P.PORTO ESCOLA SUPERIOR DE SAÚDE

3 EBtM

III ENCONTRO DE
BIOTECNOLOGIA
MEDICINAL

I IBERIAN CONGRESS ON
MEDICINAL
BIOTECHNOLOGY

BOOK OF ABSTRACTS

ENCONTRO DE BIOTECNOLOGIA MEDICINAL

18 DE MAIO DE 2018

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IBERIAN CONGRESS ON MEDICINAL BIOTECHNOLOGY

MAY 18TH, 2018

SCHOOL OF HEALTH
POLYTECHNIC OF PORTO

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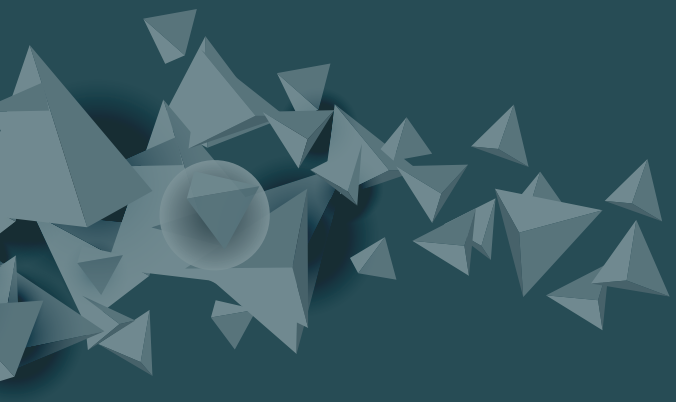
CONTEÚDOS
CONTENTS



PREFÁCIO
FOREWORD

Caros colegas,

É com grande prazer que vos recebemos no III Encontro de Biotecnologia Medicinal (IIIEBtM) e no I Encontro Ibérico de Biotecnologia Medicinal (IEIBtM).

Estes encontros de Biotecnologia Medicinal iniciaram-se em 2015 e sentimos que estamos a crescer, tal como a Biotecnologia Medicinal. Esta área da Biotecnologia tem ganho relevância, quer em empresas, quer a nível académico, criando interfaces entre a biologia molecular, a microbiologia e a química medicinal.

O interesse destes encontros tem atraído a atenção dos nossos vizinhos ibéricos e, por isso, este ano também acolhemos o I Encontro Ibérico de Biotecnologia, para poder estreitar colaborações entre Portugal e Espanha e contribuir, nesta área em grande desenvolvimento, para um melhor e maior enquadramento da Biotecnologia Medicinal a nível internacional.

À semelhança dos anos anteriores estes Encontros pretendem promover a importância da Biotecnologia Medicinal no futuro da economia e a sua relevante aplicação às Ciências da Vida e da Saúde.

Esperamos que este Encontro possa contribuir para o desenvolvimento de contactos, projetos e colaborações e constitua uma oportunidade para uma discussão frutífera em prol da Biotecnologia da Saúde e do seu futuro.

Sejam Bem-vindos a este Encontro e já estamos à vossa espera para o IV Encontro de Biotecnologia Medicinal.

A Comissão Organizadora

Dear Colleagues,

It is with great pleasure that we welcome you at the III Meeting of Medicinal Biotechnology (IIIEBtM) and at the I Iberian Congress on Medicinal Biotechnology.

These Medicinal Biotechnology meetings began in 2015 and we feel we are growing just like Medical Biotechnology. This branch of Biotechnology has gained relevance in industry and academic level and is creating interfaces between molecular biology, microbiology and medicinal chemistry.

The interest of these meetings has attracted attention from our Iberian neighbors and so this year we also hosted the I Iberian Congress on Medicinal Biotechnology. This pretends to strengthen collaborations between Portugal and Spain and to contribute, in this highly developed area, to a better and broader network of Medicinal Biotechnology at international level.

As in previous years, these Meetings intend to promote the importance of Medicinal Biotechnology in the future of the economy and its relevant application to the Life and Health Sciences.

We hope that this Meeting will contribute to the development of connections, projects and collaborations and will be an opportunity for a fruitful discussion on Medicinal Biotechnology and its future.

Welcome to this Meeting and we are already waiting for you at the IV Meeting of Medicinal Biotechnology.

The Organizing Committee

PROGRAMA
PROGRAMME

13:30 Registration

14:00 Opening Session

14:30 Panel I

"FairJourney Biologics - Antibody Tailoring for Success"
TIAGO SILVA
INVITED LECTURER 1

"Evaluation of Mucoadhesiveness of Semisolid Gels For Buccal Administration of Lipid Nanoparticless"
ANA CAMILA MARQUES
ORAL COMMUNICATION 1

"Development of new anti-bacterial Immunotherapies"
PEDRO MADUREIRA
INVITED LECTURER 2

15:30 Coffee Break and Poster Visit

16:00 Panel II

"Purification of biopharmaceuticals using ionic-liquid-based strategies"
MARA G. FREIRE
INVITED LECTURER 3

"Exploring peptide-based coatings as promising biomaterials with antibacterial properties"
MARIANA BARBOSA
ORAL COMMUNICATION 2

"Non-invasive Analysis of the Breast Cancer Biomarker HER2-ECD Trough Electrochemical Biosensing Strategies"
MARIA CASTRO FREITAS
ORAL COMMUNICATION 3

"Development of an infection prevention coating based on a bacterial extracellular polymer"
BRUNA COSTA
ORAL COMMUNICATION 4

17:00 Conference

"The past and future of Health Biotecnology"
VÍTOR VASCONCELOS
PLENARY LECTURE

18:00 Closing Session

ORADORES CONVIDADOS
GUEST SPEAKERS

PEDRO MADUREIRA
CSO IMMUNETHEP

Pedro Madureira holds a degree in Biochemistry from the Faculty of Sciences of the University of Porto (FCUP), as well as a PhD in Biomedical Sciences from the Institute of Biomedical Sciences Abel Salazar of the University of Porto (ICBAS). The work he has been developing since the beginning of his scientific career follows a research line that was initially started a few decades ago by Prof. Mário Arala Chaves, with the discovery of immunosuppressive molecules excreted by bacterial pathogens, the so called virulence-associated immunomodulatory proteins (VIP). Currently, his main research interest is focused on the role of a specific VIP in host-pathogen interactions that are responsible for the high susceptibility of neonates to bacterial sepsis. This research led to the development of a maternal vaccine that is now awaiting approval to start human clinical trials. Pedro Madureira, along with Venture Catalysts, founded Immunethep. Immunethep is a biotech company focused on the development and commercialization of immunomodulatory products.

TIAGO SILVA
FAIRJOURNEY BIOLOGICS

Tiago Silva took his first degree in Biochemistry at the Faculty of Sciences of the University of Porto. He then obtained an MSc from the Institute of Biomedical Sciences Abel Salazar of the University of Porto (ICBAS). During his MSc project, Tiago Silva developed a thesis entitled "The role of Syndecan-4 in gastric cancer cell biology", which was developed at the Institute of Molecular Pathology and Immunology of the University of Porto (IPATIMUP) in the "Glycobiology in Cancer" group. He then moved to Fairjourney Biologics where he is currently a Junior Research Associate.

VÍTOR VASCONCELOS
CIIMAR

Vítor Vasconcelos holds a PhD in Biology from the Faculty of Sciences of the University of Porto (FCUP), and is currently a Full Professor at the same institution. Vítor Vasconcelos is also the Director of the Interdisciplinary Center of Marine and Environmental Research (CIIMAR), where he leads the Group of Blue Biotechnology and Ecotoxicology (LEGE lab), as well as the Responsible for the LEGE culture collection, which comprises more than 400 cyanobacteria strains. His main research focus has been cyanobacterial secondary metabolites, namely toxins and other molecules with biotechnological applications. Vitor Vasconcelos holds a wide scientific curriculum, has published 280 papers in the fields of Toxicology and Biotechnology, participated in more than 40 research projects, and at the moment coordinates three research projects (one national and two H2020).

MARA FREIRE
PATH

Mara G. Freire is graduated in Chemistry from the University of Aveiro, and was awarded with the "Best Chemistry Student Award" from Dow Portugal. By the end of 2007 she completed her PhD in Chemical Engineering at University of Aveiro. Part of her PhD work was also developed at Federal University of Rio de Janeiro, Brazil, and at Claude Bernard University, Lyon, France. Between 2008 and 2013, Mara Freire was a post-doctoral researcher at the Institute of Chemical and Biological Technology, New University of Lisbon. In 2012, she was also an Invited Professor at Tiradentes University, Sergipe, Brazil. From June 2013 to January 2014, Mara Freire was an Assistant Researcher, and since February 2014 has been Coordinator Researcher at CICECO (Aveiro Institute of Materials), Chemistry Department, University of Aveiro. Until now, she has participated in 18 research projects, and is currently Principal Investigator and the holder of a Starting Grant from the European Research Council (ERC).

SESSÃO PLENÁRIA
PLENARY LECTURE

O passado e o presente da Biotecnologia Medicinal

VÍTOR VASCONCELOS

Professor Catedrático – Faculdade de Ciências da Universidade do Porto (FCUP)
Diretor do Centro Interdisciplinar de Investigação Marinha e Ambiental (CIIMAR)

As aplicações biotecnológicas à medicina têm raízes ancestrais, mas a descoberta acidental da penincilina por Alexander Fleming em 1928 foi um marco fundamental. Outros exemplos podem ser citados como a aplicação das vacinas contra a raiva e antrax por Pasteur e testada com sucesso em 1885. No entanto, a utilização de produtos naturais tem raízes mais antigas, com o desenvolvimento do comércio de ervas cultivadas pelos romanos (50 DC), a utilizaçãpo de plantas pelos gregos como melhoria do estado de vida (400 AC) e os registos mais antigos de uso de plantas na medicina data de 2800 AC pelos chineses. O uso de produtos naturais e mais recentemente de sintéticos químicos no combate a doenças e à melhoria do estado da saúde humana tem vindo a ser crescente. O aumento da esperança de vida conduz ao aumento da incidência de doenças como o cancro; a má alimentação conduz a uma epidemia de obesidade com as consequentes doenças associadas; as dificuldades económicas de muitos países em regiões tropicais tem conduzido a um aumento das doenças causadas por parasitas e o abuso de antibióticos tem levado a um aumento da resistência de bactérias em especial em ambiente hospitalar. Há assim a necessidade de criar novas drogas, novas vacinas e também novas técnicas de diagnóstico, para combater com eficácia infeções hospitalares decorrentes de microorganismos multiresistentes, cancro, obesidade e infeções por parasitas, entre outras. Nesta apresentação será feita uma pequena resenha histórica sobre as aplicações biotecnológicas na medicina bem como os esforços atuais no sentido de desenvolver novos fármacos, em especial com origem em microorganismos. Serão dados exmeplos de novas moléculas que têm vindo a ser descobertas por equipas do CIIMAR a partir de cultivos de fungos e cianobactérias com aplicações em diversos alvos.

III ENCONTRO DE
BIOTECNOLOGIA
MEDICINAL

I IBERIAN CONGRESS ON
MEDICINAL
BIOTECHNOLOGY

ORADORES CONVIDADOS
INVITED LECTURES

IL1

FairJourney Biologics - Antibody Tailoring for Success

TIAGO SILVA
FairJourney Biologics

Fairjourney Biologics, an established company with an outstanding track record of biotech and Big pharma partners, offers a range of assets and services on antibody engineering, discovery and production.

Being a profitable biotech company, Fairjourney Biologics has implemented a Co-Development and R&D arm to complement its fee-for-service main unit. The company has developed a full suite of primers for generation of antibody libraries from different species and has validated various naïve human Fab and llama VHH phage display libraries.

Fairjourney Biologics has one of the biggest in the world antibody discovery teams available to partner and it has entered into agreements with academia, biotech companies and Big pharma to systematically explore ground breaking targets and creating new ventures.

