochemistry and Molecular Biology, University of Leeds, and later (2004-2005) as Visiting Scholar at Chemical Engineering Department in University of California, Berkeley in USA.
Current research efforts focus in the development of nano/micro-biocatalysts (biocomposites) mostly based on hydrogels containing protein/cell assemblies and encapsulating nano-magnetic particles. The resulting biocomposites are intended to be used as nano/micro-bioreactors. The other area of research is on the development of nanoparticles mainly based on nanostructured lipid carrier and their application on the delivery of cosmeceuticals, nutraceuticals and pharmaceuticals.
As a result of his research, he was advisor of 6 Post-Docs and 14 PhDs that led to publication, as author and co-author, of more than 110 papers and 8 book chapters.
Actually, Luis P. Fonseca is participating in the Editorial Board of Microbial Cell Factories, The Open Catalysis Journal and Journal of Industrial Microbiology & Biotechnology and as Associated Editor of Biocatalysis and Biotransformations, and Journal of Integrated OMICS. Furthermore, he is member of the Scientific Committee of the European Section of Applied Biocatalysis (ESAB), member of the Steering Committee of the Bioencapsulation Research Group (BRG), member of Scientific Committee of International Conference for Implementation of Microreactor Technology in Biotechnology – IMTB, and participating in the Chemistry and Molecular Sciences and Technologies COST Action CM1303 "Systems Biocatalysis".
Since 2012, he is Coordinator of Master Course of Bioengineering and Nanosystems of DBE/IST and he is also member of FCT-PhD doctoral programme in Advanced Integrated Microsystems.

C.04 - Targeted and local delivery of nucleic acids
Administração vectorizada e local de ácidos nucleicos

Speaker / *Prelector*

Elias Fattal



Resumé / *Currículo Resumido*
Elias Fattal is professor of Pharmaceutical Technology at the University of Paris-Sud-France and the head of the Institut Galien Paris Sud. He received his Ph.D. from the University of Paris-Sud after which he visited UCSF for a post-doctoral position. He has made fundamental and applied contributions to the fields of drug delivery using nanotechnologies for targeted or local delivery of drugs and nucleic acids. Prof. Fattal has authored more than 220 refereed articles and 30 book chapters. He has issued 10 patents and has received the PSWC Research Achievement Award. He serves in the editorial board of several journals.

Abstract / *Resumo da Comunicação*
Hyaluronic acid (HA) is a glycosaminoglycan, the main constituent of the extracellular matrix and the natural ligand of CD44 receptor. The association of HA with nanotechnology allow to target the cancer stem cells through the CD44 receptor overexpressed on the surface of these cells. Lipoplexes containing a HA-dioleoyl phosphatidylethanolamine conjugate (HA-DOPE) were designed for this purpose. They were prepared from cationic liposomes and used to complex small interfering RNA (siRNAi). Targeting the CD44 receptor on lung cancer cells was shown to improve the inhibition effect of siRNA using the luciferase gene as a target. The internalization mechanism of the lipoplexes was shown to be mediated by both the CD44 receptor and caveolae. This approach has been applied successfully to deliver the same siRNA anti-luciferase in a mouse model of lung metastases demonstrating a higher inhibition than the non-targeted formulation. We have also associated an aptamer antiCD44 to the surface of liposomes. These vectors have also shown great potential for siRNA delivery in vitro and in vivo to cells overexpressing CD44 avoiding the toxicity problems related to lipoplexes. These data validates the relevance of CD44 as a target for the delivery of macromolecular drugs by nanotechnology. Finally, we have designed inhaled formulation for siRNA delivery in the treatment of lung injury disease, to reduce inflammation. Dendriplexes delivery of an antiTNF siRNA has shown, after intratracheal administration, a potent inhibition of this cytokine demonstrating the potential of nanotechnologies to also deliver locally the siRNA.

1st Session (cont.) | Sessão 1(cont.)
Nanomedicine | *Nanomedicina*

C.05 - Gold precision: Gold nanoparticles for precise delivery
Precisão de ouro: Nanopartículas de ouro para administração precisa

Speaker / *Prelector*

Pedro Viana Baptista

Resumé / Currículo Resumido
Pedro Viana Baptista has a degree in Pharmacy (FFUL) and completed his PhD in Human Molecular Genetics (School of Pharmacy, University of London). He is now Associate Professor of Molecular Genetics and Nanomedicine at the Department of Life Sciences (Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa), where he attained his Agregação in Biotechnology-Nanobiotechnology. He has published more than 100 papers in peer reviewed international journals and serving as an editorial board member of many others. He coordinates several National and International projects relating to nanotechnology for biosensing and for therapy. He is co-author of more than 5 patents and has founded a nanodiagnostics start-up.

Abstract / *Resumo da Comunicação*
Nanotechnology has been continuously challenging the way we perceive diagnostics and therapy, which has been impacting the way we diagnose, image and treat cancer. Novel concepts arise every day that show promise to deliver one or more treatment solutions to a specific type of cancer and doing so by simultaneously allowing visualization of the therapeutic effects. Some of these nanotheranostics strategies have relied on the use of gold nanoparticles for conjugating a multitude of molecules for the precise delivery of anti-cancer treatment. Starting from the use of Gold nanobeacons, we shall discuss the use of gold nanoparticles vectorisation of therapeutic molecules, alone or in combinatory delivery.

C.06 - Phytosome as leading strategy for natural compounds delivery
Fitossomas como estratégias de administração de compostos naturais

Poster selected for oral communication / *Poster selecionado para comunicação oral*

Diogo Matias

Resumé / *Currículo Resumido*
Integrated master in Pharmaceutical Sciences (FFUL) and PhD student in Health Sciencies (ULHT/UAH) in partial time. Have developed its professional background in the pharmacy and in academic research passing for the iMed.UL and IMM/FMUL. His main research interests were at the identification of natural compounds with anticancer and antibacterial activity and their delivery in nanoparticle formulations. He is the author of some articles and book chapters in his areas of interest. Have also functions as adjunct pharmacist into a local pharmacy and is member of the OF, SPFito and SPQ.

Abstract / *Resumo da Comunicação*
Introduction: The wound healing process is complex and could become impaired by the presence of pathogenic bacteria. Topical antibiotics have been useful controlling infections but the emergence of new drug resistant bacteria threatens their efficacy. This highlights the need for alternative antibacterial therapeutics. Natural products have been an interesting source of antibacterial components. Plectranthus madagascariensis (PM) is a Lamiaceae plant with ethnopharmacological uses as anti-infective and skin conditioning [1]. The extracts of PM have been investigated due to their potent antibacterial activity namely against Staphylococcus spp. including methicillin resistant strains (MIC=1.95 µg/mL). Some of their intrinsic properties such as the oil in water diffusion coefficient, molecular size and/or stability in physiologic media, lead, however, to limited bioavailability. Phytosomes may overcome those limitations [2]. Nanoparticles like phytosomes consist in a close to equimolar amorphous dispersion of natural drugs in a phospholipidic matrix that assume a micellar shape in aqueous medium [2].
Objectives, Material and Methods: In this study, a potent antibacterial extract from PM was incorporated into phytosomes obtained by a modified solvent evaporation method, which were further coated with chitosan-TPP (tripoliphosphate) complexes (Chi-PS-PM).
Resuls and Discussion: The primarily obtained phytosomes show a mean particle size of 191 ± 75 nm with neutral surface charge. The subsequent chitosan coating resulted in an increase of the size (1082 ± 363 nm) and polydispersity index (0.224) and also in a clearly inversion of the zeta potential (+21 ± 12 mV). The encapsulation efficiency of PM was 58 ± 6%. The chitosan coating was confirmed through AFM, DRIFT spectroscopy and DSC. The particles showed a sustained release of PM over 10h and slower skin permeation fluxes. The antibacterial activity of the extract was maintained after the encapsulation process.
Conclusions: These results showed that Chi-PS-PM is a promising antibacterial ingredient for topical formulations and stress the importance of nanotechnology to enhance natural drug efficacy.
Acknowledgments: PADDIC grant 2013/14 and FCT grant UID/MULTI/00612/2013.
References: [1] Lukhoba, C.W., Simmonds, M.S.J. and Paton, A.J.J. Ethnopharmacol. 103, 1-24, 2010. [2] Khan, J., Alexander, A., and Saraf, S. J. Control. Rel. 168, 50–60, 2013.

2st Session | Sessão 2
Biomedical nanotechnologies | *Nanotecnologia Biomédica*

Chairman / *Moderador*

Hugo Alexandre Ferreira

Resumé / *Currículo Resumido*
Hugo Alexandre Ferreira holds a medical degree from the Faculty of Medicine of the University of Lisbon and a physics engineering degree from Instituto Superior Técnico (IST) of the Technical University of Lisbon. He also holds a PhD degree in physics from IST, whereas his doctoral studies were done in magnetoresistive biochips at INESC-Microsystems and Nanotechnologies and at the BioEngineering Research Group – IST. Additionally, he has a post-graduation in Entrepreneurship and Innovation Management from the School of Business and Economics of the Portuguese Catholic University. He was a founder and the CEO of Haloris Nanotechnologies, a biosensor start-up and later he was a clinical education and application specialist for magnetic resonance imaging and computed tomography at Siemens Healthcare. He then returned to academia as a researcher on neuroimaging at the Institute of Biophysics and Biomedical Engineering (IBEB) of the Faculty of Sciences of the University of Lisbon (FCUL). Now he is an assistant professor at FCUL where he teaches Nanotechnologies in Biomedicine, Tissue Engineering and Artificial Organs, Medical Robotics, Neurosciences, and Mechanisms of Disease. At IBEB his current research topics include brain connectivity and classification of neuroimaging and neurophysiological signals, physiological computing and magnetic resonance imaging-guided Nanorobotics.

C.07 - Functionalisation of gold nanoparticles for biomedical application
Funcionalização de nanopartículas de ouro para aplicação biomédica

Speaker / *Prelector*

Sónia Fraga

Resumé / *Currículo Resumido*
Sónia Fraga completed her PhD degree in Human Biology from the Faculty of Medicine of the University of Porto (FMUP) in 2006. Her doctoral and postdoctoral research was in the field of the regulation of epithelial transport and electrolyte balance in health and disease. Currently, she is researcher at the Institute of Public Health of the University of Porto, Portugal and Invited Assistant Professor at the Faculty of Medicine of the University of Porto, Portugal. Her main research interests and activities focus on the applications and safety of manufactured nanomaterials. Sónia Fraga has presented more than 46 papers at national and international scientific meetings. She is author/co-author of 27 full papers in international peer-reviewed journals with over 342 citations.

Abstract / *Resumo da Comunicação*
Nanotechnology is expected to have great impact in the biomedical field through the design and development of innovative nanoscaled devices and systems for tissue engineering, diagnostic and therapeutic applications. Owing to their physicochemical properties, ease of synthesis and versatility, gold nanoparticles (AuNPs) are promising agents for diagnosis, drug/gene delivery, imaging and targeted photothermal destruction of cancer cells. Despite enormous potential, safety of AuNPs remains a controversial topic. From the large number of studies conducted to evaluate the toxicity of AuNPs, no definitive conclusions have been drawn due to the complexity of factors involved in their biological effects. Nanoparticle surface modification and/or functionalisation are important determinants of its interaction with the surrounding environment. These interactions also affect the colloidal stability of the particles, and may yield to a controlled assembly or to the delivery of nanoparticles to a specific target. In vivo studies are important to understand the contribution of different parameters such as physicochemical properties (including surface chemistry), dose, route and duration of exposure for the kinetics, biological fate and physiological responses of AuNPs.
This work will focus on the impact of AuNPs surface modification/ functionalisation on their biodistribution and toxicity.