IL2

Development of new anti-bacterial Immunotherapies

PEDRO MADUREIRA
Immunethep

Immunethep is developing anti-bacterial immunotherapies based on the discovery that five different bacteria share a common mechanism to immunosuppress the host and induce severe disease, such as pneumonia, meningitis, septicaemia and in the worse-case scenario death by septic shock. This mechanism consists on the excretion of GAPDH, a highly immunosuppressive protein. Immunethep's pipeline consists on a vaccine program and a monoclonal antibody program, both targeting bacterial GAPDH.

IL3

Purification of biopharmaceuticals using ionic-liquid-based strategies

MARA G. FREIRE

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Over the past decades, ionic liquids (ILs) have been described as "greener" solvents over conventional volatile organic solvents due to their non-volatile nature at ambient conditions. Furthermore, the large number of possible ions' combinations, with highly distinct chemical structures, allows their tuning, and thus, ILs can be designed for a particular application or to present a specific set of thermophysical properties. Due to these features, ILs have been largely investigated as promising media in separation processes. ILs have been studied as solvents, co-solvents, co-surfactants, electrolytes, and adjuvants, in liquid-liquid systems, as well as in the creation of IL-supported materials [1-2]. In this work, the main results achieved by the use of IL-based processes in the separation/purification of biopharmaceuticals, particularly antibodies and nuclei-acid-based products, will be overviewed. The key accomplishments and future challenges to the field will be highlighted.

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[2] Ventura, S. P. M.; Silva, F. A.; Quental, M. V.; Mondal, D.; Freire, M. G.; Coutinho, J. A. P., Chem. Rev. 117 (2017) 6984–7052.

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III ENCONTRO DE
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MEDICINAL
BIOTECHNOLOGY

OC

COMUNICAÇÕES ORAIS
ORAL COMMUNICATIONS

OC1

Evaluation of Mucoadhesiveness of Semisolid Gels For Buccal Administration of Lipid Nanoparticles

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Introduction

Drug administration in specific sites in the oral cavity constitutes the common treatment for local infections or systemic diseases. Moreover, in last decades, buccal mucosa has also been recognized as an attractive site for systemic drug delivery. Nanostructured Lipid Carriers (NLC) as drug delivery systems have been investigated for several years. One of the delivery routes for which these carriers can be applied is buccal administration. However, formulations can be easily removed from oral cavity through saliva action and involuntary swallowing. Poor retention of the hydrogels at the site of application has been overcome by using mucoadhesive polymers.
The aim of this work was to develop mucoadhesive buccal hydrogels with NLC, using ibuprofen as a model drug, and to compare the obtained results for three polymers.

Material and Methods

Hydrogels were performed with three acrylic acid derivatives: Carbopol® 980, Carbopol® 974P or Polycarbophil. Firstly, placebo and ibuprofen-loaded NLC were produced by high shear homogenization followed by sonication. Then, the obtained dispersions were incorporated in hydrogels with 1.5% (w/w) of polymers in a 50:50 proportion, by mechanical stirring. The obtained hydrogels were submitted to texture properties (firmness and adhesiveness) analysis and rheological characterization. Finally, a mucoadhesiveness evaluation in a simulated saliva fluid and a mucin dispersion was performed, by calculating the maximum force and adhesion work.

Results and Conclusions

Higher values of firmness and adhesiveness were observed in the hydrogels of Carbopol® 980 (HG-C980), comparing to those of Carbopol® 974P (HG-C974P) or Polycarbophil (HG-Poly). Regarding the rheological analysis, the rheograms of all tested semisolid formulations revealed a pseudoplastic behaviour and the majority exhibited no thixotropy. HG-C980 present higher viscosities than HG-C974P or HG-Poly. Concerning the mucoadhesiveness assay, the adhesion force and work values of HG-C980 are also higher than those obtained with HG-C974P or HG-Poly.
Comparing the obtained results for the three polymers, C980 is considered to be the most promising, by increasing the residence time and easiness for topical application in the buccal mucosa.

Keywords: Buccal mucosa, Mucoadhesiveness, Hydrogels

OC2

Exploring peptide-based coatings as promising biomaterials with antibacterial properties

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Introduction: Bacterial colonization and establishment of resistant biofilms remain a major health care problem. Therefore, research for new and effective antibacterial therapeutics is crucial. Antimicrobial peptides (AMP) emerge as promising alternatives to conventional antibiotics. Hence, the development of AMP-based materials is a highly attractive field of research. In this connection, the main goal of our work is to evaluate the effect of surface immobilization of Dhvar-5, an AMP with head-to-tail amphipathicity, on its antibacterial activity. To this end, Cu(I)-catalyzed azide-alkyne cycloaddition (CuAAC) "click" reactions were explored for covalent immobilization of the peptide onto chitosan.

Materials and methods: Dhvar-5 grafting onto chitosan by CuAAC was carried out either on (i) ground chitosan powder (type 1 thin films), or (ii) pre-formed chitosan films (type 2 thin films). Additionally, peptide tethering was tested in both possible orientations, i.e., the peptide was covalently immobilized via either its *N*- or its *C*-terminus. Antimicrobial activity assays were carried out using a combination dye of the LIVE/DEAD® Bacterial Viability Kit (Baclight™) for quantifying the viability of adherent bacteria.

Results and discussion: The bacterial adhesion studies demonstrated that the AMP-chitosan thin films had antibacterial effects whose potency depended on the strategy of immobilization and which region of the peptide was exposed. As such, type 1 thin films displayed bactericidal effects, whereas anti-adhesive properties were exhibited by type 2 thin films. Also, higher antimicrobial activity was observed when Dhvar-5 was immobilized through its cationic *C*-terminus, i.e., exposing its hydrophobic domain.

Taken together, results obtained thus far suggest that "click" chemistry, namely CuAAC, is an attractive chemoselective approach to synthesize AMP-chitosan thin films suitable for use as antimicrobial coatings with potential biomedical interest.

Keywords: bacterial infection; antimicrobial peptide; immobilization; biomaterial

OC3

Non-invasive analysis of the breast cancer biomarker HER2-ECD through electrochemical biosensing strategies

M. FREITAS*, H.P.A. NOS, C. DELERUE-MATOS

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Introduction

Screening and diagnosis of early-stage oncological diseases and adequate follow-up are critical for successful patient management, promoting public health and increasing survival rate. Breast cancer is a worldwide health concern and one of the leading cancer-related mortality in women. The gold standard procedures for the screening and detection of breast cancer are performed using imaging tools, such as mammography. However, it only allows the visualization of the tumour and cannot predict its behaviour and biological evolution. The development of clinical and analytical tools for non-invasive detection using cancer biomarkers is critical for disease diagnosis. The principal breast cancer protein biomarkers for non-invasive clinical tests are human epidermal growth factor receptor 2 (HER2) and cancer antigen 15-3 (CA 15-3). In the clinical field, a prominent alternative to the traditional methods is the development of biosensors. In addition, the use of nano- and micro materials can increase the sensitivity of the analysis

Material and methods

Distinct electrochemical immunosensing strategies were successfully developed for the analysis of the extracellular domain of HER2 (HER2-ECD). A sandwich assay was performed using (i) SPCEs modified with gold nanoparticles and multiwalled carbon nanotubes or (ii) magnetic beads. The antibody-antigen interaction was detected using a secondary antibody labelled with alkaline phosphatase and 3-indoxyl phosphate and silver ions as the enzymatic substrate. The electrochemical signal of the enzymatically generated metallic silver was recorded by linear sweep voltammetry.

Results and conclusions

The calibration plot for both sensing strategies was obtained between 7.5 - 50 ng/mL achieving limits of detection below the established cut-off value (15 ng/mL).

Spiked human serum samples were used to test the sensor's applicability and the selectivity was confirmed through the analysis of other biomarkers and possible serum interferents.

Keywords: Breast cancer, electrochemical immunoassay, nanomaterials, SPCEs.

Acknowledgments

Maria Freitas is grateful to FCT-Fundação para a Ciência e a Tecnologia for her PhD Grant (SFRH/BD/111942/2015). This work received financial support from the European Union (FEDER funds through COMPETE) and National Funds (FCT, Fundação para a Ciência e a Tecnologia) through project UID/QUI/50006/2013.

OC4

Development of an infection prevention coating based on a bacterial extracellular polymer

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Introduction: Medical devices are widely used in modern medicine, improving patients' overall health and quality of life, often acting as lifesavers. However, they are susceptible to bacterial contamination originating infection. Most device-related infections are caused by biofilms that are very difficult to eradicate. Current treatments have proven to be insufficient, and sometimes even promote bacterial resistance and increased toxicity. Therefore, new biotechnologies capable of preventing biofilm formation represent a promising way to fight the infection onset. The use of natural polymers to produce antibiofilm coatings is a new trend for a sustainable solution. Here, a marine bacterial extracellular polymer is being explored for the development of an anti-adhesive coating. This coating has the potential to be applied to a wide number of medical devices, however, in this work the proof-of-concept is being applied in catheter-related surfaces.

Methods: The bacterium was grown in conditions that enhance the production of extracellular polymeric substances and the polymer was isolated and extracted following an optimized protocol. For the coating development, the natural polymer was processed and applied in model pre-activated medical grade biomaterial through spincoating. Coating surface characterization was obtained by goniometry, ellipsometry and FT-IR. Coating biological performance was done following ISO 22196 (coating bacterial anti-adhesive performance) and ISO 10993-5 (coating biocompatibility regarding fibroblast cells).

Results and Conclusions: The coating revealed to be a very promising technology as it significantly reduced the adhesion of the most frequently isolated device-related etiological agents (e.g. Staphylococcus epidermidis) and not being cytotoxic.

Keywords: Anti-adhesive coating; Extracellular polymer; Marine bacteria; Medical-device infection

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BIOTECNOLOGIA
MEDICINAL

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BIOTECHNOLOGY

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POSTER COMMUNICATIONS

P1

Antimicrobial activity of *Chritmum maritimum L.* essential oils from Northern Coast of Portugal

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Crithmum maritimum L. (Apiaceae), usually known as sea fennel, is an halophyte plant typical of rocky coastal ecosystems, that grows wild on maritime rocks, piers and breakwaters and sandy beaches in the Atlantic coast of Portugal and Azores. The plant is edible and its leaves are traditionally used as appetizer, tonic, carminative, diuretic and vermifuge. Portuguese sailors used to consume it as food preparations, namely sea fennel pickles, as protection against scurvy due to its high content on vitamin C. Nowadays, it is most consumed in vinegar preparations. Several studies reported α-terpinene, α-felandrene, sabinene and p-cymene as the major compounds of C. maritimum essential oils. In order to study the antibacterial activity of *C. maritimum* essential oils aerial parts of the plant were submitted to hydrodestillation in a Clevenger apparatus. Four bacterial species were tested (*Staphylococcus aureus*, *Enterococcus faecalis*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Bacillus cereus*) using the disk diffusion method on Mueller-Hinton culture media for 48 h. After 24h of incubation the antibacterial activity of pure or diluted essential oils was only registered for *P. aeruginosa* while for *S. aureus* and *E. faecalis* species inhibition hales were only observed after 48 h. On the other hand no antibacterial activity was observed for E. coli and B. cereus during the incubation period. The results suggest the potential use of *C. maritimum* essential oils as an antibacterial agent particularly on *P. aeruginosa*. Further studies are in progress to test the potential of other *C. maritimum* extracts as antimicrobial agents.

Keywords: *Crithmum maritimum L.*; essential oils; antibacterial activity; *Pseudomonas aeruginosa*

P2

Glycine-betaine ionic liquid analogues as novel phase-forming components of aqueous biphasic systems (ABS)

MATHEUS M. PEREIRA[1], MAFALDA R. ALMEIDA[1], JOANA GOMES[1], ANA F. C. S. RUFINO[1], MARGUERITA E. ROSA[1], JOÃO A. P. COUTINHO[1], AMINOU MOHAMADOU[2] AND MARA G. FREIRE[1]

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The enhanced production of value-added biocompounds has increased the need of finding new cost-effective purification and recovery techniques. One of the biocompatible purposes of extraction platforms are the aqueous biphasic systems (ABS), in which the use of imidazolium-based ILs has been preferred as phase-forming agents. Nevertheless, this ILs have some associated toxicity that can compromise the biological activity of molecules, so novel ABS composed of ILs analogues of glycine-betaine (AGB-ILs) are proposed and investigated. For this purpose, five AGB-ILs were synthesized, characterized in terms of ecotoxicity, and applied toward the development of novel ABS formed with Na_2SO_4, and for comparison were also used three commercial ILs. The respective ABS ternary phase diagrams, as well as the tie-lines and tie-line lengths, were determined at 25°C. For the evaluation of extraction strategies were used five amino acids (L-tryptophan, L-phenylalanine, D-phenylalanine, L-tyrosine and L-3,4-dihydroxyphenylalanine/L-dopa). The amino acids preferentially migrate to the IL-rich phase in all studied systems, where amino acid extraction efficiencies were achieved for the IL-rich phase ranging between 65-100%, in a single-step using AGB-ILs. The aminoacids preferentially migrate to the IL-rich phase in all studied systems, where amino acid extraction efficiencies were achieved for the IL-rich phase range between 65-100%, in a single-step with AGB-ILs. In addition, the five AGB-ILs display a higher ability to form ABS and extract amino acids when compared to ABS composed of more traditional and commercial ILs. Thus, AGB-ILs can form novel ABS that can be used as separation of value-added compounds of biotechnological interest.

Keywords: Aqueous Two-Phase Systems, Ionic Liquids, Glycine-Betaine, Amino acids purification

P3

Screening of positive urines using Sysmex UF-1000i flow cytometer and identification by MALDI-TOF mass spectrometer

JOANA SANTOS-OLIVEIRA[1,2], RITA TEIXEIRA-SANTOS[3], SOFIA COSTA-DE-OLIVEIRA[2,3], ANA SILVA-DIAS[3], INÊS MARTINS-OLIVEIRA[3], ACÁCIO G RODRIGUES[2,3], CIDÁLIA PINA-VAZ[2,3]

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Introduction: Urinary tract infections (UTIs) are the most common infections both in hospitalized and community patients often leading to severe complications. Gram-negative bacilli are the most prevalent microorganisms often resistant to main antimicrobial agents. Microbiology diagnosis is necessary but take at least 48 hours to provide results. Sysmex UF-1000i is an automated urine flow cytometer that have been described as able to avoid the culture on negative samples. We evaluated this equipment in order to screen positive urines with gram-negative bacilli >1 x 105/mL comparing it with the routine method. Additionally, we have developed an extraction protocol for identification of the strains directly from urine on MALDI-TOF.

Material and methods: One-hundred thirty-nine urine samples from routine microbiology laboratory were analyzed on Sysmex UF-1000i. Those showing values > 1 x 105/mL rods were centrifuged according a developed protocol and analyzed on MALDI-TOF (4 spot for each sample) from Bruker. All the results were compared with routine analysis: semi-quantitative smear in CLED agar (BioMérieux), overnight incubation at 37ºC and identification from the colonies by MALDI-TOF. The quantification of microorganisms was recorded and compared to CFU determination using a paired samples t-test ($p < 0.05$). A proportion of agreement (PA) was determined between MALDI-TOF performed directly from urine samples and from colonies. When Sysmex UF-1000i detected cocci/mixed population or negative (<1 x 104/mL) no further studies were done.

Results and conclusions: Eighty-two urine samples screened as positive for rods from Sysmex UF-1000i were positive on classic method (≥ 1 x 105 bacteria/mL). Regarding quantification, significant differences ($p = 0.013$) between Sysmex UF-1000i and routine were found however not exceeding ± 1 log, with no clinical meaning. MALDI-TOF was able to identify directly from urine (at least in one spot) 95.3% of the isolates, being the majority (70.4%) *E. coli*, *Kl. pneumoniae* (8.2%), *Proteus mirabilis* (3.3%), Enterobacter spp. (6.6%), *Pseudomonas aeruginosa*, *Providencia* and *Aeromonas* (1 isolate for each). The 4 cases (6.6%) without identification directly, did not had pellet after extraction protocol (1 Salmonella, 3 E. coli). The PA between the identifications obtained directly from urines and from colonies was 100%. Therefore, 57 samples analyzed on Sysmex weren't considered for identification on MALDI-TOF, because they were negative (14), showed cocci/mixed population (22) or presented values > 1 x 105 bacteria/mL but without clarifying if they were rods or cocci/mixed (21).

In conclusion, the Sysmex UF-1000i revealed to be a useful tool for screening and quantification positive urines. Additionally, MALDI-TOF directly from urine showed to be accurate and fast (few minutes versus 24h), representing a step forward on UTIs diagnosis.

Keywords: Urinary tract infections; Gram-negative bacilli; Flow cytometry; Mass spectrometry

P4

Flow cytometric antimicrobial susceptibility test for gram-negative bacilli directly from positives urines

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Introduction: Urinary tract infections (UTIs) are the most frequent infections in both hospitalized and community patients. These infections may provoke serious complications especially in children, pregnant human and elderly. The rate of recurrent UTIs is high and the increasing number of resistant bacteria is intensified by incorrect or excessive use of antimicrobial drugs. Consequently, rapid antimicrobial susceptibility tests are urgent. FASTinov® kits allow the determination of antimicrobial susceptibility profile directly from biological products (time-to-result, TTR, 2h). In this work, we evaluated the performance of FASTinov® gramneg kit for AST on gram-negative bacilli directly from urine.

Material and methods: A total of 13 quality control strains (ATCC 35218, ATCC 25922, ATCC 8739, IMP NCTC 13476 *E.coli*, BAA 1705, BAA 1706, ATCC 700603, OXA-48, NCTC 13443 NDM-1 *K. pneumomiae*, CCUG 59627 *Enterobacter cloacae*, ATCC 27853, IMP-8 *Pseudomonas aeruginosa* and ATCC 13048 *E. aerogenes*) were included in this study. Bacteria were inoculated into 5 mL Mueller-Hinton Ca2+ broth and incubated overnight (37°C; shaking at 130 rpm). Human urine from healthy donors was collected and filtered (0.45 µM pore size). A total of 20 mL of urine was inoculated according with Roos V. et al. (2006), and incubated for ±120 min (until exponential growth phase). Bacteria was extracted from urine according to IFU, inoculated in a FASTinov® gramneg kit containing the main antimicrobial drugs, and incubated for 1h, at 37ºC; afterwards, the kit was analyzed BD AccuriTM C6 Flow Cytometer. EUCAST and CLSI protocols were selected on the FASTinov dedicated software. Results obtained from FASTinov® kit and microdilution broth reference method were compared. All experiments were performed twice.

Results and conclusions: The overall agreement between FASTinov® gramneg kit and broth microdilution reference method was 96.1% for EUCAST and 95.5% for CLSI protocols. One major discrepancy was detected for gentamicin and ciprofloxacin, and one minor discrepancy was observed for amikacin and piperacillin/tazobactam. For imipenem were detected 3 minor discrepancies for EUCAST and 1 major discrepancy for CLSI protocol. There were no very major discrepancies. Regarding the screening for detection of enzymatic resistance mechanisms, the concordance was 100%.

We hereby presented a fast flow cytometric protocol which revealed to be an excellent tool for AST on gram-negative bacilli directly from positive urine in a TTR of 2 hours versus the 48h requires by the routine method, with high agreement with broth microdilution reference method.

Keywords: Antimicrobial susceptibility testing; Urine; Gram-negative bacilli; Flow cytometry

P5

Influence of KRAS activation in the colorectal cancer immunosurveillance escape

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Introduction: The immune system as a host defense system watches the cell growth and division, eliminating cells with antigens different from those present in healthy cells. However, some transformed cells have the capacity, through various mechanisms, to escape the immune system. Genomic instability and some mutations are pointed as possible mechanisms supporting the immunosurveillance escape, as is the case of KRAS mutation. This oncogenic mutation is present in about 30% of cases of colorectal cancer and confers to the tumor a greater potential for malignancy. It is known that KRAS mutant cancer cells regulate the recruitment, activation, and differentiation of immune cells, promoting tumor evolution by ensuring leakage to the immune system and increasing the proliferative potential. Few evidence highlights an association between a KRAS mutation and myeloid cells, mainly macrophages and neutrophils infiltration. However, the mechanism which determines this interaction remains unclear. Due to the growing knowledge of different immunosuppressive molecules, it became interesting to investigate if there is an alteration in these molecules related to the KRAS activation.

Matherials and Methods: In our work, a series of these molecules were analyzed by flow cytometry in a panel of KRAS mutant colorectal cancer cells in which KRAS was silenced by small interfering RNA.

Results and Conclusions: Preliminary results suggest that the silencing of this oncogene lead to the alteration of some molecules involved in the crosstalk with the immune system cells, such as macrophages. In conclusion, the KRAS activation seems to be capable to regulate the expression of surface markers which can regulate and suppress the immune response of the tumor infiltrated immune cells.

KeyWords: colorectal cancer, KRAS mutation, immune surveillance, macrophages.

P6

Etidronate-based ionic liquids: in vitro effects on bone metabolism

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Introduction: The Bisphosphonates have been used for various purposes since the middle of the 19th century, but the propriety that elicited more curiosity was their ability to regulate bone mineralization. This characteristic made this drug the elite choice for treatments of several pathologies associated to increased bone resorption.

Although they present a low bioavailability, their preferential uptake is localized in regions of active bone remodeling or accelerated bone turnover, where they exert their biological effects.

Methods: It was developed ionic liquids (ILs) containing etidronate, aiming to improve not only its chemical properties, but also its efficacy in the modulation of cellular behavior, particularly on human osteoclasts and osteoblasts. The ionic liquids were prepared with two different superbases as cation and different stoichiometric combinations were tested.

Results: It was observed that some of the developed compounds presented increased water solubility, with diminished or absent polymorphism. Several of them, also appeared to be more cytotoxic against some of the human cancer cells analyzed (T47D, A549 and MG63). Regarding bone cells, a promotion of an anabolic state was observed, meaning an inhibition of osteoclastogenesis and an increase in osteoblastogenesis. These effects resulted from differential modulation of intracellular signaling pathways by the Eti-ILs.

Conclusion: Taken together, some of the developed ILs revealed improved chemical and biological properties, when compared to etidronate alone; which means that the synthesis of etidronate-containing ILs may represent a potential strategy to create new drugs with better physical, chemical and biological properties.

Keywords: Bisphosphonates; Etidronate; Ionic liquids; Osteoclastogenesis;

P7

Insights into the peptide hybrid constructs approach

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The invention of peptide synthesis around the sixties prompted the development of synthetic peptides for different applications, including the study of protein functions, the identification and characterization of proteins, the development of epitope-specific antibodies against antigens from pathogens, the development of novel antimicrobial agents, and their use as intracellular delivery of a wide variety of exogenous molecules. Furthermore, synthetic peptides are used to study enzyme-substrate interactions within important enzyme classes such as kinases and proteases, which play a crucial role in cell signalling.

Peptides can be obtained chemically by solution-phase synthesis, by solid-phase peptide synthesis, or by a combination of both methods, which can involve various ligation strategies. The benefit of peptide synthesis approaches today is that, in addition to the ability of obtaining peptides that are found in nature, one can use imagination and creativity to make unique non-natural peptides with a desired and optimized biological response. For example, chemical strategies for the design of multifunctional peptides can include a hybrid of two peptides being bound together like modules either directly or via a linker. As such, this work will focus on the success case of CA(1-7)M(2-9), an hybrid of cecropin A (CA) and melittin (M) that shows powerful antibacterial activities with a wider spectrum and improved potency relative to cecropin A without the undesirable cytotoxic effects of melittin.

Acknowledgements

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P8

Generation of glycoengineered Nicotiana tabacum for production of therapeutic protein

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Introduction: Plants have a great potential to be used in the production of therapeutic proteins. N-glycosylation in plants is slightly different from that in mammals, due to modifications occurring in the Golgi apparatus. So, the glycans of the proteins produced in plants can give rise to allergic reactions and decrease of biological activity in animals, when tested *in vivo*. The N-glycan core structure synthesized by plants lacks β1,4-galactose, sialic acid and core α1,6 fucose and contains a α1,3 linked core fucose and β1,2-xylose residues not found in mammals. The current study was undertaken to generate a *Nicotiana tabacum* plant expressing the catalytic domain of human β1,4-galactosyltransferase fused to the transmembrane domain of the β1,2-xylosyltranferase from tobacco plant, providing an enzyme with galactosyltranferse activity and localization of the xylosyltransferase tobacco as a first step towards the obtaining of "humanized" plants.