2st Session | Sessão 2
Biomedical nanotechnologies | *Nanotecnologia Biomédica*

C.08 - Light-activatable biomaterials for biomedical applications
Biomateriais fotoactivados para aplicações biomédicas

Speaker / *Prelector*

Lino Ferreira

Resumé / *Currículo Resumido*

Lino Silva Ferreira holds a Ph.D. in Biotechnology from the University of Coimbra (Portugal). He did postdoctoral work at INEB and MIT (USA) in the areas of stem cells, micro- and nanotechnologies. He joined the Center of Neurosciences and Cell Biology (CNC, University of Coimbra) in 2008. He has published more than 80 peer-reviewed papers and has 20 issued or pending patents– 8 of which have been licensed to companies in the biomedical industry. He is the director of the Biomaterials and Stem Cell-Based Therapeutics research group, CNC coordinator of the MIT-Portugal Program and the founder of the biotech company Matera. In 2012, he was awarded with a prestigious European Research Council starting grant. His research group has two main avenues of research: (i) development of bioengineering platforms to modulate the differentiation and maturation of stem cells, (ii) development of nanomedicine platforms to modulate the activity of stem cells and their progenies. The seminar will focus in the use of light to trigger biological events in cells or tissues for Therapeutic/Regenerative Medicine applications.

Abstract / *Resumo da Comunicação*

The advent of molecular reprogramming and the associated opportunities for personalised and therapeutic medicine requires the development of novel systems for on-demand delivery of reprogramming factors into cells in order to modulate their activity/identity. Such triggerable systems should allow precise control of the timing, duration, magnitude and spatial release of the modulator factors. Furthermore, the system should allow this control even in vivo, using non-invasive means. Nanoparticles (NPs) are very promising for the intracellular delivery of transcription factors. They offer several advantages over the viral vectors including high biomolecule carrying capacity, low risk of immunogenicity, low cost and ease of production. In addition, the possibility of making NPs that disassemble by light make them excellent candidates as triggerable systems. During my talk, I will give several examples of technologies that under development to address this challenge in the context of therapeutic and regenerative medicine.

C.09 - 7α-acetoxy-6β-hidroxyroyleanone derivatives and its topical application through delivery nanosystems
Derivados da 7α -acetoxi-6β-hidroroileanona e sua aplicação tópica através de nanossistemas

Poster selected for oral communication / *Poster selecionado para comunicação oral*

Filipe Pereira

Resumé / *Currículo Resumido*

Currently as a PhD student at the Lusófona University, where I have developed work on the research of new therapeutic molecules with microbiological activity and its application in drug delivery nanosystem. I started my academic career in applied chemistry, in biotechnology field at FCT- New University of Lisbon, followed by master's degree in pharmaceutical and therapeutics chemistry in FF- University of Lisbon. Throughout this period, I got passion and knowledge in the synthesis and functionalization of metal nanoparticles and research of new molecules with microbiological activity obtained from plants.

Abstract / *Resumo da Comunicação*

Introduction: Bacterial infection is one of the leading causes of mortality. The phenomenon of antibiotic resistance, the lack of new structures with therapeutic effect and the misuse of antibiotics are some of the causes to a high mortality. Nanotechnology allowed the development of new tools for the diagnosis and treatment of infections with significant benefits for patients when compared to traditional methods.

The 7α-acetoxy-6β-hidroxyroyleanone is a natural diterpene isolated from Plectranthus species with an abietane scaffold [1]. This compound is associated with a strong antibacterial activity against Gram-positive strains, especially against resistant Staphylococcus aureus strains (MRSA with MIC values between 3.12 to 15.63 µg.mL-1) [2].

Objectives, Material and Methods: In the present work the mechanism of action of 7α-acetoxy-6β-hidroxyroyleanone was studied in a bacterial strain MRSA /VISA (CIP 106760) by analysis of the growth curve, cell leakage, morphology, influence in surface charge and lytic capacity of the bacteria when exposed to different concentrations of compound (2×MIC, MIC and MIC/2). The royleanone reactivity and derivatization was studied using hydrogenation and Mitsunobu reaction. The derivatives structural characterization was confirmed by spectroscopic methods. Also, a delivery system using silver nanoparticles (AgNP) was optimized using royleanone as a reducer agent. This type of synthesis results in silver nanoparticles with the active compound at surface. Physico-chemical characterization (mean particle size, polydispersity index (PI) and morphology) and spherical nanoparticles was characterized through atomic force microscopy (AFM).

Results and Discussions: Particles were very small (around 699 nm) with PI of 0.309. The colour of nanoparticle suspension changed after Roy attachment (from yellow to brown). Efficacy of the resultant nanosystem demonstrated a high efficacy against bacteria.

Conclusions: The resulting nanoparticles are a potential tool for the development of a new transdermal delivery strategy against dermal infections.

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

Chairman / *Moderador*

José Paulo Farinha

Resumé / *Currículo Resumido*
José Paulo Farinha completed his PhD in Chemical Engineering in 1996 (Technical University of Lisboa) and a postdoc in Polymer and Colloid Chemistry (University of Toronto, 1997-1999). He is Professor of Physical Chemistry Materials and Nanosciences at Instituto Superior Técnico, University of Lisbon. He authored over 20 patents, 80 papers in ISI-listed journals and 130 in conference proceedings (1200 citations and h-factor 23). His main scientific interests are in the areas of polymers, colloids, nanoparticles and nanostructured materials, for optical and biomedical applications.

C.10 - Thin-film silicon: From large area electronics to MEMS/NEMS and lab-on-chip applications

Filme de silicone: Da grande área da eletrónica a MEMS/ NEMS e aplicações lab-on-chip

Speaker / *Prelector*

João Pedro Conde

Resumé / *Currículo Resumido*
João Paulo Conde is currently a Professor in the Department of Bioengineering of Técnico, a Senior Researcher and Co-Group Leader of INESC MN, and the Coordinator of the Institute of Nanoscience and Nanotechnology. His current research focuses on: (i) thin-film silicon micro and nanoelectromechanical systems; (ii) microfluidic biochips for biosensing; and (iii) cell chips for medical research.

Abstract / *Resumo da Comunicação*
Thin-film amorphous and nanocrystalline silicon can be deposited at low temperatures on large area substrates such as polymers, glass and steel. These materials have found important applications in solar cells, thin-film transistor (TFT) backplanes for flat-panel displays, and in matrix-addressed photosensor arrays for digital X-rays. In addition, thin-film silicon devices have been used on flexible and stretchable substrates.
In this talk, I will discuss two novel applications of thin-film silicon:
- the first is to fabricate micro and nanoelectromechanical systems (MEMS and NEMS). Thin-film silicon brings the performance of crystalline and polycrystalline silicon MEMS to applications in which low-temperature processing or large area substrates are required, as for CMOS integration, or when flexible substrates are needed. I will discuss the electromechanical properties of the structural materials, MEMS and NEMS processing, and bridge and bulk resonator characteristics.
- the second is the integration of thin-film optical sensors in microfluidic devices for biosensing. The aim is to have a "sample-to-answer" system that integrates fluidic handling, sample preparation, target detection, transduction, and signal processing. I will discuss our current research on the detection of toxins for food safety applications, and of cancer biomarkers.
I will conclude with two points for discussion: (i) can thin-film silicon MEMS and NEMS be successfully integrated in Lab on Chip devices for increased sensitivity? (ii) what are potential innovative or disruptive applications of thin silicon devices in MEMS and Lab on Chip?

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

C.11 - Nanochemistry applied to fight cancer - case study metallodendrimers as anticancer drugs
Nanoquímica aplicada à oncologia - estudos de caso de metalo dendrímeros como citostáticos

Speaker / *Prelector*

João Rodrigues

Resumé / *Currículo Resumido*

João Rodrigues (JR) received his Ph.D. degree (1999) in Inorganic Chemistry from the University of Lisboa (Portugal). He is a staff member of the University of Madeira (Madeira Island/Portugal), the head of Centro de Química da Madeira (FCT National Research Unit) and, since 2012, the director of the Master in Nanochemistry and Nanomaterials. He is a visiting scientist in several universities abroad and invited Professor of the Biomedical Engineering Master Degree at Donghua University, Shanghai, China. JR has authored 54 peer-reviewed articles in high impact journals (h index= 15, i10 index = 24), 1 book chapter, and 7 proceeding papers. He presented more than 25 invited talks and 60 other oral presentations around the world.

Abstract / *Resuno da Comunicação*

Cancer in its different forms is a deadly global disease. According to the American Cancer Society, cancer will kill ca. 0.6M of citizens this year in the USA, while more than 1.7 new cancer cases are expected to appear – 3 new cases and 1 death every minute.

Nowadays, just a few number of metallodrugs reached the market and are currently in clinical use as anticancer drugs.

The search for metallodrugs displaying less secondary effects, and enhanced anticancer activity, especially for cancer types that do not respond well to Platinum compounds, is of high interest. Ruthenium complexes, due to the variety and accessibility (under physiological conditions) of oxidation states (Ru(II), Ru(III) and Ru(IV)), rich synthetic chemistry, and proposed capability to preferentially accumulate in neoplastic tissues present an alternative approach to the clinically used Platinum chemotherapeutic agents.

The fact that cationic dendrimers, a type of hyperbranched nanoscale polymer, can easily interact with the cell surface, enter into the cytoplasm and reach, in short time, the nucleus of the cells, prompt us to develop low-generation poly(alkylidenamine) dendrimers functionalized with nitriles coordinated to the organometallic moiety [Ru(η5-C5H5)(PPh3)2]+, for use as chemotherapeutic agents. The degradation behavior of the metallodendrimers was studied by 31P NMR in [D6]DMSO, along time, as well as their cytotoxic behavior towards six cancer cell lines, revealing the potential of these compounds for biomedical applications.

C.12 - Supercritical fluids as an important tool to produce pharmaceutical nanoparticles
Fluidos supercríticos como uma ferramenta importante para a produção de nanopartículas

Poster selected for oral communication / *Poster selecionado para comunicação oral*

Beatriz Nobre

Resumé / *Currículo Resumido*

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

Abstract / *Resumo da Comunicação*

Particle size of pharmaceuticals compounds can play a significant role in the amount of the active principle absorbed by the human body and with many compounds it is possible to provide dosages well below the toxicity threshold.

Supercritical fluid anti-solvent processes (SAS) were recently proposed as alternative to liquid anti-solvent ones. The SAS process works similarly, but instead of the use of a liquid solvent, in which the compound to be micronized is insoluble, it is used a supercritical fluid. The combination of the high solvent power of supercritical fluids to dissolve the organic solvent and the low solubility of the pharmaceutical compounds in the supercritical fluids makes this technique the most suitable for the precipitation of pharmaceutical compounds. On the other hand, it is possible to recover the supercritical anti-solvent by simple decompression, avoiding complex treatments typical of the liquid process.

Supercritical CO2 is the most used antisolvent in SAS processes. In addition to the advantage of replacing toxic solvents, CO2 has also the capability of producing pure particles with special morphologies. Moreover, a wide range of compounds can be processed using this solvent.

SAS micronization of pharmaceuticals and bioactives, such as sodium fusidate, fusidic acid and astaxanthin, has been carried out in our laboratories. Nano and micro particles with morphology and particle size suitable for its use in pharmaceutic formulations were obtained.

The most interesting results of these studies will be presented and discussed, with special focus on the effect of SAS operation parameters in the particle size and particle size distribution.

Panel Comunications | *Comunicações em Posters*

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P.02 **AN UPDATE ON PRE-CLINICAL SAFETY OF SOLID LIPID NANOPARTICLES AND NANOSTRUCTURED LIPID CARRIERS: REVIEW AND ANALYSIS OF CURRENT *IN VITRO* AND *IN VIVO* DATA.** S. Doktorovová, A.B. Kovačević and E.B. Souto.

P.03 **ANTIFUNGAL ACTIVITY OF SANGUISORBA HYBRIDA EXTRACTS AND EVALUATION OF EFFICIENCY WHEN ENCAPSULATED INTO SILVER NANOPARTICLES.** C. Ferro, A.M. Madureira, A. Duarte, M.M. Lopes, G. Teixeira, C.P. Reis and P. Rijo.

P.04 **DEVELOPMENT OF NANOSTRUCTURED LIPID CARRIERS (NLC) CONTAINING LINALOOL FOR TOPICAL DELIVERY.** S.S.C. Filho, S. Silva, F.J. Veiga, E.B. Souto and S. Doktorovová.

P.05 **NOVEL POLYMERIC COATED GOLD NANOPARTICLES LOADED WITH THE ANTITUMORAL 6,7-DEHYDROROYLEANONE.** C. Garcia, C. Silva, A.S. Viana, T. Stanković, M. Pešić, C.P. Reis and P. Rijo.

P.06 **LIGHT-ACTIVATABLE POLYMERIC NANOPARTICLES FOR INTRACELLULAR DELIVERY OF PROTEINS AND SMALL MOLECULES.** A. Jiménez-Balsa, E. Costa and L. Ferreira.

P.07 **LIGHT CONTROLLED RELEASE OF PROTEINS FROM GOLD NANORODS.** M.M. Lino, S. Simões, S. Pinho and L. Ferreira.

P.08 **PLGA NANOPARTICLES WITH ANTI-INFLAMMATORY AGENTS OBTAINED FROM *PLECTRANTHUS* PLANTS.** J. Marçalo, B. Mourato, M. Nicolai, C.P. Reis, C. Faustino and P. Rijo.

P.09 **TABLETING NLC FOR ORAL DRUG DELIVERY.** M. Mendes, M.B. Vaz, H.T. Soares, J.J. Sousa, A.A.C.C. Pais and C. Vitorino.

P.010 **NEW FORMULATION OF ORAL INSULIN.** J. Moreira, I.V. Figueiredo, M. Silveira, P. Gonçalves, M. Nicolai, T. Almeida, N. Saraiva, M. Eduardo-Figueira, P. Faísca, I. Correia, L. Ascensão, P. Rijo and C.P. Reis.

P.011 **SELF MICROEMULSIFYING DRUG DELIVERY SYSTEMS (SMEDDS) OF S-NITROSOGLUTATHIONE (GSNO)/ SMEDDS OF GSNO.** A.H. Mota, M. Socha and P. Maincent.

P.012 **ANTIMICROBIAL ACTIVITY OF NOVEL ABIETANES CATIONIC AMPHIPHILES ENCAPSULATED IN ALGINATE MICROSPHERES.** I. Neto, C.P. Reis, C. Faustino and P. Rijo.

P.013 **DESIGN AND CHARACTERIZATION OF SUNFLOWER OIL NANOSTRUCTURED LIPID CARRIERS FOR RETINYL PALMITATE DELIVERY.** F. Pinto, D.P.C. Barros and L.P. Fonseca.

P.014 **BIOCOMPATIBLE ANTIMICROBIAL PEPTIDE CONJUGATED GOLD NANOPARTICLES WITH ANTIMICROBIAL EFFICACY A SYSTEMIC INFECTION MODEL.** A. Rai, S. Pinto, T. Velho, A. Ferreira, C. Moita, U. Trivedi, M. Evangelista, M. Comune, K. Rumbaugh, P.N. Simoes, L. Moita and L. Ferreira.

P.015 **INNOVATIVE ANTIMICROBIAL METALLIC-BASED NANOFORMULATION CONTAINING HERBAL EXTRACTS.** L. Roque, A. Cândido, F. Rodrigues, I. Gomes, N. Cristo, R. Duarte, P. Rijo and C.P. Reis.

P.016 **INNOVATIVE NANOCARRIER-BASED TOPICAL FORMULATIONS FOR ALOPECIA TREATMENT.** L. Roque, I. Dias, L. Palma, P. Rijo, A. Roberto and C.P. Reis.

P.017 **NEW FORMULATION FOR ONYCHOMYCOSIS TREATMENT.** L. Roque, A. Pereira, A. Santos, M. Simões, V. Demchenko, P. Rijo and C.P. Reis.

P.018 **NOVEL GEL FORMULATION FOR FUNGAL INFECTIONS.** L. Roque, A. Pereira, A. Santos, M. Simões, V. Demchenko, P. Rijo and C.P. Reis.

P.019 **BSA NANOPARTICLES WITH PARVIFLORON D FOR PANCREATIC CANCER TREATMENT.** A. Rebelo, A.S. Viana, P. Rijo and C.P. Reis.

P.020. **FUNCTIONAL PROTEIN NANOPARTICLES FOR REDUCTION OF CARDIOVASCULAR DISEASES RISK.** A. Silva, J.T. Marquês, A.S. Viana, R. Pacheco and M.L.M. Serralheiro.

P.021 **DEVELOPMENT OF NANOSTRUCTURED LIPID CARRIERS (NLC) BY FACTORIAL DESIGN.** M.C.T. Truiti, S. Doktorovová, M. Mendes, M. Vaz, C. Vitorino and E.B. Souto.

P.022 **MICRONIZATION OF ASTAXANTHIN BY SUPERCRITICAL ANTISOLVENT PRECIPITATION.** J. Costa, B.P. Nobre, J. P. Farinha and A.F. Palavra.

P01. A state-of-art on gels technologies for transdermal drug delivery

S. Antunes[1, 2], S. Doktorovová[1, 2] and E.B. Souto[1, 2]
[1]Department of Pharmaceutic Technology, Faculty of Pharmacy, Coimbra University, Azinhaga de Santa Comba 3000-548, Coimbra, Portugal.
[2]Center for Neuroscience and Cell Biology, University of Coimbra, Pólo das Ciências da Saúde, Azinhaga de Santa Comba, 3000-548 Coimbra, Portugal.

Gels have consistently been studied for their role in topical and transdermal drug delivery systems as a non-invasive technique for pharmaceutical and cosmetics application. These formulations are semi-solid tridimensional structures, porous, with unique characteristics, such as rigidity and elasticity at the same time [1]. Because of their high aqueous phase content, gels permit a greater dissolution of drugs through the skin and enhance skin hydration by retaining a significant amount of transepidermal water, in contrast to creams and ointments [2]. Conventionally, gels are differentiated into two types according to the nature of their liquid phase: hydrogels, which contain a polar solvent (water) and organogels, which contain an organic/non-polar solvent, as external phase [3].

Hydrogels consist of polymeric materials that exhibit the ability to swell and retain a large amount of water or other biofluids in its structures. Despite its great affinity for water, they only possess a swelling behavior without dissolving in water. This proves its high flexibility, similar to natural tissue [4].

Organogels, or oleogels, consist of a network of self-assembled molecules which forms a thermally reversible gel upon cooling, immobilizing a non-aqueous liquid. They are mainly composed by lipids (organic phase), so they easily interact with the lipid skin surface and enhance the drug permeation through the skin [5]. The most widely used lipids are based on edible oils, such as soybean oil, sunflower oil, sesame seed oil or olive oil, due to their high biocompatibility [6]. Organogels form viscoelastic structures through non-covalent associations with gelling agents in low concentrations. These superstructures, often form long fibers or needle-shaped structures, which entangle into pseudocrystalline regions, immobilizing the liquid largely by surface tension and forming a gel of variable consistency. Lecithin organogels are a special type of organogels that do not require addition of any additional surfactant or penetration enhancer, as lecithin serves both the purposes. Recent studies have reported other types of gels for dermal drug application, such as proniosomal gels, emulgels, bigels and aerogels, combining features of conventional hydrogels and organogels [7].

In conclusion, further studies in gel technologies are essentials to overcome the drawbacks of each gel system and for developing cost effective delivery systems for transdermal applications.

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P02. An update on pre-clinical safety of solid lipid nanoparticles and nanostructured lipid carriers: Review and analysis of current in vitro and in vivo data

S. Doktorovová1, [2+*], A.B. Kovačević[1, 2+] and E.B. Souto[1, 2]
[1]Department of Pharmaceutical Technology, Faculty of Pharmacy, University of Coimbra Azinhaga de Santa Comba, 3000-548 Coimbra, Portugal.
[2]Center for Neuroscience and Cell Biology, University of Coimbra, Pólo das Ciências da Saúde, Azinhaga de Santa Comba, 3000-548 Coimbra, Portugal.
+ These authors contributed equally

Solid lipid nanoparticles (SLN) and nanostructured lipid carriers (NLC) were invented 25 years ago as innovative colloidal carriers based on well-known, safe lipid materials, intended primarily for delivery of poorly soluble drugs. SLN and NLC reached cosmetic market quickly, but until now, there are no marketed medicines using lipid nanoparticle technology. Several patents held by pharmaceutical companies illustrate that the technology is considered promising, especially for dermal delivery or lipophilic drugs.

In this study, we present data on pre-clinical safety collected from scientific publications focused on in vitro and in vivo studies. Special attention was focused on skin compatibility data. An overview of experimental data obtained in the skin irritation studies with SLN and NLC performed in accordance with internationally accepted test methods validated by ECVAM (in vitro methods based on the RhE technology and in vivo animal test – Draize rabbit test) is presented. An updated set of data of impact of SLN/NLC on cytotoxicity, cell membrane damage and oxidative stress induction is also included. Data were collected from PubMed indexed publications released between 2014 and January 2016 (in vitro and ex vivo data) and without publication date restriction for in vivo data.

The results confirmed previous evidence [1] that dermal route is the safest route of SLN/NLC administration, but good safety profile is found also for other routes. For in vitro tests, SLN/NLC are generally acceptable at concentrations <1 mg/mL total lipids, with rare exceptions of more compatible formulations. Induction and/or promotion of oxidative stress in cultured cells were identified as possible mechanism of cell damage, with implications on inflammatory reaction induction capacity in vivo. The collected in vivo safety data are still limited, despite large number of in vivo studies reported in literature. Lipid dose-dependent adverse reactions were reported in orally administered SLN/NLC. Reports of inflammatory reactions in adipose tissue in mice were identified. Isolated reports of adverse effects on the nasal mucosa were identified, but were attributed to inadequate excipient selection.