Matherial and methods: The plant expression vector was created by fusing the nucleotids encoding for the initial 27 amino acids of the β 1,2-xylosyltransferase from *N. tabacu*m (GenBank: DQ192540) to the nucleotids encoding for aminoacids 42 to codon stop of 1,4- galactosyltransferase of human gene from commercial vector pUNO1-hB4GALT1a (Invivogen) by PCR and introducing the construction in the pMDC32 vector by Gateway cloning System (Invivogen). An *Agrobacterium*-mediated leaf disc transformation system was used to create stable transgenic plants. All plants were screened for the presence of the transgene by genomic PCR and RT-PCR. Plants were self-*pollinated* and subjected to lectin blotting using biotinylated *Ricinus communis* agglutinin I (RCA_{120}) for detection of β1,4-galactosylated N-glycans.

Results and Conclusions: Transgenic tobacco plants that stably express a modified version of human β1,4-galactosyltransferase grew normally. The human β-1,4 galactosyltransferase was capable to galactosylate endogenous tobacco glycoproteins. Confirmation of the results by MALDI-TOF and analyses of the influence of N–terminal cytoplasmic transmembrane and stem region of plant xylosyltransferase on N-linked glycans, should be carried out.

Keywords: β1,4-Galactosyltransferase, transgenic plant, *Nicotiana tabacum*, glycoengineering

P9

Bioinformatic research of the gene "pregnane X receptor" (PXR) homologues in marine invertebrates

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Introduction: the xenobiotic detoxification enzymes are regulated, in mammals and other animals, by a specific group of nuclear receptors. In particular pregnane X receptor (PXR) has shown incredible versatility in recognizing a broad range of xenobiotics and natural compounds which function as agonists of the protein. However this regulatory mechanism is poorly characterized in marine invertebrates including bivalves. The objective of this work was, 1) to collect sequence information from PXR homologous genes already discovered, 2) to search for putative PXR homologous sequences in other marine species and in the marine mussel *Mytilus galloprovincialis*.

Materials and methods: We firstly conducted a literature search using PUBMED (https://www.ncbi.nlm.nih.gov/pubmed/) and SCOPUS (https://www.scopus.com) online search tools for published PXR gene sequences in marine invertebrates. Subsequently a search of homologous sequences deposited in NCBI (https://www.ncbi.nlm.nih.gov/) and UNIPROTKB (http://www.uniprot.org/) genomic databases was carried out using the respective BLAST tools of these databases. Finally, the transcriptome of the marine mussel *Mytilus galloprovincialis* was used to search for homologous PXR sequences in this particular species using the BLAST2GO tool.

Results and conclusions: At least 3 PXR genes have been so far identified and characterized from the marine species *Crassostrea virginica, Scrobicularia plana* and *Ciona intestinalis*. Our bioinfomatic analysis allowed to select 7 gene sequences from different marine invertebrates, highly homologous to the *Scrobicularia plana* and *Crassostrea virginic*a PXR genes. It was still possible to identify an homologous sequence of *Scrobicularia plana* gene in the transcriptome of the marine mussel *Mytilus galloprovincialis*. This work thereby supports previous studies that showed that PXR are conserved genes along the animal kingdom.

Keywords: Xenobiotic Metabolism, Marine Invertebrates, Nuclear Receptors

P10

Evaluation of quinoxaline-1,4-dioxide and derivatives Biological Activity in normal and tumour cell

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Introduction: Quinoxaline derivatives are synthetic heterocyclic compounds with multiples pharmacological applications and biological effects. Previous studies of our group about the biological activity in eukaryotic and prokaryotic microbial models with quinoxaline-1,4-dioxide (QNX) derivatives concluded a selective antimicrobial activity in gram negative strains. To further evaluate these compounds' potential, we investigated the biologic activity *in vitro* of this chemical structures in normal and tumour cells lines.

Material and Methods: The compounds in study were gently provided by the Centre of Investigation in Chemistry of the University of Porto. Quinoxaline-1,4-dioxide (QNX); 2-amino-3-cyanoquinoxaline-1,4-dioxide (2A3CQNX); 2-methylquinoxaline-1,4-dioxide (2MQNX); 3-Methyl-2-quinoxalinecarboxamide-1,4-dioxide (3M2QNXC) and 2-Hydroxiphenazine-N-dioxide (2HF), were tested in sixteen different final concentrations. Mouse fibroblast 3T3-L1 (ATCC® CL-173™), human dermal microvascular endothelial cells (HMVEC-D), mouse melanoma B16-F10 (ATCC® CRL-6475™), human malignant melanoma MeWo (ATCC® HTB-65™), mouse Glioma cell line GL-261 and mouse brain tumour BC3H1 (ATCC® CRL-1443™) were used in this work. The cell viability was determinate used MTT Assay (4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) from (Sigma-Aldrich, Co., USA). The IC_{50} was calculated using the GraphPad software with the results of cell viability assay, for all line cells and concentrations in study.

Results and Conclusions: The IC_{50} concentrations of QNX, 2MQNX and 3M2QNCX presented high values compared with other compounds. 2A3CQNX and 2HF IC_{50} concentrations exhibited a strong influence in all cell lines. In conclusion, our results demonstrated the influence of quinoxaline-1,4-dioxide (QNX) and derivatives in cell viability in normal and tumour cells. 2A3CQNX and 2HF induced a modulative action in cells survival mechanisms, with IC50 concentrations calculated in all cell lines in study.

Keywords: quinoxaline-1,4-dioxide and derivatives, normal and tumour cell, IC_{50}

P11

Development of a method based on HPLC-DAD for the detection and quantification of glutamate and gamma-aminobutyric acid

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Introduction: With the increasing incidence of neurodegenerative diseases, the need arose to study several molecules that may be useful to the study of new therapies and to treat the symptoms that come with these diseases, as is the case of depression. GABA and glutamate have a very important role in the homeostasis of the organism and molecules of interest and potential from the point of view of diagnosis are revealed. HPLC is a method that allows the detection and quantification of various analytes in different biological matrices, allowing rapid results with high precision, sensitivity and specificity.

Materials and methods: All chromatographic assays were performed using a Hitachi LaChrom Elite® HPLC system, with separation on a Lichrospher LiChroCART ®

250-4 100 (5 μm) RP-18 column and with DAD detection. The parameters of flow rate, mobile phase composition, temperature and wavelenght settings were varied during the process of the development of the method. The validation of the method was performed according to ICH guidelines, which was tested in standard solutions of GABA and glutamate and in samples of serum, urine and yeast extract.

Results and conclusions: At the end of several assays, the chosen method allowed for a 10 minutes analysis with specific detection of GABA and glutamate and a quantification in the order of μg/mL. Even though the quantification was possible in the standard solutions, the same did not occur in the biological matrices tested. Although it still needs to be optimized for biological matrices, the method developed allows an easy, fast and economically sustainable analysis of GABA and glutamate. It is, to date, the only method with DAD detection that allows the simultaneous detection of GABA and glutamate without recourse to derivatization of the sample.

Keywords: Neurodegenerative diseases, depression, GABA, glutamate, HPLC-DAD

P12

Noradrenergic descending pain modulation in a Kaolin-induced hydrocephalus rat model

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Pain transmission at the spinal cord is modulated by descending actions that arise from several supraspinal areas which collectively form the endogenous pain control system.

In the context of neurological defects, several diseases may affect the structure and function of the brain. Hydrocephalus is a congenital or acquired disease characterized by an enlargement of the ventricles which leads to a distortion of the adjacent tissues. Usually, patients suffering from hydrocephalus present dysfunctions in learning and memory and also have motor deficits.

The effects of hydrocephalus in pain modulation from the brain was here evaluated in a validated animal model of hydrocephalus induced by kaolin injection into the cisterna magna. Due to its periventricular location and key role in descending modulation of nociceptive transmission, through direct noradrenergic projections to the spinal cord, we focused our study in the pontine locus coeruleus (LC).

Three weeks after hydrocephalus induction immunodetection of the noradrenaline-synthetizing enzyme tyrosine hydroxylase (TH) was performed in the LC and Spinal Cord (SC). In general, rats with kaolin-induced hydrocephalus presented a higher dilatation of the 4 th ventricle. Increases in the levels of TH in the LC, were detected in hydrocephalic animals. The following pain-related parameters were measured, namely 1) pain behavioural responses in a validated pain inflammatory test (the formalin test) and 2) the nociceptive activation of SC neurons. A decrease in behavioral responses was detected in rats with kaolin-induced hydrocephalus, namely in the second phase of the test (inflammatory phase).

Collectively, the results of the behavioral studies indicate that rats with kaolin-induced hydrocephalus exhibit hypoalgesia. A decrease in Fos expression was detected at the superficial dorsal layers of the spinal cord in rats with kaolin-induced hydrocephalus, further indicating that hydrocephalus decreases nociceptive responses. Since the LC has higher levels of TH in rats with kaolin-induced hydrocephalus, which also appears to increase the noradrenergic innervation in the spinal dorsal horn, it is possible that an increase in the release of noradrenaline at the spinal cord accounts for pain inhibition.

P13

A microfluidics technique to evaluate *Staphylococcus aureus* adhesion to micropatterned silica surfaces

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Postoperative complications like poor osteointegration or bacterial adhesion to bone implants are the most common causes that lead to implant failure.

Micropatterned surfaces have been used to improve implant osteointegration as they have shown to promote enhanced cellular attachment, proliferation and differentiation. It is well established that modifying the surface topography of a biomaterial has a positive effect on regeneration.

However, a concern related to using implants with greater surface area and roughness is that bacterial adhesion might be increased.

This study intends to evaluate the bacterial adhesion of S. aureus to micropatterned silica surfaces, proposed to be applied as bioactive coatings for bioinert hard-ceramic implants.

A combined methodology of sol-gel and soft-lithography was used to produce SiO2 thin films with micropatterns in the shape of lines and pillars, with 10um of interspacing. Spin-coating was used to create flat SiO2 surfaces, to be used as controls. Materials were characterized by SEM, FTIR and contact angle.

For a more effective evaluation, bacterial adhesion will be assessed under different conditions through two distinct techniques: static and microfluidics. The technique of microfluidics has the advantage of simulating the biological phenomena with physiological flow velocities, being able to provide a more accurate quantification. The results from this method will be compared to the results obtained with the standard method, under static conditions. On both, the adherent bacteria will be quantified by colony forming units (CFU), Live/Dead Staining and SEM.

We expect with this study to perform a more precise evaluation of bacterial adhesion on microstructured silica thin films, which have previously shown to improve cell proliferation and osteogenic differentiation.

P14

The negative impact of cosmetics

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Cosmetics are widely used by infants, children, adults and workers of salon beauty with different purposes. However, they can cause adverse effects due its composition. This review is focused in the potential adverse effects to the human and other organism's health and to the environment of some of the ingredients used in the formulation of cosmetics. In order to prevent these harmful effects, there are also alternatives to the potential hazardous substances that should be taken in account by consumers and manufactures when choosing a product in order to enhance the quality of life, avoiding health risks.

Keywords: cosmetics; health; environment; formulations

P15

New biotechnological perspectives for identification of Antioxidant Peptides from biodiversity: Case study of Antioxidin-I

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Introduction. The amphibian skin plays an important role protecting the organism from external harmful factors such as microorganisms or UV radiation. Based on biorational strategies, many studies have investigated the cutaneous secretion of anurans as a source of bioactive molecules.

Material and Methods. By a peptidomic approach, a novel antioxidant peptide (AOP) with *in vitro* free radical scavenging ability was isolated from *Physalaemus nattereri*. Characterization of the antioxidin-I was determined by RP-HPLC and MALDI-TOF/MS systems. Design of specific oligonucleotides targeting relative gene expression assays by means of RT-PCR. DFT calculations were performed to optimize the structures. Peptide was assembled solid-phase peptide synthesis assisted with microwave. Cell viability assay was carried out using NIH-3T3. Hypoxia-induced ROS production in living microglia with the microglial cell line CHME3 by transfection with a plasmid encoding for the large T antigen of SV40.

Results and conclusion. The gene encoding the antioxidin-I precursor was expressed in the skin tissue. Antioxidin-I presented a low cytotoxicity and suppressed menadione-induced redox imbalance when tested with fibroblast in culture. In addition, it had the capacity to substantially attenuate the hypoxia-induced production of ROS when tested in hypoxia exposed living microglial cells, suggesting a neuroprotective role for this peptide. The bioprospection of this class of molecules is of great interest due to its wide applications in different industries, including cosmetics or food industry to control lipid oxidation that produce undesirable off-flavors and potentially toxic reactions products. Moreover, they might also be used as leading templates for designing novel molecules for preventing neurodegeneration. AP is grateful for the FCT grant (SFRH/BD/97995/2013) financed by POPH-QREN (subsidized by FSE and MCTES).

Key-words: Antioxidants, bioactive peptides, microglia, neuroprotection.

P16

Development of an electrochemical biosensor for an Alzheimer biomarker detection in Point-of-Care

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Introduction: Alzheimer's disease (AD) is the dominant dementia condition of our society with great social and economic impact. One of the major pathological changes is the formation of extracellular insoluble deposits of β-amyloid protein (Aβ) in plaques. Pathological changes are often accompanied by neuroinflammation, abnormal activity of the neural network, dysfunction, degeneration and loss of neurons.

Moreover, there is no current test capable of providing accurate diagnosis of AD and, besides that there is no treatment of prevention available, only a way to delay its progression, so the early diagnosis is the key in present. Research activities targeting such possibility include the identification of biomarkers in several biological fluids that may turn out an important means to diagnosis within future, mostly if these are found in peripheral blood (avoiding more invasive procedures).

Thus, the main goal of this proposal is to develop novel, low cost (bio)sensing-devices with an aptamer as a biorecognition element of AD biomarker detection, Aβ42, in point-of-care.

Material and Methods: The electrochemical measurements were conducted with a potentiostat/galvanostat from Metrohm Autolab and a PGSTAT302N, equipped with a FRA module and controlled by Nova software. The development of the biosensor makes use of two different strategies: covalent and physical adsorption of the aptamer probe onto a carbon screen printed electrode (SPE), modified by drop-casting biographene, on work electrode area. Then, the aptamer modified electrode was incubated with L-asparagine to prevent unspecific links.

The surface modification and the ability of the biomaterial to rebind Aβ42 oligomers was measured by electrochemical techniques, namely electrochemical impedance spectroscopy (EIS), square wave voltammetry (SWV), and cyclic voltammetry (CV). RAMAN analysis was performed in order to control the surface modification of the carbon electrode.

Results and conclusion: The biorecognition element was successful immobilized with different strategies: covalently and physically adsorbed on the modified SPE with a biocompatible nanostructured material, biographene. The control of the surface modification were evaluated electrochemically and by RAMAN techniques. Further tests are progressing to couple the target analyte and the aptamer modified biosensor.

Keywords: Alzhemier's disease ; Aβ-42 ; electrochemical biosensor.

P17

Development of an anti-adhesive coating based on a marine bacterium-produced polymer – towards industrial application

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Introduction: Hospital-acquired infections arising from bacterial accumulation on indwelling medical devices are still a major challenge for society. These infections are frequently related to biofilm development being difficult to eradicate. Current treatment consists in high antibiotic dosages and device removal. Cost-effective and non-toxic natural polymers arise as an alternative promising solution. Cyanobacteria are excellent producers of extracellular polymeric substances (EPS) with high application potential. Therefore, a cyanobacterial polymer (EPS) was evaluated as a bacterial anti-adhesive coating for preventing catheter-related infections. The aim of the current work is to (i) scale-up cyanobacterium culture conditions and (ii) optimize coating production under industrial-like processes.

Methods: The cyanobacterium culture was scaled-up from 1L to 5 L bioreactors. The EPS secreted to the medium was extracted and purified. For EPS based-coating linkage to substrate (medical grade polyurethane used in catheters) different strategies were tested: polydopamine layer (pDA) application and plasma and ozone surface activation under industrial environment. In addition, distinct coating techniques were performed such as dip-coating and spin-coating. The obtained coated surfaces were characterized before and after accelerated coating degradation conditions. Finally, bacterial anti-adhesive performance was tested.

Results and Conclusions: Culture scale-up did not affect cyanobacterial growth and EPS production. Different techniques produced coatings with low wettability and good stability (particularly with pDA layer and plasma treatment). Preliminary assays showed low adherence of *Staphylococcus aureus* in the tested coatings. Nevertheless, further optimization is being carried out in order to infer the best technique for the industrial application of this natural polymer as a coating on catheters.

Keywords: Anti-adhesive coating, Biofilm, Industrial, Natural polymer

P18

Bioactivity screening of marine cyanobacteria for the isolation of novel compounds for hepatic steatosis

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Introduction: Nonalcoholic fatty liver disease (NAFLD) is a common chronic liver disease and effective therapeutics has been widely study. Cyanobacteria, previously known as blue-green algae, are known to produce many secondary metabolites including toxins, with applications to the pharmaceutical industry. The aim of this study is to investigate the effect of extract from cyanobacteria to reduce lipid accumulation in a model of hepatic steatosis using HepG2 cells in vitro.

Material and methods: HepG2 cells were treated with 62 µM oleic acid (OA) to induce lipid accumulation and co-exposed to cyanobacterial extracts. 6 hours later, cells were stained with Nile Red (1:400) to quantify lipid levels in cells in a fluorescence plate reader. Subsequently, sulforhodamine B (SRB) assay was performed to the same cells to measure cellular protein content that indicates the toxicity of the compounds. To validate our protocol, we exposed HepG2 cells to resveratrol (REV) at 25 and 50 µM. Extraction of cyanobacterial biomass was performed with hexane, ethyl acetate and methanol, resulting in fractions A, B and C respectively. These fractions were prepared in dimethyl sulfoxide (DMSO) in a concentration of 10 mg/mL.

Results and conclusions: In the present study, an assay was optimized to quantify the reduction of lipid accumulation in HepG2 cells. REV reduced the lipid level about 20%, to similar values as described in the literature. Extracts from 27 cyanobacteria strains were tested and positive results were obtained in some fractions from different cyanobacteria. In the ongoing work, we intend to isolate novel bioactive compounds to elucidate their structures.

Acknowledgement: This work was supported by the European ERA-NET Marine Biotechnology project CYANOBESITY (ERA-MBT/0001/2015), financed by national funds through FCT (Fundação para a Ciência e a Tecnologia, Portugal).

Key words: Steatosis; Cyanobacteria; HepG2 cells; Bioactive compounds

P19

Anomalias Citogenéticas associadas ao aumento da Translucência da Nuca

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Introdução: A translucência da nuca (TN) traduz-se numa zona hipoecogénica de líquido acumulado no triângulo posterior do pescoço do feto, avaliada entre as 11 a 13 semanas gestacionais, aumentada se ≥ percentil 95 (p95). É o marcador ecográfico de maior sensibilidade no rastreio de anomalias cromossómicas, sendo associado o seu aumento a achados citogenéticos como Trissomia 21,18 e 13, Monossomia do X e triploidias.

Objetivos: Determinar a prevalência de anomalias citogenéticas em fetos com TN acima do p95 no rastreio ecográfico, referenciadas num período de 20 anos para estudo no Centro de Genética Médica Doutor Jacinto Magalhães.

Metodologia: Efetuou-se um estudo descritivo transversal de 927 amostras de fetos referenciadas para estudo citogenético por TN acima do p95 entre janeiro de 1997 e dezembro de 2016. Determinaram-se as frequências absolutas e relativas das variáveis em estudo e realizou-se uma comparação de proporções para verificar a relação entre o aumento da TN e da incidência de anomalias citogenéticas.

Resultados: Das 927 amostras de fetos com TN aumentada, 11,4% apresentaram anomalias cromossómicas. Verificou-se que a proporção de cariótipo anormal nos fetos com TN entre o p95 e 99 (0,014) é significativamente inferior à dos fetos com TN acima do p99 (0,048). A Trissomia 21 foi a anomalia mais prevalente com 63,2%, seguida da Trissomia 18 (10,4%) e da Trissomia 13 (5,7%).

Conclusão: Concluiu-se que existe uma associação entre o aumento da TN e cariótipos anormais, consolidando a medida da TN como um marcador eficaz no rastreio de alterações cromossómicas.

Palavras-Chave: TN, Anomalias Citogenéticas, Rastreio Ecográfico.

P20

Estudo de dois *hotspots* mutacionais no gene *TTN* em doentes com miopatia

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As doenças neuromusculares são um grupo heterogéneo entre as quais se destacam as miopatias de origem genética, associadas a variantes patogénicas no gene da titina (TTN). Este gene não é totalmente analisado na rotina laboratorial, contudo recentemente têm sido descritas diversas variantes associadas a diversos fenótipos. Este estudo teve por objetivo rastrear dois hotspots mutacionais nucleotídicos no gene TTN: um correspondente ao exão 343, em doentes diagnosticados com miopatia progressiva, e outro localizado no intrão 359 (c.106531+1G>A), em doentes com miopatia congénita e miopatia progressiva, em doentes referenciados ao Centro de Genética Médica Jacinto Magalhães (CGMJM).

Para estudar o exão 343 usou-se sequenciação convencional (método de Sanger) e pesquisou-se a variante c.106531+1G>A por análise de alta resolução de curvas de melting (hrMCA). Neste estudo foi identificada uma variante patogénica, c.95195C>T (p.Pro31732Leu), no exão 343 do gene TTN em dois doentes, sugerindo que o rastreio deste hotspot é útil em doentes que apresentem falência respiratória precoce, miopatia e corpos citoplasmáticos na biópsia muscular, sintomas caraterísticos de Miopatia Hereditária com Insuficiência Respiratória Precoce (HMERF). Concluiu-se que o rastreio do exão 343 apresenta utilidade clínica, tendo sido dois casos de HMERF em Portugal. Relativamente à variante c.106531+1G>A, que é extremamente rara, não foi detetada em qualquer doente. A técnica utilizada revelou-se bastante sensível e reprodutível além de ser a opção menos dispendiosa, pelo que evidencia ser uma metodologia a escolher para pesquisa desta variante como teste de rastreio.

Palavras-chave: Titina; HMERF; Miopatia Congénita; Sequenciação

P21

Probiotic properties of lactic acid bacteria from unconventional origin

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Introduction: Recently, probiotics have been evaluated for use in different medical and pharmaceutical fields. Potential medical benefits of probiotics include management of lactose intolerance, improved digestion, lowering of cholesterol and resistance to infections of the gastrointestinal tract. Also, anti-inflammatory, anti-obesity, anti-diabetic and antihypertensive effects have been demonstrated for probiotics. Traditionally, isolation and selection of potential probiotic bacteria have been achieved from feces, breast milk, and dairy products. Nowadays there is considerable interest in new probiotics from non-conventional sources. Particularly, probiotics of vegetable origin are actually of great interest due to the growing demand for non-dairy probiotic products. In this work, we present a preliminary study on the probiotic potential of two different *Lactobacillus* strains isolated from ripe fruits.

Materials and Methods: Isolation was done on MRS agar. Identification was carried out by sequencing the 16S rDNA gene. Isolates were screened on the basis of their probiotic attributes: acid, phenol and bile salt tolerance, resistance to various enzymes, hydrophobicity, auto-aggregation ability, bile salt hydrolysis capacity and antioxidant and antimicrobial activities.