Investigation on dermal SLN/NLC, both in vitro and in vivo, suggested good skin tolerability, assigned predominantly to the use of biodegradable, well-tolerated and physiological excipients. Relevant literature also indicated that numerous drugs, causing skin irritation reactions, revealed reduced local concentration and skin irritation after encapsulation in SLN/NLC. Despite efforts of establishing SLN/NLC as drug carriers for other than topical routes, the dermal route remains the most safe and adequate route of SLN/NLC administration. This is largely due to excipients available for SLN/NLC formulation that are approved or recommended for topical, a limited number also for oral, but not parenteral administration.

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References: [1] Doktorovová, S., Souto, E.B. and Silva, A.M. Eur. J. Pharm. Biopharm. 87(1), 1-18, 2014.

P03. Antifungal activity of Sanguisorba hybrida extracts and evaluation of efficiency when encapsulated into silver nanoparticles

C. Ferro[1], A.M. Madureira[1], A. Duarte [1], M.M. Lopes[1], G. Teixeira[2], C.P. Reis[3] and P. Rijo[3]
[1]Universidade de Lisboa, Faculdade de Farmácia de Lisboa, iMed Universidade de Lisboa, Av. Prof. Gama Pinto, 1649-003, Lisboa, Portugal.
[2]Universidade de Lisboa, Faculdade de Farmácia de Lisboa, Center for Ecology, Evolution and Environmental Changes (CE3C), Av. Prof. Gama Pinto, 1649-003, Lisboa, Portugal.
[3]Universidade Lusófona de Humanidades e Tecnologias, CBIOS – Center for Research in Biosciences and Health Technologies, Campo Grande, Lisboa, Portugal.

Introdution: The genus Sanguisorba L. (Rosaceae) is distributed throughout the northern hemisphere and has long been recognized to have medicinal properties [1, 3]. S. hybrida (L.) Nordborg is endemic in Portugal [4] and in previous studies the antimicrobial activity of its different extracts has been tested against reference and multiresistant bacterial strains [5]. MIC values of 3.50-1.75 μg/mL were obtained with polar extracts against Staphylococcus aureus, including MRSA strains. This activity is probably related to the high content of those extracts on phenolics and terpenoids [5].

Objectives: The present study aimed to elucidate about the antifungal activity of the same extracts and also test their antimicrobial efficiency when encapsulated into silver nanoparticles.

Material and Methods: S. hybrida was collected in SW Portugal (3008' N – 8033' W), during July 2010. Plant material is deposited at the Herbarium of University of Lisboa (LISU). The extracts (n-hex, CH2Cl2, AcOEt, MeOH and H2O) antifungal activities were tested against several fungi (Geotrichum spp., Candida kruzei, Candida glabrata, Candida dublininensis, Saccharomyces cerevisae, Candida albicans, Candida tropicalis, Candida parapsilosis, Aspergillus niger, Trichosporon cutaneum, Cryptococcus neoformans and Rhodotorula rubra). MICs were evaluated by the micromethod. The silver nanoparticles (AgNP) were functionalized with the extracts according to Brown et al. method [6].

Results and Discussion: The most polar extracts were those who presented biggest antifungal activity, in particular the aqueous extract (MIC, 16-62 μ g/mL). Those results might be correlated with the presence of flavonoids and other phenolic compounds. The most sensitive fungi were A. niger (MIC 31μg/mL) and Geotrichum spp. (MIC 16 μg/mL). The encapsulation of S. hybrida extracts into silver nanoparticles did not change the previously values of antifungal activity obtained with the no encapsulated extracts. Other authors [7] also found good results with the use of silver nanoparticles against some fungi. Considering that encapsulation can contribute to the stabilization of plant compounds [8], our results are encouraging as improving sample stability and its bioavailability.

References: [1] Camejo-Rodrigues et al. J. Ethnopharmacol. 89, 199-209, 2003. [2] Kokoska, L. et al. J. Ethnopharmacol. 82, 51-53, 2002. [3] Bedoya, L.M. et al. J. Ethnopharmacol. 77, 113-116, 2001. [4] Navarro, C. and Garmendia, F. Flora Iberica, Sanguisorba. Vol. VI. Real Jardín Botánico, CSIC. Madrid, 1998. [5] Madureira, A.M. et al. Rev. Ciências Agrárias 37, 482-490, 2014. [6] Brown, A.N. et al. Appl. Environ. Microbiol. 78, 2768–2774, 2012. [7] Lara, C. et al. J. Nanobiotechnol. 13, 91-103, 2015. [8] Guterres, S.S. et al. Drug Target Insights 2, 147-157, 2007.

P04. Development of nanostructured lipid carriers (NLC) containing linalool for topical delivery

S.S.C. Filho, S. Silva, FJ. Veiga, E.B. Souto and S. Doktorovová
Department of Pharmaceutic Technology, Faculty of Pharmacy, Coimbra University, Azinhaga de Santa Comba 3000-548, Coimbra, Portugal.
Center for Neuroscience and Cell Biology, University of Coimbra, Pólo das Ciências da Saúde, Azinhaga de Santa Comba, 3000-548 Coimbra, Portugal.

Introduction: Linalool is small terpenic compound whose inhibitory effect on the growth of melanoma cells has shown promising results in in vitro studies even at concentrations as low as 0.56 μM [1].

Objectives: Our goal was to develop a stable formulation for the delivery of linalool into these cells, starting from raw GRAS materials.

Materials and Methods: In an initial approach, we tested the solubility of linalool in 14 different lipids, which were chosen based on our solubility prediction calculations and data from literature. With the results of these screening tests, we were able to narrow down our options to only two of these compounds (Glycerol Monostearate type I and II) which we then used to formulate different solid lipid nanoparticles (SLN) using different surfactants. All screening and final formulations were prepared by high-shear homogenization (Ultra-Turrax), using Imwitor 900K (BASF GmbH) as solid lipid, and Cetomil (Fabriquimica SA) at 2.5% as surfactant and linalool at 20% relative to lipid matrix.

Results and Discussion: A mean particle size of 417 ± 3 nm and polydispersity index of 0.29 ± 0.088 were obtained for this formulation.

SD is recipient of postdoctoral scholarship from FCT under ref. BPD/101650/2014.
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P05. Novel polymeric coated gold nanoparticles loaded with the antitumoral 6,7-dehydroroyleanone

C. Garcia[1,2], C. Silva[1,2], A.S. Viana[3], T. Stanković[4], M. Pešić[4], C.P. Reis[1,5], and P. Rijo[1,6]

[1]Center for Research in Biosciences & Health Technologies (CBIOS), Universidade Lusófona de Humanidades e Tecnologias, 1749-024 Lisboa, Portugal.
[2]Dpto. Ciencias Biomédicas, Universidad de Alcalá, Plaza de San Diego, s/n, 28801 Alcalá de Henares, Madrid, Espanha.
[3]CQB, Faculdade de Ciências, Universidade de Lisboa, Campo Grande 1749–016, Lisboa, Portugal
[4]Institute for Biological Research, "Siniša Stanković", University of Belgrade, Despota Stefana 142, 11060 Belgrade, Serbia.
[5]IBEB, Faculdade de Ciências, Universidade de Lisboa, 1749–016, Lisboa, Portugal.
[6]Instituto de Investigação do Medicamento (iMed.ULisboa), Faculdade de Farmácia, Universidade de Lisboa, 1649-003 Lisboa, Portugal.

Introduction: Natural compound based green chemistry process has recently been emerged as one of the active areas of current Nanobiotechnology research. On the other hand, biomedical applications using gold nanoparticles (GNPs) have been extensively studied in recent years.
Plectranthus genus (Lamiaceae family) is widely used in traditional medicine, and it is known as a source of bioactive natural products. Diterpenes are frequently found in Plectranthus genus, and are compounds of considerable interest, namely due to their anti-tumor properties. 6,7-Dehydroroyleanone, the major diterpene isolated from P. madagascariensis essential oil, has recently demonstrated antitumoral activity [1].
Material and Methods: In this work, the new antitumoral properties of 6,7-dehydroroyleanone were evaluated. In order to do so, the model system of sensitive non-small cell lung cancer cell line (NCI-H460) and its resistant counterpart (NCI-H460/R) were used, along with normal human embryonal bronchial epithelial cells (MRC-5).
Results and Discussion: The diterpene was cytotoxic against these cancer cell lines, demonstrating selectivity towards them (IC50 of 14 ± 2; 11 ± 1; 24 ± 2 µM, respectively). In order to increase the activity of 6,7-dehydroroyleanone, polymeric coated gold nanoparticles were produced according to Silva et. al [2] and then characterized through atomic force microscopy (AFM). Nanoparticles showed a spherical form and a very small size (19.05 ± 2nm).
Conclusions: It seems plausible that combining a cancer cell-selective drug, such as 6,7-dehydroroyleanone, with GNPs could be a powerful tool for the development of novel treatment options in cancer. New nanoparticles will be evaluated in in vitro models of cancer multidrug resistance.
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P06. Light-activatable polymeric nanoparticles for intracellular delivery of proteins and small molecules

A. Jiménez-Balsa, E. Costa and L. Ferreira
Biomaterials and Stem Cell-Based Therapeutics Group @ Center for Neuroscience and Cell Biology, University of Coimbra, Rua Larga FMUC 1º Piso, 3004-504 Coimbra, Portugal.

Introduction: In the last 5 years, a new discipline has been proposed called "Optogenetics" which combines the use of light and genetically encoded light sensitive proteins to control the behavior of living cells and organisms [1]. Light-sensitive ion channels and pumps are activated by a millisecond precision laser, which allows the precise delineation of specific cellular activity, signaling, etc. Some optical tools for gene activation and silencing have been proposed also in recent years. In most cases, the tools consist in a light-sensitive antenna domain and an expression regulator domain [2]. However, there are two major limitations in these approaches. First, the current methods rely in the constitutive and not transient expression of the protein. Second the system is not extensive to small molecules. In the last 10 years, we have developed nanoparticulate-based systems to release proteins and small molecules to modulate (stem) cell biology [3, 4]. However, so far, we were unable to provide a nanoparticulate-based system that is degradable, biocompatible and effective in providing spatial-temporal controlled delivery of small molecules and proteins.
Objectives, Materials and Methods: Here, we present a non-leaky, photo-triggerable polymeric nanoparticle for the intracellular delivery of small molecules and proteins. We have selected UV/blue light as the stimuli for the light disassembly of the NP, as this is universal and easy to apply. In order to conjugate different types of molecules we have synthesized a set of photo-cleavable linkers (PCL) bearing diverse functional groups: amine, acid, etc. After covalent ligation of the bioactive compound (i.e. protein or small molecule) to the PCL and subsequent purification, the resulting conjugate was attached to the polymeric carrier by click reaction under mild conditions. Purification of the corresponding nanomaterials was done by dialysis and characterization of the final nanosystems by DLS.
Results and Discussion: In this work, we show the suitability of acid (all-trans Retinoic acid, atRA, as a proof of concept of a small molecule) and amine (lysozyme as a proof of concept of a protein) conjugation to a PCL, and so, their immobilization on a light-responsive nanoformulation. An enzymatic test or a reporter cell line monitored the release of lysozyme and atRA, respectively.
Conclusions: Our results show that we are able to have spatial-temporal control in the intracellular delivery of bioactive compounds.
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P07. Light controlled release of proteins from gold nanorods

M.M. Lino[1,2], S. Simões [1,2], S. Pinho [1,2] and L. Ferreira[1,2]
[1]CNC, Center for Neurosciences and Cell Biology, Rua Larga FMUC 1º Piso, 3004-504 Coimbra, Portugal.
[2]Biocant, Center of Innovation in Biotechnology, Cantanhede, Portugal.

Introduction: Intracellular delivery of proteins is extremely useful for the manipulation of cellular processes and cell reprogramming [1]. Although some formulations have been described in the last years for the successful intracellular delivery of proteins [2], so far no formulation has the capacity to orchestrate the delivery of multiple proteins. This is an important issue in many biological applications. For example, lineage-switching experiments in the hematopoietic system have shown that the order in which two transcription factors become expressed in a progenitor can decide lineage outcome and thus timing is of utmost importance [3].
Objectives: Here we describe a formulation that can orchestrate the delivery of multiple proteins by light. The user can control the delivery of a specific protein immobilized on a nanoparticle by controlling the power of a near-infrared light source.
Material and Methods: Our formulation is based on gold nanorods (NR) conjugated with more than one oligonucleotide having different physico-chemical properties. These oligonucleotides were used for the attachment of proteins to the gold surface via DNA directed immobilization. Proof of concept experiments were performed with β-galactosidase and with BSA labelled with DyLight fluorophores. NR-BSA-Dylight were used to test the sequential release of fluorescent proteins with three laser powers at 785 nm. In order to release both proteins independently from the same nanorod, the samples were irradiated with a lower power stimulus, releasing only one of the proteins and then with a higher energy stimulus, releasing the other protein.
Results and Discussion: In fibroblasts incubated with NR-BSA-DyLight, the protein fluorescence increased, the signal was more diffuse and the colocalization with NR decreased significantly in the irradiated samples, which indicates that the protein was released from the NR. Moreover, transmission electron microscopy studies revealed that, upon irradiation, the number of NR in the endosomes decreased, suggesting a laser-induced endosomal escape.
In fibroblasts incubated with NR-DNA-βGal, the fluorescence from X-Gal staining increased 1.2 and 1.5 fold when the cells were irradiated at 0.57 and 1.25 W/cm2, respectively. The protein was distributed homogeneously in the cytosol and the colocalization with NR decreased. The same level of enzymatic activity was achieved when the photo-activation was done up to 24 hours post incubation.
Conclusions: We report a light-activatable nanomaterial that efficiently escapes the endolysomal compartment and allows precise control over the release of more than one protein from a single nanomaterial using an external near infrared laser.
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P08. PLGA nanoparticles with anti-inflammatory agents obtained from Plectranthus plants

J. Marçalo[1,2], B. Mourato[1], M. Nicolai1, C.P. Reis[1,3], C. Faustino[2] and P. Rijo[1,2]
[1]CBIOS - Research Center for Health Sciences & Technologies, Ulusófona de Humanidades e Tecnologias, Campo Grande 376, 1749-024 Lisboa, Portugal.
[2]iMed.ULisboa - Research Institute for Medicines, Faculdade de Farmácia, Universidade de Lisboa, Av. Prof. Gama Pinto, 1649-003 Lisboa, Portugal. 3 IBEB – Institute of Biophysics and Biomedical Engineering, Faculdade de Ciências, Universidade de Lisboa, 1749-016 Lisboa, Portugal.

Introduction: Recent emerging nanotechnologies have revelead attractive possibilities for controlled and advanced drug delivery systems, using nanoparticles, particularly the poly(lactide-co-glycolide) (PLGA) nanoparticles [1].
Objective: This study aims to formulate a poly(lactide-co-glycolide) (PLGA) nanoparticle and its inclusion into a topical gel with the most anti-inflammatory bioactive agents from Plectranthus spp. compounds and extracts. Although NSAID efficiency is well established, preceding studies reveal that the main inhibition of COX-1 enzyme from these drugs mechanism of action, cause major side effects (gastric ulceration and risk of cardiovascular events). Therefore, we hope to find bioactives acting solely by inhibiting cyclooxygenase-2 (COX-2 EC 1.14.99.1) [2].
Material and Methods: The medicinal plants under study were P. grandidentatus, P. ecklonii, P. ornatus, P. madagascariensis, P. porcatus, P. neochilus and P. porcatus prostratus. The corresponding isolated compounds: 7α-acetoxy-6β-hydroxyroyleanone, parviflorón D, 11R*-acetoxy-halima-5,13E-dien-15-oic acid, 6β,7α-dihydroxyroyleanone, (13S,15S)-6β,7α,12α,19-tetrahydroxy-13β,16-cyclo-8-abietene-11,14-dione, 1α,6β-diacetoxy-8α,13R*-epoxy-14-labden-11-one and 6,7-dehydroroyleanone. The organic extracts (methanol, ethyl acetate and acetone) and aqueous extracts were obtained by ultrassound and microwaves assisted-extraction methods, respectively. The anti-inflammatory activity assay by an COX-2 inhibition spectrophotometric assay is under optimization [2,3].
Results and Discussion: A nanoparticle formulation based in PLGA with the most anti-inflammatory extracts and /or their isolated compounds were produced. The production of this topical dosage form is under optimization by previously described methods [1] and characterized physical- and chemically, for further application to an in vivo model (Wistar rats) using the carrageenan-induced rat paw oedema method.
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P09. Tableting NLC for oral drug delivery

M. Mendes[1], M.B. Vaz.[1], H.T. Soares[2], J.J. Sousa[1, 3], A.A.C.C. Pais[2] and C. Vitorino[1, 3]
[1]Faculty of Pharmacy, University of Coimbra, Portugal.
[2]Department of Chemistry, University of Coimbra, Portugal.
[3]Pharmacometrics Group of the Centre for Neurosciences and Cell Biology (CNC).

Introduction: Nanostructured lipid carriers (NLC) are nanosystems (40-1000 nm) considered a second and smart generation of lipid nanoparticles, consisting of a matrix composed of a blend of solid and liquid lipids (oils), stabilized by an aqueous emulsifier solution [1]. In the present work, NLC were used to simultaneously convey olanzapine (OL) and simvastatin (SV). OL is an atypical antipsychotic drug, extensively used in the treatment of schizophrenia and bipolar disorder, but long-term treatment is associated with metabolic adverse effects, in particular the alterations in the lipid profile. Its association with simvastatin would prevent dyslipidemia and reduce cardiovascular risk [2].
Objectives: The aim of this work was the development and modulation of oral formulations based on NLC. Their oral administration allows to eliminate the drug first pass metabolism, due to the shunt pathway promoted by the NLC to lymphatic system, thus improving bioavailability.
Materials and Methods: Simvastatin was kindly provided by Labesfal (Santiago de Besteiros, Portugal). Olanzapine was purchased from Zhejiang Myjoy (Hangzhou, China). All other reagents were from analytical or HPLC grade. NLC were prepared by the hot high-pressure homogenization (HPH) technique, as described elsewhere [2]. The NLC dispersion was subsequently converted into a solid formulation, either by freeze-drying or spray-drying. Tablets based on a combo-NLC formulation (OL-SV-NLC) with different coatings were further prepared by direct compression. Characterization was performed in terms of particle size, morphology and in vitro dissolution tests. Finally, the cytotoxicity and cellular uptake of the optimal formulations was assessed using Caco-2 cells. The influence of the secondary process upon Combo-NLC was firstly evaluated, based on particle size analysis. As protector, trehalose 5% (w/V) was used in both cases.
Results, Discussion and Conclusions: Spray-drying allowed to obtain particles with smaller size than freeze-drying, 3.25±2.01 µm and 4.01±2.23 µm, respectively. SEM images were in good agreement with the particle size results. In addition, it should be noted that this is a procedure that requires solely one step, conversely to freeze-drying. For these reasons, spray-drying was chosen to proceed with polymer coating screening. The dissolution profiles revealed different behaviours, according to the polymers and drugs used. Regarding the different particle coatings employed, Eudragit L30D allowed the slowest release for both drugs, despite the higher particle size observed. SEM images showed a continuous and smooth particle surface, supporting the sustained release observed. Sureteric led to a delayed release, having been more pronounced for SV. In the case of PVP k30, no significant differences were observed compared with commercial reference. Such behaviour could be attributed to some discontinuities on the coating layers, as elucidated by SEM images. Irrespectively of the coating, olanzapine, the less lipophilic drug, was released faster than simvastatin. Combo-NLC:Sureteric and combo-NLC:PVP exhibited lower cytotoxicity than NLC dispersions, with a cell viability approximately of 80% when concentrations were below 35uM. The cellular uptake studies corroborated the previous results. This work allowed to demonstrate the versatility in the application of NLC for oral administration, in tablets as dosage form. This could be a valuable preliminary basis for a future in vivo approach.
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P010. New formulation of oral insulin

J. Moreira[1], I.V. Figueiredo[2, 3], M. Silveira[2], P. Gonçalves[2], M. Nicolai[4], T. Almeida[4], N. Saraiva[4], M. Eduardo-Figueira[5], P. Faísca[4], I. Correia6, L. Ascensão[7], P. Rijo[1, 4, 5] and C.P. Reis[1, 4, 8]
[1]Universidade Lusófona de Humanidades e Tecnologias, 1749-024 Lisboa, Portugal.
[2]Faculty of Pharmacy, Universidade de Coimbra, Portugal.
[3]IBILI, Institute of Biomedical Imaging and Life Sciences, Coimbra, Portugal.
[4]Center for Research in Biosciences & Health Technologies (CBIOS), Universidade Lusófona de Humanidades e Tecnologias, 1749-024 Lisboa, Portugal.
[5]iMed.ULisboa, Faculty of Pharmacy, Universidade de Lisboa, Lisboa, Portugal.
[6]Centro de Química Estrutural, Instituto Superior Técnico, Lisboa, Portugal.
[7]CESAM, Faculty of Sciences, Universidade de Lisboa, Lisboa, Portugal.
[8]IBEB, Faculty of Sciences, Universidade de Lisboa, Lisboa, Portugal.