Results and conclusions: Two different strains of lactic acid bacteria (LAB) isolated from fruits were identified as belonging to the genus *Lactobacillus* and screened for their probiotic potential. Both strains showed good growth capacity and survival under simulated gastrointestinal conditions (acidic pH of 3 and 2,5 and 0,3% of bile salts), being as well resistant to the treatment with lysozyme, pepsin, and pancreatin. The studied strains exhibited also good ability to auto-aggregate, which may contribute to their adhesion to host cells. In addition, they showed bile salt hydrolase activity that plays an important role in the reduction of cholesterol. Antioxidant and particularly antimicrobial activities against different pathogens were also demonstrated for the analysed strains. The results indicate that the new LAB isolates have good potential to be used as probiotics.

Key words: probiotics, *Lactobacillus*, non-conventional origin

P22

Devlopment of New GPE Conjugate with Applications in Neurodegenerativa Diseases

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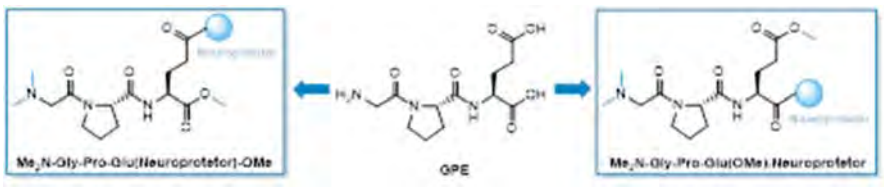
Neurodegenerative diseases result in progressive degeneration and/or death of neurons. This fact can affect movement (ataxias) or brain functioning leading to dementia, with an estimated 48.1 million people in the world suffering from dementia by 2020. Glycyl-L-prolyl-L-glutamic acid (**GPE, Scheme 1**) is a neuropeptide obtained by the *N*-terminal cleavage of insulin-like growth factor 1 (IGF-1), which is found in brain tissue. Although its *modus operandi* remains unknown, *in vitro* and *in vivo* studies have demonstrated that this tripeptide is capable of stimulating the release of acetylcholine and dopamine and acting as neuroprotective against several neurotoxic agents. However, GPE displays unfavorable pharmacokinetic properties, namely low oral absorption.

Thus, the main objectives of this work are to synthesize and evaluate novel conjugates of GPE by increasing its lipophilicity by masking exposed functional polar groups (amine and carboxylic acid) and coupling with neuroactive amines to create a synergic effect of neuroprotective action while increasing the permeability through the blood-brain barrier of the conjugates, potentiating their pharmacological action. Since it is known that GPE is metabolized by carboxypeptidases (from *C*-terminal to *N*-terminal), glutamate functionalization was initially performed by peptide coupling with aminoindane (metabolite with neuroprotective properties obtained from Rasagiline, which is used in the treatment of PD), memantine (used in the treatment of AD) and amantadine (used in the treatment of PD). Subsequently, the functionalized glutamates were coupled to the remaining GPE residues using a one-pot method of peptide synthesis developed by our research group.

The proposed synthesis is now successfully completed, yielding in good yields, 12 final compounds which are now in the biological evaluation step in i3S to determine their potential neuroprotective potential using the SH-SY5Y neuronal cell line under conditions of H_2O_2-induced oxidative stress.



Scheme 1: Chemical structure of GPE and global structure of its conjugates with neuroactive amines.

Key words: Neurodegenerative diseases; GPE; GPE conjugates; pseudopeptides.

P23

Serine-based surfactants active against antibiotic-resistant bacteria

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Bacterial resistance to antibiotics has been a recognized reality almost since the dawn of the antibiotic era, but only within the past twenty years has the emergence of dangerous, resistant strains occurred with a disturbing regularity. As no new antibiotics have been developed in the last twenty years, existing drugs are quickly becoming ineffective.

We have previously developed biocompatible serine-based surfactants whose ability to form liposomes has been demonstrated. Following these findings, we decided to evaluate antibacterial activity *in vitro* of the serine-based surfactants, as this may be the basis for a novel strategy to fight infection: encapsulation of antibiotic drugs in liposomes that show antimicrobial properties *per se*.

Four serine-based surfactants were synthesized and their antimicrobial activity against Gram negative and Gram positive bacteria was determined using a standard microdilution method. Minimum Inhibitory Concentration (MIC) values were determined according to the methodology of the Clinical Laboratory Standards Institute (CLSI), against both antibiotic-sensitive and resistant bacterial strains. The influence of surfactant concentrations on bacterial growth rate was also evaluated.

The tested surfactants were active against antibiotic-resistant Gram positive and Gram negative bacteria, which holds great promise on their future contribution towards antimicrobial stewardship, either as good alternatives to classic antibiotics or, more likely, as antibiotic nanocarriers with intrinsic antibacterial activity.

Keywords: surfactants, nanocarriers, bacterial resistance, antibacterial activity.

P24

Diabetic neuropathy and oxidative stress: a systematic review focused on oxidative stress biomarkers analysis.

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Introduction: Diabetic neuropathy is a worldwide disease with great impact in modern society. Diabetes may lead to the overproduction of reactive oxygen species, resulting in an imbalance in body's redox homeostasis. Oxidative stress is pointed as an important phenomenon associated with several disease states, including diabetic neuropathy.

Material and Methods: A PubMed search using the MeSH terms "Oxidative Stress" [AND] "Diabetic Neuropathies" was conducted, with no linguistic restriction, to collect the studies that relate oxidative stress and diabetic neuropathy between 1994 and 2017. The main inclusion criterion was the abstract, including keywords, presents oxidative stress and diabetic neuropathy or related words. Being a review article or articles that were not written in English were exclusion criteria.

Results and Conclusions: The search originated 310 studies and 189 had matched the first inclusion criterion. From those, 53 are reviews and 4 are not written in English. 2008 and 2015 are the years with more publications (n=27) and in the last 5 years were published 105 papers, representing almost 1/3 of the total. Mice were the preferred biological model (n=100), thus Wistar (n=37) and Sprague-Dawley (n=37) were the most used. Lipid peroxidation, an oxidant biomarker (n=54), and the enzyme superoxide dismutase (n=32), an antioxidant biomarker, were the biomarkers assessed more frequently. Thus, this data search indicated that the evaluation of oxidative stress biomarkers may have clinical significance and may be useful in diabetic neuropathy diagnostic, representing a possible relation between both and having valid biotechnological interest/application.

Keywords: diabetic neuropathy, oxidative stress and oxidative stress biomarkers.

P25

Identification of new candidate genes for retinopathy in type 2 diabetics

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Introduction: The cases of diabetes mellitus (DM) are constantly increasing worldwide. Therefore, early detection programs and the development of validated biomarkers are necessary to transfer molecular and genetic studies to the clinic, to prevent the presentation of DM and the complications derived from the chronicity of the process, including diabetic retinopathy. Although in the etiopathogenesis of DM2 various mechanisms are involved, such as inflammation, angiogenesis, and apoptosis, the genetic factor is essential to complete the knowledge of the molecular base of this disease.

Objectives: To identify genes involved in the pathogenic mechanisms of non-proliferating diabetic retinopathy (DR), such as oxidative stress, alteration of the extracellular matrix and / or apoptosis, to assess the risk of developing it in a population of type 2 diabetics (DM2).

Material and Methods: This study was carried in 81 participants, both genders and ages 25-85 y, classified in: 1) group DM2 (n = 49), with DR (+ DR ; n = 14) and without DR (-DR; n = 35), and 2) control group (GC; n = 32). A personal interview, ophthalmological examination and blood extraction were performed and processed to analyze the DNA and determine the gene expression of: TP53, MMP9 and SLC23A2 in all the participants. It was used SPSS v22.0 to perform statistical analyses.

Results: The TP53 and MMP9 genes increased their expression in the DM2 group compared to the GC, although only significantly in the MMP9 gene (TP53: 10.40 ± 1.20 vs. 8.23 ± 1.36, p = 0.084; MMP9: 1.45 ± 0.16 vs. 0.95 ± 0.16, p = 0.036) and the SLC23A2 gene significantly decreased its levels in DM2 vs. GC (5.58 ± 0.64 vs. 11.66 ± 1.90, p = 0.026). When subdividing the DM2 group according to the presence of DR, the expression of the TP53, MMP9 and SLC23A2 genes showed significant differences between the DM2-DR, DM2 + DR and GC groups (TP53: 9.95 ± 1.47 vs. 11.52 ± 2.05 vs. 8.23 ± 1.36, p = 0.038, MMP9: 1.47 ± 0.20 vs. 1.41 ± 0.27 vs. 0.95 ± 0.16, p = 0.021; SLC23A2: 5.61 ± 0.77 vs. 5.51 ± 1.21 vs. 11.66 ± 1.90, p = 0.018).

Conclusions: The regulatory genes of apoptosis (TP53) and extracellular matrix integrity (MMP9) could be involved in the susceptibility for the development / progression of DR, as well as the SLC232A2 gene (transporter of ascorbic acid) can behave as a risk protector for this pathology.

Keywords: Diabetic Retinopathy; Genes; Molecular Diagnosis

P26

Dermocosmética: qualidade na produção e análise comparativa

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Introdução: Os cosméticos podem ser utilizados na higiene pessoal ajudando a melhorar a aparência. Para a sua produção, é necessário o cumprimento de normas específicas que garantam a qualidade do produto, pelo que o design da estrutura onde estes são produzidos é um fator determinante.

Material e métodos: Realizou-se primeiramente o planeamento e design de uma sala funcional para a produção de dermocosméticos. Em seguida, foram realizados testes comparativos de um sabão líquido de mãos e de um gel de banho produzido pela A_2BRIOS com outros produtos semelhantes. Assim, após aprovação da comissão de ética local, foi pedido a voluntários do curso de Biotecnologia Medicinal, que (i) não estejam grávidos ou a amamentar, (ii) que não possuam historial de reações adversas pelo uso de produtos cosméticos de higienização, (iii) que não estejam a utilizar medicamentos que possam produzir uma resposta cutânea anormal e (iv) que não apresentem doenças dermatológicas localizadas, para realizarem uma avaliação quantitativa e qualitativa, através de um questionário, confidencial, acerca de uma amostra cega do grupo de géis de banho e de sabonetes líquidos de mãos em teste, que inclui a avaliação da hidratação da pele dos voluntários, com o auxílio de uma sonda não invasiva, antes e após a lavagem das mãos com sabão líquido de mãos ou gel de banho, e análise do cheiro, cor, textura, efeito na pele, sensação de hidratação e remoção de sujidade aparente do produto em teste. Adicionalmente, foram determinados os valores de pH, viscosidade, produção de espuma e tensioativos aniónicos. Os dados foram analisados estatisticamente.

Resultados: Espera-se obter uma sala funcional, que evite contaminações cruzadas, assegurando a qualidade dos produtos cosméticos a serem produzidos na empresa e que estes agradem os consumidores em geral.

Conclusão: Em suma, espera-se, contribuir para a determinação da perceção do consumidor relativamente aos produtos cosméticos testados e, assim, poder ajustar a razão qualidade/preço, um fator de relevante importância na indústria cosmética e, em particular, para a A_2BRIOS.

Palavras-chave: Dermocosmética, gel de banho, sabonete líquido

P27

Anticancer potential of spores of the soil fungus *Pisolithus tinctorius*

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INTRODUCTION : Cancer is one of the major causes of death worldwide being the search for new anticancer drugs essential for the treatment of this disease. The sporocarps of the soil fungus *Pisolithus tinctorius* contain pisosterol, a triterpene that has been shown to have antitumor activity against some cancer cell lines. Nevertheless, no studies have focused on the anticancer potential of other structures such as spores, and so the anticancer potential of *P. tinctorius*, remains largely unknown. The main objective of this study was to evaluate the potential of *P. tinctorius* spores as a source of anticancer compounds

MATERIALS AND METHODS: A crude extract of spores was prepared with dichloromethane/methanol (DCM/MeOH-2:1). From the crude extract, 11 fractions were prepared with increasing polarity. The viability of the colon adenocarcinoma cell line RKO, breast carcinoma cell line T47D, osteocarcinoma cell line MG63 and the normal cell hCMEC/D3 exposed to crude extract and fractions was assessed by the 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide reduction assay.