Introduction: Diabetes mellitus is a chronic disease caused by inherited and/or acquired deficiency in production of insulin by the pancreas, or by the ineffectiveness of the insulin produced. People with Type 1 diabetes are usually totally dependent on insulin injections for survival and require daily administration of insulin. Orally administered insulin should improve the patient compliance. In addition, intestinally absorbed-oral insulin actually mimics insulin's natural location and gradients in the body by first passing through the liver [1].
Objectives: The aim of this study was to prepare and characterize a new polymeric-based formulation of oral insulin.
Materials and Methods: Insulin polymeric nanoparticles (NPs) were produced by spontaneous emulsification method with solvent dispersion followed a specific coating. Mean particle size, polydispersity index of the NPs and zeta potential were measured in a Particle Analyser. Morphology was assessed by scanning electron microscope. Free insulin present in the supernatant was analyzed by HPLC method and Encapsulation Efficiency (EE%) was determined. The secondary structure of insulin released from NPs was analyzed by circular dichroism and quantified by ELISA. Ex-vivo permeation study was performed using segments of excised intestine of Wistar rats. In vivo efficacy studies were performed using STZ-induced diabetic Wistar male rats. Empty NPs and loaded NPs were orally administered through gavage and glycemic values were measured every 15 minutes during the first hour, then every half an hour for the next two hours and, at last, every hour until the 8th hour post-administration.
Results and Discussion: Results showed that these NPs had a mean diameter of 613.3 ± 76.7 nm with a narrow size distribution and homogeneous particle production. Zeta potential was -14.93 ± 0.01 mV. Particles were spherical and encapsulation efficiency of insulin was 59.8 ± 2.56%. The circular dichroism analysis showed that the secondary structure of insulin was unchanged after the encapsulation process. Insulin release was 93 ± 3% (ELISA data). The histological analysis showed that the intestine remains viable after administration of the NPs. In vitro release further indicated that entrapment of the insulin into those nanoparticles causes a reduction in the release rate of insulin. Preliminary efficacy studies confirmed the effectiveness of insulin-loaded NPs. The blood glucose levels of these rats decreased more than 40%.
Conclusions: NPs were capable of releasing bioactive insulin in vivo at a slower release rate. All excipients used are well accepted in the pharmaceutical field and thus this formulation is able to markedly improve the intestinal absorption of insulin and will be of interest in the treatment of diabetes with oral insulin.
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P011. Self microemulsifying drug delivery systems (SMEDDS) of S-nitrosoglutathione (GSNO)/ SMEDDS of GSNO

A.H. Mota[1, 2], M. Socha[1] and P. Maincent[1]
[1]EA3452 CITHEFOR «Cibles Thérapeutiques, Formulation et Expertise préclinique du médicament», Faculté de Pharmacie, Université de Lorraine, 5, rue Albert Lebrun, BP 80403, 54001 Nancy, France.
[2]Escola de Ciências e Tecnologias da Saúde, Universidade Lusófona de Humanidades e Tecnologias, Campo Grande 376, 1749-024 Lisboa, Portugal.

Introduction: The principal reasons of death in western world are the cardiovascular diseases with 31% [1]. Among current treatment, the NO exhibits a vascular function at smooth muscle (vasodilatation) and a relaxation proliferation of cells (inhibition of activation and aggregation platelets; inhibition of adhesion of leucocytes at endothelium) [2]. The donors of Nitric Oxide (RSNO) are physiologic components and endogenous forms such as GSNO [3]. The RSNOs present cardiovascular homeostasis function, have a vasodilator action (local and systemic) and also an antiplatelet effect [3]. However, RSNO are very sensitive compounds, which have very short half-life after administration. Thus, it is very important to create news formulations in this therapeutic field. Among the innovative dosage forms, SMEDDS are microemulsions systems, which are known to increase solubility, bioavailability, and to potentiate action of drugs [4-5].
Objectives: The purpose of the current study was to prepare and characterize SMEDDS of GSNO (in terms of size, stability, HLB, kinetic, cytotoxicity and incorporation efficiency), with a view to protecting GSNO and to increasing its bioavailability after oral administration.
Materials and Methods: SMEDDS with GSNO were prepared through the self-emulsifying systems. Briefly, Labrafac ALF and Transcutol HP that represent 70% of formulation, Capryol 90 (10%) and solution of GSNO (20%) at two concentrations (0.01 M and 0.029 M) were used. Blank SMEDDS were prepared accordingly with KH2PO4 aqueous solution instead of GSNO. NO assay during the in vitro release study was performed by Griess-Saville and DAN-DAN Mercury methods. For cytotoxicity study, the MTT test was performed in Caco-2 cells.
Results and Discussion: Mean particle size was 210 ± 63 nm (n = 8) and 200 ± 21 nm (n = 4) for SMEDDS without and with drug, respectively. These formulations demonstrated a satisfactory stability during 9 days at room temperature when protected from light. The value of HLB was 3.879 ± 0.093 (n = 9) and 3.835 ± 0.126 (n = 5) for SMEDDS without and with drug, respectively. The results of release kinetic were not concluded. In the cytotoxicity study, it was observed that the increase of GSNO amount in formulation decreases the toxicity of this formulation; for GSNO (0.029M) ± 50% of cell viability and GSNO (0.01 M) ± 2%. The incorporation efficiency was between 3-8%.
Conclusions: Based on the preliminary results obtained so far, SMEDDS look like a promising system for the oral administration of GSNO. However, it will be necessary to optimize release kinetic and enhance the incorporation efficiency. In addition, it has the advantage that it should be easily scaled up at the industrial level.
Acknowledgments: The authors would like to thank to ANRF by financial support and Gattefosse for providing the excipients.
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P012. Antimicrobial activity of novel abietanes cationic amphiphiles encapsulated in alginate microspheres

I. Neto[1, 2], C.P. Reis[1, 3], C. Faustino[2], and P. Rijo[1, 2]
[1]CBIOS, Universidade Lusófona de H e Tecnologias, Campo Grande 376, 1749 - 024 Lisboa, Portugal.
[2]iMed.UL, Faculdade de Farmácia da Universidade de Lisboa, Av. Prof. Gama Pinto, 1649-003 Lisboa, Portugal.
[3]IBEB – Institute of Biophysics and Biomedical Engineering, Faculdade de Ciências, Universidade de Lisboa, 1749-016 Lisboa, Portugal.

Introduction: Encapsulation technology not only allows the manipulation of molecules, as will overcome the challenges of the different types of administration. Micro and nanotechnology offers several advantages that make it a greater value compared with other types of formulations, such as increased bioavailability, drug protection and a vectorized action at the site of action [1]. Dehydroabietic acid (DHA) is an aromatic diterpenoid that has been extensively studied, and displayed a wide spectrum of biological activities such like antimicrobial, antiproliferative, anti-inflammatory and antiviral activities [2, 3]. Previous studies demonstrate that DHA has antimicrobial, but its efficacy and spectrum augmentation must be enhanced [2, 4].
Objectives: The aim of this study is to improve DHA antimicrobial activity through a synergetic effect between an antimicrobial synthetic molecule, namely a new abietane cationic amphiphile (ACA), and a protective polymer, alginate.
Materials and Methods: Microsphere production was performed by extrusion/ external gelation method using calcium salts [1]. The ACAs-alginate microspheres antimicrobial efficacy was screened in some reference strains (Staphylococcus aureus, S. epidermidis, Pseudomonas aeruginosa, Klebsiella pneumoniae, Escherichia coli and Candida albicans) by the well diffusion tests [5].
Results and Discussion: ACAs-alginate microspheres showed to be a potential strategy for antimicrobial application. Future studies will focus on their incorporation in dermatological formulations.
Acknowledgments: The authors thank Professor Aida Duarte from Faculty of Pharmacy (University of Lisboa) for providing the bacterial strains.
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P013. Design and characterization of sunflower oil nanostructured lipid carriers for retinyl palmitate delivery

F. Pinto[1], D.P.C. Barros[1] and L.P. Fonseca[1]
[1]Institute for Bioengineering and Biosciences (IBB), Bioengineering Department/Instituto Superior Técnico (IST), Universidade de Lisboa, Lisboa, Portugal.

Nanostructured lipid carriers (NLCs) are one of the main types of lipid nanoparticles representing an alternative carrier system to emulsions, liposomes and polymeric nanoparticles [1]. NLCs are submicron particles, usually with spherical shape and mean diameters ranging between 50 and 500 nm, composed of a mixture of solid and liquid lipids dispersed in an aqueous medium and stabilized by the presence of emulsifiers [1]. This allows the formation of an overall amorphous nanostructure with many imperfections within its matrix, providing NLCs with higher drug capacity and a lesser degree of drug expulsion during storage. NLCs are safe and biodegradable once they are produced using non-toxic ingredients. These benefits ensure wide applications for NLCs as delivery systems in the area of dermal cosmetics and in pharmaceutical and food industries.

Retinyl palmitate (RP) is the ester of retinol (vitamin A) combined with palmitic acid. According to some authors, once it is topically delivered and absorbed by the skin, RP is converted to retinol and then to the active component of retinoic acid [2]. The biological effects of retinoids include improvement of fine wrinkles and acne vulgaris, decrease in roughness, improvement in reducing actinic keratoses, and hyperpigmentation [2].

Currently, the research in cosmetics, pharmaceuticals and food supplements is not only restricted to the discovery of new molecules but also on the development and optimization of new delivery systems [2].

Objectives: In this perspective, the present study aimed to develop safe and effective lipid nanocarriers to successfully deliver retinyl palmitate.

Materials and Methods: The influence of the ratio % Total lipids/ % Emulsifier (2-4) and RP percentage (0.05-2% (v/v)) in the composition of the formulations were investigated on structure and on physicochemical properties of NLCs. The lipid carriers were produced through the miniemulsions methodology using only sunflower oil and lauric acid as liquid and solid lipids to minimize the ingredients and polysorbate as surfactant. The nanostructured lipid carriers loaded with retinyl palmitate (RP-NLCs) were characterized by particle size distribution, polydispersity index, viscosity, pH, Entrapment Efficiency (%EE) and Drug Loading (%DL).

Results and Discussion: The produced RP-NLCs were within the nanosized range (132 -188 nm) with relatively low polydispersity index (<0.250) and zeta potential values were around -20 mV. It was not noted a significant pH and viscosity variation by increasing the percentages of total lipids and RP in the system. The most suitable composition was obtained with a ratio of %Total lipids/ % Emulsifier equal to 1.6 and 2% of RP with an EE of 99.8% and a DL of 49.9%.

Conclusions: The results have shown that this lipid composition and especially their combination can be successfully used in designing effective nanostructured formulations to deliver retinyl palmitate.

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P014. Biocompatible antimicrobial peptide conjugated gold nanoparticles with antimicrobial efficacy a systemic infection model

A. Rai[1,2], S. Pinto[1,2], T. Velho[3], A.F. Ferreira[4], C. Moita[3], U. Trivedi[5], M. Evangelista[1,2], M. Comune[1,2], K. Rumbaugh[5], P.N. Simões[4], L. Moita[3] and L. Ferreira[1,2]
[1]BIOCANT, Parque Tecnológico de Cantanhede, Cantanhede, Portugal.
[2]Centro de Neurociências e Biologia Celular (CNC), U. Coimbra, Coimbra, Portugal.
3Instituto Gulbenkian de Ciência, 2780-156 Oeiras, Portugal.
[4]CIEPQPF, Department of Chemical Engineering, U. Coimbra, 3030-790, Coimbra, Portugal.
[5]Department of Surgery, Texas Tech University Health Sci. Center, Lubbock, TX 79424, USA.

Introduction: Antimicrobial peptides (AMPs) are integral part of innate immune system of most organisms including human and are very effective against several strains of bacteria, fungi and viruses [1]. Recently, self-assembled AMP nanoparticles have been developed for treatment of meningitis; however, the stability and activity of these nanoparticles have not been shown in the presence of serum and proteases [2,3]. The synthetic analogues of AMPs with varying chain lengths have been synthesized to prevent their degradation, although this might be a time-consuming and expensive process [4].

Objectives: Here we describe an elegant approach to generate cecropin melettin (CM) peptide conjugated gold nanoparticles (CM-SH-Au NPs) with high stability and antimicrobial activity in serum and minimum degradation of conjugated peptide after exposure to protease. CM-SH peptide is selected due to due to its broad antimicrobial property and lower hemolytic properties (>600 μM) than their counter-parts cecropin A and melittin. On the other hand, Au NPs are chosen due to their biocompatibility and easy bioconjugation.

Materials and Methods: Au NPs were synthesized using 100 mM HEPES (pH 7.5) in the presence of CM-SH peptide at 25ºC. The synthesized Au NPs were characterized using UV-vis and FTIR spectroscopy, TEM, and TGA. Antimicrobial activity of Au NPs and native CM peptide were tested against 105 CFU/mL Gram positive (S. aureus) and Gram-negative (E. coli) bacteria in PBS and 10% serum containing PBS, incubated at 37ºC for 4 h. The biocompatibility of Au NPs was evaluated on human vein endothelial cells (HUVEC) and normal dermal fibroblasts (NDHF) cells cultured on top of the surfaces using for 24 h. Annexin V-PI staining and membrane potential measurement with DiOC5(3) was performed using flow cytometry.

Results and Discussion: Spherical CM-SH-Au NPs of size ranging from 9-14 nm were synthesized in the presence of CM-SH peptide in 8 days using HEPES as reducing agent. In contrary, multibranched Au NPs were synthesized in the absence of peptide within 30 min. The critical concentrations of free Au(0), peptide and peptide-Au(0) complex play an important role in controlling reduction rate and size of NPs. Thermogravimatric analysis (TGA) shows that 50% of NP mass is organic. Antimicrobial test demonstrate that CM-SH-Au NPs higher antimicrobial activity and stability in serum and in the presence of non-physiological concentrations of proteolytic enzymes than soluble CM-SH peptide. Native CM-SH peptide is toxic against HUVECs and NDHF cells above concentration of 20 μg/mL while CM-SH-Au NPs are non-toxic to both cells up to 100 μg/mL. Our result shows that apoptosis is death pathway for 20 μg/mL while necrosis is predominant pathway for higher concentrations (50 and 100 μg/mL) of CM-SH peptide. CM-SH-Au NPs are hemocompatible and do not induce inflammatory response. CM-SH-Au NPs have antimicrobial efficacy in wound infected animal model, where a significant reduction in bacterial population is observed. Additionally, CM-SH-Au NPs have 2 logs reduction in bacterial concentration in sepsis infection animal model compared to soluble peptide [5]. CM-SH-Au NPs have been synthesized with maintenance of their antimicrobial activity in the presence of serum and proteases. Our study demostrate significant differences in terms of stability, antimicrobial activity and mechanism, cytotoxicity and in vivo efficacy between soluble CM-SH and CM-SH-Au NPs. CM-SH-Au NPs are biocompatible to human cells and have antimicrobial efficacy in systemic infection model.

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P015. Innovative antimicrobial metallic-based nanoformulation containing herbal extracts

L. Roque[1], A. Cândido[2], F. Rodrigues[2], I. Gomes[2], N. Cristo[2], R. Duarte[2], P. Rijo[1, 3] and C.P. Reis[1, 4]

[1]CBIOS, Universidade Lusófona de Humanidades e Tecnologias, Campo Grande 376, 1749 - 024 Lisboa, Portugal.
[2]ERISA, Lisboa, Portugal.
[3]iMed.UL, Faculdade de Farmácia da Universidade de Lisboa, Av. Prof. Gama Pinto, 1649-003 Lisboa, Portugal.
[4]IBEB – Institute of Biophysics and Biomedical Engineering, Faculdade de Ciências, Universidade de Lisboa, 1749-016 Lisboa, Portugal.

Introduction: Transdermal drug delivery system is being extensively investigated as a viable alternative for conventional routes of administration. It allows the use of relatively potent drug with minimal risk of system toxicity and avoids gastrointestinal degradation and hepatic first-pass metabolism. In addition, the transdermal patch can easily removed by the patient. Natural products are a common source of bioactive molecules for the treatment of bacterial infections [1]. On the other hand, synthesis of nanosized particles with antibacterial properties is of great interest in the development of new pharmaceutical products. Silver nanoparticles (NPs) are known to have inhibitory and bactericidal effects. This study was designed to prepare a non-invasive, cost effective and not painful transdermal delivery of antimicrobial extract of P.madagascariensis with polymers. PLGA is well established, safe, biocompatible and resorbable excipients commonly employed in the formulation of controlled release drug delivery systems.
Objectives: The aim of this study is to prepare and characterize NPs containing extracts for a further use as a transdermal patch.
Material and Methods: NPs were prepared using silver nitrate solution and sodium borohydride solution. Then, herbal extract was incubated overnight under magnetic stirring. The extract was obtained through ultrasounds. Briefly, ten grams of the plant in 100 mL of acetone was sonicated during 15 minutes. After this period, the mixture was filtrated through Whatman paper nr 4. Then, NPs were tested against S.epidermidis. The inoculum was obtained by suspending of S. epidermidis colonies in sterile saline solution in order to reach a density equivalent to the 0.5 McFarland Standards. This inoculum was spread in Mueller-Hinton agar media plates (wells of approximately 5mm diameter) and filled with NPs with extract.
Results and Discussion: NPs presented a diameter of 836.2 ± 1.0 nm and 686.4 ± 1.0 nm before and after the association of the extract, respectively. The efficacy tests of the NPs with extract are still ongoing.
Conclusions: This study suggests that polymeric carriers could be exploited to improve the delivery of therapeutic molecules. It also suggests that this delivery system could offer a better and more promising approach for the treatment of topical infections than the commercially available topical cream.
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P016. Innovative nanocarrier-based topical formulations for alopecia treatment

L. Roque[1], I. Dias[2], L. Palma[1], P. Rijo[1, 3], A. Roberto1 and C.P. Reis[1, 4]

[1]CBIOS - Research Center in Biosciences and Health Technologies, Universidade Lusófona de Humanidades e Tecnologias, Campo Grande, 376, 1749-024 Lisboa, Portugal.
[2]Universidade Lusófona de Humanidades e Tecnologias, Campo Grande, 376, 1749-024 Lisboa, Portugal.
[3]iMed.UL, Faculdade de Farmácia da Universidade de Lisboa, Av. Prof. Gama Pinto, 1649-003 Lisboa, Portugal.
[4]IBEB – Institute of Biophysics and Biomedical Engineering, Faculdade de Ciências, Universidade de Lisboa, 1749-016 Lisboa, Portugal.

Introduction: Androgenetic (or pattern) alopecia is a genetically disorder characterized by the gradual loss of hair. It generally involves some changes in the protein synthesis of follicular cells such high levels of dihydrotestosterone (DHT) or 5α-dihydrotestosterone (5α-DHT) [1].
Finasteride (FNS) is a 5-alpha reductase type 2 inhibitor that was found that its oral administration may be useful in the treatment of various dermatological and follicular disorders, particularly, in androgenetic alopecia. However, the oral administration of FNS causes some side effects such as impotence and erectile dysfunction.
Objectives: The objective of this study was to produce and characterize PLGA NPs with FNS and its inclusion of these NPs in three different formulations (shampoo, lotion and solution) for the used in the treatment of alopecia [2].
Material and Methods: NPs were prepared through emulsification/ solvent diffusion method. Encapsulation efficiency (EE) of FNS was measured in supernatant after encapsulation using a UV-visible spectrophotometry method (at 210 nm). Mean particle size, polydispersity index (PI) and zeta potential of the particles were measured with a Coulter Nanosizer Delsa Nano™C. Morphology of the NPs was conducted using scanning electron microscopy. In vitro drug release tests were also assessed.4 After inclusion of the FNS-loaded NPs into the three different formulations, permeation fluxes were accessed using Franz-type static glass diffusion cells with PBS pH=7.4 and at 32ºC. Preliminary toxicity studies were firstly performed using S. Cerevisae. Thereafter, human safety studies were made to evaluate the formulation excipients by the Occluded Patch Test method.
Results and Discussion: Resultant mean particle size was 316.5 nm ± 14.4 (PI 0.114) and 185.3 nm (PI 0.122) for loaded NPs and unloaded, respectively. Zeta potential was negative with a mean value of -5.71 ± 0.43 mV. NPs showed an EE value around 79.5 ± 0.5%. Drug release profiles indicated a controlled release profile of FNS from the particles. Permeation studies revealed that the permeation fluxes of all formulations were very similar until 8h. No toxic effect of the PLGA NPs was observed in terms of cell viability and during human safety trials.
Conclusions: FNS-loaded NPs may be considered an efficient and safe drug delivery system, obtaining high values of EE. The permeation tests showed that FNS permeates the skin. Further studies will be developed in order to test the efficacy of this nanosystem.
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P017. New formulation for onychomycosis treatment

L. Roque[1], A. Pereira[2], A. Santos[2], M. Simões[2], V. Demchenko[2], P. Rijo[1, 3] and C.P. Reis[1, 2, 4]

[1]CBIOS, Universidade Lusófona de Humanidades e Tecnologias, Campo Grande 376, 1749 - 024 Lisboa, Portugal.
[2]ERISA, Lisboa, Portugal
[3]iMed.UL, Faculdade de Farmácia da Universidade de Lisboa, Av. Prof. Gama Pinto, 1649-003 Lisboa, Portugal.
[4]IBEB – Institute of Biophysics and Biomedical Engineering, Faculdade de Ciências, Universidade de Lisboa, 1749-016 Lisboa, Portugal.

Introduction: Onychomycosis accounts for one third of fungal skin infections [1]. On the other hand, patients with chronic mucocutaneous candidiasis may develop candidal infection of the nails. Paronychia is usually caused by Candida albicans and occasionally other Candida species. Candidal paronychia more commonly affects the hands and usually occurs in persons who frequently immerse their hands in water. Candida paronychia, when mild and localized, will usually respond to imidazole or terbinafine cream or nystatin ointment applied topically for 1–3 weeks.
Objectives: The goal of this study is to prepare and characterized a topical formulation of an antifungal agent encapsulated into polymeric nanoparticles.
Material and Methods: PLGA nanoparticles (NPs) containing an antifungal drug were produced by spontaneous emulsification method with solvent dispersion. Average particle size, polydispersity index (PI) of the NPs and zeta potential were measured in hydrated systems with a Coulter Nanosizer Delsa NanoTMC (Fullerton, CA). Morphology was assessed by scanning electron microscopy (SEM). After the preparation of the particles, the supernatant was filtered and the amount of free drug present in the supernatant was analyzed by a spectrophotometric method at 267 nm (UV–visible spectrophotometer, Evolution 300, Hertfordshire, England) in order to determine the encapsulation efficiency (EE). Nanoparticles were also tested against Candida albicans. The antifungal activity of NPs was evaluated by the well diffusion assay, against C. albicans that was grown in Sabouraud culture media.
Results and Discussion: The resultant mean particle size was 350.7 nm ± 1.00 (PI 0.164) and 235.7 nm ± 1.00 (PI 0.073) for the NPs with and without the drug, respectively. NPs showed an EE value around 90.7 ± 1.0%. The inhibition zone of C. albicans obtained was 21 mm and 18 mm for the drug-loaded NPs and the free drug, respectively.
Conclusions: These results showed that the encapsulation of an antifungal drug successfully occurs and drug maintained its bioactivity. Future efficacy and release studies will be made after the incorporation of those nanoparticles into a cream formulation.
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P018. Novel gel formulation for fungal infections

L. Roque[1], A. Pereira[2], A. Santos[2], M. Simões[2], V. Demchenko[2], P. Rijo[1,3] and C.P. Reis[1, 2, 4]

[1]CBIOS, Universidade Lusófona de Humanidades e Tecnologias, Campo Grande 376, 1749 - 024 Lisboa, Portugal.
[2]ERISA, Lisboa, Portugal.
[3]Med.UL, Faculdade de Farmácia da Universidade de Lisboa, Av. Prof. Gama Pinto, 1649-003 Lisboa, Portugal.
[4]IBEB – Institute of Biophysics and Biomedical Engineering, Faculdade de Ciências, Universidade de Lisboa, 1749-016 Lisboa, Portugal.