RESULTS AND CONCLUSIONS: The results concerning the cytotoxicity of the crude extract showed that this extract was able to inhibit cell viability in all cancer cell lines. The concentration test of 0.1 mg/ml showed a reduction in cell viability of around 95% in all cancer cell lines in the fractions D, F, G and H, without significant reduction in viability of hCMEC/D3 cells. *P. tinctorius* spores exert cytotoxic activity in cancer cell lines, while having little effects on normal cells, which highlights their anticancer potential. Further studies are on-going such as the evaluation of the effects of different concentrations of the fractions in the cancer cell lines and the identifications of the compounds with anticancer activity that are present in the obtained *P. tinctorius* fractions, more specifically, in the fractions D, F, G, and H.

Keywords: *Pisolithus tinctorius* spores; Crude extract, Fractions, Anticancer potential; Cytotoxicity

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P28

Evaluation of the bacterial activity of natural compounds

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Introduction: Medicinal plants with therapeutic properties are an important source of new biologically active compounds. They present great potential for therapeutics and prevention applications. The use of plant extracts and phytochemical products, both with known antimicrobial properties, is of great importance in the treatment of infectious diseases, where resveratrol, propolis and chamomile stand out.

Material and methods: The bacterial activity of resveratrol, propolis and chamomile was assessed. Firstly, was performed the compounds extraction in ethanolic medium at 70 and 30ºC and in aqueous medium at 100 and 80ºC. The crude extract was dried in a rotary evaporator at 40ºC for ethanolic extractions and at 60ºC for aqueous extractions. Bacterial activity was evaluated based on CLSI methods for antimicrobial susceptibility tests with modifications, using 5 strains, one gram-negative and 4 gram-positive strains, for both crude and concentrated extracts. It was assessed the size of the inhibition halo after 24h at 37ºC.

Results: Resveratrol extract presented the highest inhibition halos, indicating increased anti-bacterial activity. The anti-bacterial effect was higher in concentrated extracts, in both ethanolic and aqueous extracts, than in the respective crude extracts. The results of the aqueous and ethanolic extracts of propolis and chamomile were not satisfactory, since the inhibition halo was inferior than the resveratrol extracts, demonstrating a lower anti-bacterial activity. However, for these two compounds, the concentrated extracts showed a higher inhibition than the crude extracts.

Conclusion: Thus, resveratrol was the compound that showed a higher anti-bacterial activity, demonstrating its potential for the use at an industrial scale.

Keywords: anti-bacterial activity, medicinal plants, natural compounds, resveratrol.

P29

Development of a biosensor for a biomarker CA15-3 in breast cancer by electropolymerization of pyrrole

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Introduction: Breast cancer is a disease that affects millions of people in the world and a constant search for the reduction of its incidence is necessary. If a global cure is not possible, methods must be developed to allow an early diagnosis, thereby reducing the level of disease progression. In the present study, we aim to obtain a molecularly-imprinted polymer (MIP) for the detection of a breast cancer biomarker (CA15-3). The imprinted sites are generated by the electropolymerization of pyrrole, yielding a conducting polymer.

Material and Methods: In a first stage, MIP and control (non-imprinted polymer, NIP) materials were synthesized on carbon Screen Printed Eletrodes (SPEs), and evaluated by electrochemical measurements (Cyclic Voltammetry, CV, and Electrochemical Impedance Spectroscopy, EIS). For the preparation of the imprinted material, the SPEs were first cleaned using 100 mM H_2SO_4, followed by incubation of 5 KU/mL CA15-3 (MIP), for 30 minutes, in a humid environment. The was made in the same conditions as the MIP, without the presence of the biomarker. Electropolymerization with Pyrrole monomer was made in both SPEs, and the removal of the protein was performed with 100 mM H_2SO_4. Every step of the imprinting and removal was followed by measuring CVs and EIS using an Iron redox probe. Different pyrrole concentrations (5.0, 7.5 50.0, 75.0, 80.0, 85.0, 100.0 mM) were tested by electropolymerization to determine the best condition to use during this work. In a second stage, calibrations were performed with increasing concentrations of CA15-3.

Results and Conclusions: The obtained results regarding the pyrrole concentration study showed that the optimal concentration for this work was 85 mM. With 50 and 75mM the obtained polymer was too resistant, whereas with higher concentrations the polymer was too conductive. This work is in progress now, with calibrations being made to understand the response of the sensor. These calibrations, are made with increasing concentrations of CA15-3 and it is expected that there will be a linear increase in resistance until saturation. This type of early diagnosis using MIPs aims to reduce the inconveniences associated with other detection methods, which are invasive and costly.

Key Words: Breast Cancer, MIP, Biomarkers, Pyrrole

P30

Development of a biossensor for prostate cancer using sarcosine as biomarker

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Introduction: Prostate cancer is the most common type of tumor disease in men, making the development of new methods that allow an earlier detection extremely important. One of these methods concerns the use of biosensors to diagnose specific biomarkers for this type of cancer. Biomarkers could be amino acids, proteins or nucleic acids. In this work, the amino acid *sarcosine* was selected for biosensor development, making use of a molecularly-imprinted polymer (MIP) as biorecognition element. In healthy persons, sarcosine is not present or occurs in negligible concentrations in urine in heathy individuals, but individuals with prostate cancer are expected tohave higher concentrations of sarcosine. In turn, growing interest in the integration of MIP materials in biosensors has led researchers to design novel formats for electrochemical sensors. MIPs are a class of cross-linked polymers with specific recognition sites that are complimentary in shape, size and binding groups to the template.

Material and Methods: MIPs and control (non-imprinted polymers, NIPs) materials were developed in bulk, onto a carbon support (carbon black), by free radical polymerization, using acrylamide, *Bis*-acrylamide and vinylphosphonic acid as monomers. The absence/presence of radical initiators (APS and TEMED) were also evaluated.

The polymerized samples were analyzed by FTIR-ATR and RAMAN spectroscopy. The sarcosine rebinding on MIP are first analyzed using UV/Vis and chromatographic techniques. Finally, MIP materials will be inserted in a direct methanol fuel cell (DMFC), and calibrations with sarcosine will be performed.

Results and Conclusion: FTIR-ATR and Raman results evidenced that polymerization onto carbon black was successful. The use of radical initiators seemd to improve the polymerization, according the I_D/I_G ratio obtained by Raman spectroscopy studies. Detection conditions for sarcosine are currently being studied and optimized.

Key words: Prostate cancer, sarcosine, biomarkers, MIP

P31

Dual-action peptides as potential novel topical agents for treatment of skin and soft tissue infections

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Chronic skin and soft-tissue infections (SSTI) such as diabetic foot ulcers (DFU) exhibit signs and symptoms that are consistent with localized bacterial biofilms that contribute to tissue destruction, delayed wound-healing and other serious complications. As such, most current approaches for advanced wound care aim at providing antimicrobial protection to the open wound together with a matrix scaffold (often collagen-based) to boost reestablishment of the skin tissue. While efficient production of recombinant human collagen remains an unmet goal, an alternative sensible option may be the design of formulations containing collagen-boosting instead of collagen-like components. Actually, collagen-boosting peptides, e.g., Matrikines®, are already used in cosmetics to promote extracellular matrix production, rebuilding structure and restoring all functions of healthy skin. Additionally, many antimicrobial peptides (AMP) can also act as wound-healing peptides, thus displaying the dual antimicrobial and tissue-regenerating properties highly desired in novel topical agents for treatment of SSTI. With the increasing prevalence of multi-drug resistant bacteria, and considering the burden that DFU alone represents to human health and healthcare facilities, the development of novel topical agents for effective treatment for this and other severe SSTI is an urgent need.

In view of the above, our group has recently started a research project focused on the chemical synthesis, in vitro and in vivo evaluation of conjugates where collagen-boosting peptides, like KTTKS (Matrixyl®), are linked to AMPs aiming at dual-action peptides with potential interest as topical agents for the treatment of SSTI. Preliminary results thus far obtained will be communicated.

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P32

Peptides of the skin secretion from *Leptodactylus vastus* (Amphibia): Biotechnological applications as antioxidants

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Introduction: The cutaneous secretion of amphibians has bioactive compounds, such as peptides, with potential for biotechnological applications. Therefore, this work aimed to isolate and determine the primary structure, as well as to investigated peptides obtained from the secretion cutaneous of the *Leptodactylus vastus* frog as a source of bioactive molecules.

Material and methods: *L. vastus* species were collected on the Ilha Grande of Santa Izabel, Parnaiba Delta and their cutaneous secretion was extracted with a small electrical stimulation. The lyophilized total extract was made RP-HPLC on a C18 semi-preparative column and the bioactive peptides were identified by MALDI TOF-TOF and then synthesized by solid-phase chemistry by f-moc system. The secondary structure was determined by circular dichroism. Peptides were screened for antibacterial activity against strains *Escherichia coli* ATCC 25922 and *Staphylococcus aureus* ATCC 25923. The antioxidant potential *in vitro* of the peptide was evaluated against the DPPH and ABTS radical. In addition, *in vivo* antioxidant potential in the mice hippocampus with determination of malondialdehyde (MDA), glutathione (GSH), nitrite, and superoxide dismutase (SOD) levels was evaluated.

Results and Conclusions: The peptides obtained have the following amino acid sequences GVVDILKGAAKDLAGHLASKV and GVVDILKGAAKDLAGH, with corresponding molecular mass of 2062.4 Da and 1563.8 Da. The peptides has poor antimicrobial activity and poor performance in free radical scavenging assays *in vitro*. However, treatment *in vivo* with the peptide reduced nitrite and MDA content, as well as increased SOD activity in the hippocampus of mice. Further studies will be carried out in an attempt to clarify the mechanism of action associated with this peptides, as well as to observe the interaction between the level of oxidative stress and other pathological processes.

Keywords: Amphibia, Skin secretion, Peptide, Reactive oxygen species

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P33

Efficacy Of a Semi Synthetic Compounthe number of leukocytes or neutrophils were expressed d Phenol Derivative as Anti-Inflammatory Agent: New Small Molecule COX Inhibitor Without Gastric Side Effects

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INTRODUCTION: Vanillic acid is a phenolic acid that has efectiveness in the management of inflammatory or immune. In this sense isopropyl vanillate (ISP-VT), has been synthesized from chemical modifications of vanillic acid. METHODS: In vitro methods of analysis of antioxidant activity at various concentrations (0.025-5 mg/mL) were performed. Anti-inflammatory activity of the ISP-VT in the doses 0.3,1 and 3 mg /kg by measuring paw edema induced by carrageenan and phlogistic agents. We evaluated the immunohistochemical for Cox-2 and INOs. In a mouse peritonitis the number of leukocytes or neutrophils were expressed. Aliquots of these exudates were collected for determination of glutathione (GSH), superoxide desmutase (SOD), malondialdehyde (MDA) levels,myeloperoxidase (MPO) activity, cytokine levels (TNF-α and IL-6), cyclooxygenase (COX- 2),was also performed analysis by intravital microscopy of the rolling and adhesion of leukocytes. The gastric toxicity was also evaluated.

RESULTS: ISP-VT has in vitro antioxidant capacity in the dpph, ferrous ion chelation and total antioxidant capacity. Pre-treatment with ISP-VT in the doses 1 and 3 mg/kg ip produced a reduction ($p<0.05$) in paw edema formation at 3 h. Pre-treatment ISP-VT (1mg/kg) effectively inhibited paw edema ($p<0.05$) and phlogistic agents. ISP-VT decreases the immunoblotting for COX-2 and INOS. The ISP-VT exhibit a COX-2 inhibition, and COX-1significanty ($p<0.05$), when compared to carrageenan group. Pre-treatment with ISP-VT significantly reduced the leukocyte migration and neutrophil into peritoneal cavity, and significantly reduced ($p<0.05$) levels of MDA, and nitrite in the peritoneal exudates and significantly increases ($p<0.05$) GSH and SOD levels. ISP-VT pre-treatment significantly reduced the TNF-α and IL-6 levels in peritoneal fluid, compared to the corresponding levels in the carrageenan group. The administration of indomethacin (20mg/kg) promoted a significant ($p<0.05$) gastric lesion and increased MPO activity. However, pre-treatment ISP-VT (100 mg/kg) did not promote gastric damage or change in MPO activity.