Introduction: Candida Albicans is a fungi that is part of normal vaginal microflora but it may cause up to 90% of vulvovaginal Candidiasis [1]. When there is a disturbed balance between the host and the commensal flora, inflammation generally occurs.
Objectives: The aim of this study is to prepare a new formulation that allows the topical treatment of candida infections using nystatin silver nanoparticles.
Materials and Methods: Silver nanoparticles (NPs) were incubated with nystatin (Nys) overnight under magnetic stirrting. The average particle size and polydispersity index (PI) of the nanoparticles were measured using a Coulter Nanosizer Delsa NanoTMC (Fullerton, CA). After the preparation of the particles, the supernatant was filtered and the amount of free Nys present in the supernatant was analyzed by a spectrophotometric method at 267 nm (UV–visible spectrophotometer, Evolution 300, Hertfordshire, England). Those nanoparticles were also tested against C. albicans. The inoculum was obtained by suspending of C. albicans colonies in sterile saline solution in order to reach a density equivalent to the 0.5 McFarland Standards. This inoculum was spread in Sabouraud Agar media plates (wells of approximately 5mm diameter) and filled with Ag-NPs of Nys.
Results and Discussion: The resultant average particle size was 1729.4 ± 1.00 nm (PI 0.551) and 836.2 ± 1.00 nm (PI 0.355) for the NPs with Nys and the NPs without the Nys, respectively. NPs showed an association efficiency value around 74.1 ± 1.0 %. The inhibition zone of C.albicans obtained was 23 mm and 18 mm for the NPs with Nys and free Nys, respectively.
Conclusions: These results showed that the association between the NPs and the Nys is very probable since it was achieved an association efficiency value between 70-80%. The activity of Nys was maintained after the association process but future efficacy studies will be further required.
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P019. BSA nanoparticles with Parvifloron D for pancreatic cancer treatment

A. Rebelo[1], A.S. Viana[2], P. Rijo[1, 3] and C.P. Reis[1, 4]
[1]CBIOS, Universidade Lusófona de Humanidades e Tecnologias, Campo Grande 376, 1749 - 024 Lisboa, Portugal.
[2]CQB, Faculdade de Ciências, Universidade de Lisboa, Campo Grande 1749-016 Lisboa, Portugal.
[3]iMed.UL, Faculdade de Farmácia da Universidade de Lisboa, Av. Prof. Gama Pinto, 1649-003 Lisboa, Portugal.
[4]IBEB, Faculdade de Ciências, Universidade de Lisboa, 1749–016, Lisboa, Portugal.

Introduction: Pancreatic cancer is the thirteenth most common cancer and the eighth leading cause of cancer death worldwide. The prognosis of this type of cancer remains very poor with only a 5-year survival in 5% of most reports [1]. Nanotechnology can play a crucial role by targeting drugs to the malignant cells since they show a tendency for accumulation in certain tumors. In addition, the small particle size and their strong capability of easy escape through leaky endothelial tissue in the tumor can potentially result in higher intratumor concentrations of the encapsulated drug [2]. Medicinal plants studies have led to the discovery of new bioactive compounds. As example, Parvifloron D is the main component isolated from Plectranthus ecklonii Benth. (a) and has a potent antiproliferative activity [3].
Objectives: The aim of this work is to prepare and characterize albumin nanoparticles and to isolate Parvifloron D from P. ecklonii.
Material and Methods: Parvifloron D was extracted by an acetone ultrasound-assisted method. Albumin nanoparticles were produced through the desolvation method and using different cross-linking processes [4]. Particles were then characterized in terms of the stability, particle size and shape.
Results and Discussion: The resultant nanoparticles showed a very small and spherical size (766.2 nm +/- 63.1) and high stability. The extraction and Parvifloron D isolation yields were also high, 14.45 % (w/w) and 0.87 % (w/w), respectively.
Conclusions: This study confirms the feasibility of producing albumin nanoparticles and isolation of a Parvifloron D. Further studies will be performed with Parvifloron D and the activity against tumor cells will be accessed.
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P020. Functional protein nanoparticles for reduction of cardiovascular diseases risk

A. Silva[1], J.T. Marquês[1], A.S. Viana[1], R. Pacheco[1, 2] and M.L.M. Serralheiro[1]
[1]Centro de Química e Bioquímica, Faculdade de Ciências, Universidade de Lisboa, 1749-016 Lisboa, Portugal.
[2]Area Departamental de Engenharia Química, Instituto Superior de Engenharia de Lisboa, Av. Conselheiro Emídio Navarro, 1959-007 Lisboa, Portugal.

Introduction: Cardiovascular diseases are among the highest causes of death in EU [1], being the high cholesterol level in the blood one of the risk factors. The treatment often consists in the administration of drugs that are reported as presenting side effects. Previous work showed that plant infusions and decoctions containing flavonoids and other polyphenols can reduce the serum cholesterol levels [2, 3]. However, some of the compounds tested demonstrated low permeability (e.g. Rutin) and also low stability and solubility in water [4]. Encapsulating these flavonoids in proteins can improve this, increasing the uptake by the cells which is expected to have an effect on cholesterol reduction.
As a vital macronutrient in food, proteins possess unique functional properties including the ability to form emulsions which allow them to be an ideal material for the encapsulation of bioactive compounds [5], therefore proteins are suited for the development of delivery systems for bioactive compounds in the form of nanoparticles (NPs). Furthermore, these are considered safe and have nutritional value, and hence the biological products obtained will be applied as functional foods.
Materials and Methods: The synthesis of the protein based NPs was made using bovine serum albumin (BSA) loaded with rutin (Rut), as standard, and Annona cherimola leaves decoctions. Nanoparticles were prepared in a molar proportion of 10:1 of flavonoid (Rut) to BSA and mixed on a vortex [4]. For NPs loaded with Annona cherimola leaves decoctions, the extract stock concentration was adjusted in order for the prepared NPs to have the same Rut concentration as standards [3]. Obtained NPs were characterized through Atomic Force Microscopy (AFM), their permeability study was conducted using an epithelial cell line (Caco-2), normally used to stimulate the intestinal barrier, grown in Transwell plates. Citotoxicity was determined with MTT viability test, using the same cell line.
Discussion and Conclusions: In the characterization of each different nanopreparations, AFM has shown small nanoparticles of BSA with loaded Rut (from 20 nm up to 40 nm) and larger aggregates which corresponds to their association. The preparation with the greater dispersion of aggregates was found to be BSA/Annona, possibly due to the variety of the compounds present in Annona cherimola decoctions.
Conclusions: Future work is expected to increase the uniformity of the nanopreparations. The results have demonstrated that the NPs are not toxic. Our work has accomplished a great improvement in the cells uptake of rutin when encapsulated in BSA in the intestinal barrier, this is expected to contribute in the increase of the benefits of decoctions in cholesterol reduction as well as in the supply of protein for the development of new food supplies.
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P021. Development of nanostructured lipid carriers (NLC) by factorial design

M.C.T. Truiti[1, 2], S. Doktorovová[2, 3], M. Mendes[2], M. Vaz[2], C. Vitorino[2, 3] and E.B. Souto[2, 3]
[1]Department of Pharmacy, State University of Maringá, Avenida Colombo 5790, Maringá, Paraná, Brazil.
[2]Department of Pharmaceutic Technology, Faculty of Pharmacy, Coimbra University, Azinhaga de Santa Comba 3000-548, Coimbra, Portugal.
[3]Center for Neuroscience and Cell Biology, University of Coimbra, Pólo das Ciências da Saúde, Azinhaga de Santa Comba, 3000-548 Coimbra, Portugal.

Introduction: NLC have been thoroughly described as suitable carriers for a wide range of drugs [1] and their development include several variables that must be standardized.
Objectives: The aim of this work was to investigate the effects of lipid:surfactant ratio (LSR) and homogenization time (HT) on the particle size.
Material and Methods: Thus, a 22 factorial design was carried out on two levels (+1 and -1) with one center point, leading to 5 runs. The SLN were prepared using the high-pressure homogenization (EmulsiFlex®-C3, Avestin). The lipids and surfactants used were caprylic / capric triglyceride and glyceryl palmitostearate (3:1) and Tween® 80, Span® 80 (2.6:1), respectively. The levels were: LSR (+1= 2:1; 0= 1.5:1; -1= 1:1) and HT (+1= 8 min; 0= 5 min; -1= 2 min). According to the adjusted model obtained for particle size (nm), Y = 115.78-34.92xHT+11.42xLSRxHT (R2= 0.99879), the dependent variable may be improved with decreasing LSR and increasing HT.
Results and Discussion: The results showed that the statistical experimental design used was important to determine the best conditions for development of NLC, which has already been demonstrated for solid lipid nanoparticles [2].
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P022. Micronization of astaxanthin by supercritical antisolvent precipitation

Joana Costa[1, 2] , B.P. Nobre[1], J.P. Farinha[3] and A.F. Palavra[1]
[1]Centro de Química Estrutural, Instituto Superior Técnico, Universidade de Lisboa, Av. Rovisco Pais 1, 1049-001 Lisboa, Portugal.
[2]ISEL, IPL, Rua Conselheiro Emídio Navarro 1, Lisboa, Portugal.
[3]CQFM, Instituto Superior Técnico, Universidade de Lisboa, Av. Rovisco Pais, 1 1049-001 Lisboa, Portugal.

Introduction: Particle design is a subject increasingly discussed nowadays and with extreme importance for compounds produced by pharmaceutical, cosmetic and nutraceutical industries.
Objectives: In the present work, a factorial design was investigated for the micronization of synthetic astaxanthin by supercritical antisolvent technique (SAS). The objectives were accomplished using CO2 as antisolvent and THF as solvent.
Materials and Methods: Design of experiences was applied in a fractional factorial design at 4 factors, pressure (100 to 150 bar), concentration (0.5-3 mg/mL), temperature (40-60°C) and solution flow rate (0.5-1.5 mL/min) and at 2 responses (yield of micronized product and mean particle size).
Results and Discussion: Screening analysis showed higher significance to pressure, concentration, and temperature. Two experiments were run in order to assess the temperature effect. It was verified that temperature influenced the morphology of the micronized particles and that at higher temperatures smaller particles with a sphere like morphology were obtained. Central Composite Design (CCD) was used for optimization of the process. The evaluated factors were pressure (100-150 bar) and concentration (1-3 mg/mL), being the mean particle size of the micronized compound the response. Minimum mean particle size obtained was 0.182 □m at 100 bar, 60° C, 0.5mL/min and 3 mg/mL.
Conclusions: This result is in agreement with that predicted by the CCD.