CONCLUSION: The results showed that ISP-VT demonstrated anti-inflammatory by decreasing oxidative stress, by acting via inhibiting COX-2. Financial Support: CNPq. Protocol No. 06/14

P34

Mechanisms of Paroxysmal Nocturnal Hemoglobinuria clonal expansion

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Paroxysmal Nocturnal Hemoglobinuria (PNH) is a rare, acquired clonal disease of bone marrow stem-cells, genetically characterized by the somatic mutation of the phosphatidylinositol glycan class A (PIG-A) gene. That leads to defective synthesis of glycosylphosphatidylinositol (GPI) responsible for anchorage and fixation of surface proteins like complement decay-accelerating factor (DAF/CD55) and membrane inhibitor of reactive lysis (MIRL/CD59). These proteins protect red blood cells from lysis by activated complement, leading to intravascular hemolysis.

Despite basis hemolysis in PNH is already explained, mechanisms promoting expansion of the PNH clone have yet to be elucidated. PIG-A mutation is essential, but not sufficient, to cause PNH: rare PIG-A mutations have been found in a very small proportion in healthy individuals. It seems that clonal dominance in PNH occurs due to an intrinsic growth advantage and/or to a imune escape ability of PIG-A mutant cells. PNH clones with multiple mutations are present at substantially higher frequencies than those of clones with only mutations in PIG-A. Decrease of PNH clone expression can also occur concomitantely with spontaneous remission of the disease in 15% of the cases.

The aim of this project is to discover mechanisms of clonal expansion and remission that may be useful in revealing the possible mechanisms of proliferation of PNH clones and developing therapeutic strategies for PNH.

Keywords: Paroxysmal nocturnal hemoglobinuria; PIG-A gene; clonal expansion

P35

PET aplicada à Medicina Moderna: prova de conceito baseada na produção de Titânio-45 (^{45}Ti)

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Introdução – Os paradigmas modernos da Medicina baseada em Evidência e/ou Ciência,bem como os conceitos relacionados com a Medicina Personalizada, têm levado aocrescente uso de meios complementares de diagnóstico, cada vez mais sensíveis eespecíficos. Nesse contexto, as metodologias de imagem médica têm especial relevo.Dentro destas, vários exemplos podem ser destacados, mas a Medicina Nuclear, baseadano uso de radiofármacos, é especialmente importante ao nível do estudo funcional emolecular dos mais diversos processos patológicos.

Material e Métodos – Partindo-se da caracterização das técnicas de imagem em Medicina Nuclear, em especial da Tomografia por Emissão de Positrões (PET), e assumindo que a investigação em Medicina Nuclear visa, habitualmente, a criação de soluções tecnológicas para um problema clínico/científico, será descrito o processo de investigação inerente à produção de um radionuclídeo não convencional para formulação de radiofármacos para uso em PET, como prova de conceito. Várias reacções nucleares foram estudadas para avaliar o potencial de se produzir Titânio-45 (^{45} Ti) em ciclotrões (aceleradores de partículas) de baixa energia. Seguidamente, a produção de ^{45}Ti foi testada num ciclotrão de 18 MeV da IBA ® . Várias potenciais aplicações biomédicas foram ainda exploradas com base em revisão bibliográfica e desenvolvimento de propostas inovadoras.

Resultados e Conclusões – A produção de ^{45}Ti revelou-se possível em ciclotrões de baixaenergia. Por seu lado, o desenvolvimento de radiofármacos marcados com ^{45}Ti parece apresentar potencial, dadas as características físicas inerentes ao decaimento do ^{45}Ti, bem como a possibilidade de formular diversos ligandos com afinidade para o mesmo. Por fim, dado o interesse actual das nanopartículas de dióxido de titânio para fins médicos, propõe-se a marcação directa destas nanopartículas com ^{45}Ti (com recurso ao método do precursor radioactivo). Deste modo, pretende-se apresentar todo o processo inerente à investigação fundamental em Medicina Nuclear com vista à criação de soluções tecnológicas para as mais diversas aplicações biomédicas.

P36

Desenvolvimento e aplicação de modelos biológicos para estudo de efeitos da radiação ionizante

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Introdução - Com o crescente uso médico das radiações ionizantes, tanto ao nível dos meios complementares de diagnóstico (ex: Medicina Nuclear e Radiologia), como ao nível terapêutico (ex: Radioterapia), as preocupações inerentes ao aumento da exposição da população a este tipo de radiações têm aumentado. Apesar dessas preocupações, a literatura carece ainda de evidência científica suficiente para esclarecer a relação entre a irradiação com baixas doses de radiação ionizante e os efeitos despoletados.

Material e Métodos – Ao longo dos últimos anos vários modelos biológicos têm sido desenvolvidos e aplicados para colmatar as carências dos modelos mais tradicionais. Neste trabalho dar-se-á especial importância à descrição dos aspectos técnicos inerentes ao uso de modelos celulares avançados (culturas celulares tridimensionais com técnicas de agar/agarose, encapsulação em alginato e agitação durante cultura) e de um modelo animal aquático (*danio rerio*, peixe-zebra), bem como às técnicas de irradiação actualmente disponíveis para estes fins.

Resultados e Conclusões – Exemplos de trabalhos realizados recentemente com os modelos biológicos avançados aqui em apreço serão apresentados, começando por comprovar a adequabilidade desses modelos. Em geral, a evidência recolhida tem apontado para: i) a existência de uma relação entre as alterações à proliferação cellular induzidas pela irradiação em culturas celulares e a metodologia de cultura; ii) a diminuição aguda (24h) da proliferação celular após irradiação com baixas doses de radiação e a recuperação ao longo do tempo (72h); iii) a redução da expressão proteica ao nível muscular no *danio rerio*; entre outros.

P37

The effect of high concentrations of vitamin C on a H_2O_2 - oxidative stress induced yeast model

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Nowadays, the ageing of population is conducting to a rise in the number of individuals with age-related illnesses, in which neurodegenerative diseases (ND) are included. Regardless of many variations in etiology and diverse mechanisms of cell injury, most of ND share high levels of oxidative stress, which have been highly referred not only as an underlying factor, but also as a feature. Thus, an actual challenge is to evaluate the role of antioxidants on oxidative stress states and evaluate the magnitude of possible therapeutic effects of these agents. In the present study, the toxic effect of hydrogen peroxide (H_2O_2) and the antioxidative function of vitamin C were investigated using Saccharomyces cerevisiae as a model. Results were achieved by exposing the yeast model to different concentrations of H_2O_2 and vitamin C, itself or combined, and colony forming units counting after 48 h incubation at 30ºC in Yeast Extract Peptone Dextrose agar plates. The results demonstrated that H_2O_2 exposure decreased yeast cells viability in a dose-dependent manner and that, at an optimal concentration, vitamin C was able to revert its effects. These results improved the understanding of the reversal effect of antioxidant treatment and, therefore, may be helpful on providing insights on a natural antioxidant-based therapy for ND.

Keywords: neurodegenerative diseases, oxidative stress, Saccharomyces cerevisiae, vitamin C.

P38

Optimizing the expression of different chitinases with antifungal activity

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Introduction: Chitinases are glycosyl hydrolases (EC 3.2.1.14.) that cut the β-1,4-glycosidic bonds present in chitin which is thereby transformed into its oligo- and monomeric components. Many relevant applications have been demonstrated for chitinolytic enzymes being of particular interest their potential use as alternative antifungal compounds. However, successful exploitation of chitinases depends upon the availability of strains and expression conditions that allow the production of active forms in large quantities. *Escherichia coli* is one of the most commonly used hosts for high-level production of heterologous proteins. In this work, we determined the conditions for a high level of active protein production of two recombinant chitinases with antifungal activity: *Hs*ChiA1p (a family 18 archaeal chitinase) and *Pt*Chi19p (a family 19 bacterial chitinase).

Material and Methods: Optimization of production was carried out studying the effect of culture cell density, inducer concentration, post-induction time, induction temperatures, and *E. coli* host strains on the functional expression of the above-mentioned chitinases. Antifungal activity was tested in a plate antagonism assay.

Results and conclusions: According to the results, the effect of each analyzed parameter on the expression of both chitinases was specific to each enzyme. Testing various *E. coli* host strains compatible with the expression in pET systems, differences in the active protein produced were also observed. A significant increment in expression was demonstrated for the active *Hs*ChiA1p chitinase when using BL21 Star (DE3) strain as compared to that found when using BL21 (DE3) strain, indicating that the rne131 gene mutation efficiently stabilizes the mRNA for *Hs*ChiA1p. Rare codon analysis of the chitinase genes revealed that both DNA sequences were not the more convenient for maximal expression in *E. coli*. Different host strains with extra copies of tRNA genes for rare codons were then assayed. A significant increase was reached for the activity of *Hs*ChiA1p and *Pt*Chi19p when Rosetta 2 (DE3) and the BL21 RP (DE3) strains were used. Finally, the best conditions for recovering biologically active protein from inclusion bodies were determined for each enzyme and their antifungal activity demonstrated against *Aspergillus niger*.

Keywords: chitinase, antifungal, enhanced expression

P39

Effects of embryonic exposure to venlafaxine on a zebrafish model

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Introduction: Major depressive disorder affects over 350 million people around the world, and might result from a complex interaction of epigenetic, genetic, environmental and developmental factors. Antidepressants are a class of neuroactive compounds that are used mostly in the treatment of clinically severe mood and anxiety disorders, and can be divided into three major classes: tricyclic antidepressants, monoamine oxidase inhibitors and selective serotonin reuptake inhibitors. The boost on antidepressants' prescription and consumption is related to an increase in the prevalence of psychiatric disorders and knowledge of mental health problems. These are considered emerging pollutants due to their omnipresence at trace levels in the environment. Nonetheless, the lack of knowledge concerning their impact on the environment, and consequently on aquatic species, highlights this topic as a pivotal concern. Even in low concentrations, antidepressants may cause several effects on the aquatic environment as a result of disturbing homeostasis throughout the central and peripheral nervous systems, both in vertebrates and in vertebrates, and by modifying the regulation of neurotransmitters such as serotonin, norepinephrine and dopamine. Zebrafish (*Danio rerio*), an aquatic vertebrate species, is one of the most important model organisms in developmental biology, considered extremely valuable for the study of translational neuroscience of complex human brain disorders, being particularly useful for studying genetic and pharmacological mechanisms of depression and antidepressant action.

Material and methods: In the present study we will be using a developmental zebrafish model with the purpose of evaluating the impact of venlafaxine in zebrafish embryos. Morphological and motor abnormalities, as well as oxidative stress will be evaluated in the most important key points of development.

Conclusion: Since zebrafish embryos are sensitive to venlafaxine it is important to study its impact on the organisms' development. Additionally, this study could bring insight to potential impacts on human development.

Keywords: Depression, Venlafaxine, Emerging Pollutants, Zebrafish

P40

Detecting DNA Polymorphisms using genosensors and molecular biology tools

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Introduction

Warfarin is a commonly used anticoagulant prescribed for patients with chronic atrial fibrillation, deep vein thrombosis, pulmonary embolism, recurrent stroke and prosthetic heart valves. Several factors have been reported to influence therapeutic warfarin dose requirements, including age, vitamin K intake, as well as genetic variants.
Recently pharmacogenomics studies have demonstrated that single-nucleotide polymorphisms (SNPs) in genes involved in warfarin metabolism and action may contribute to interindividual differences in patient's responses to warfarin. Thus, it is crucial to develop methodologies to predict the individual dosage of warfarin.
In this work, two analytical approaches based on molecular biology and genosensors techniques are under development to create a low-cost genotyping platform able to genotype SNPs related with the therapeutic response of warfarin.

Material and Methods

Using qPCR TaqMan assay, the *VKORC1* -1639G>A, *CYP2C9**2 and *3 SNPs (related with the biotransformation and mode of action of warfarin) were genotyped in 204 Brazilian healthy individuals (from Piauí, Northeast of Brazil).
Electrochemical genosensors for the SNPs determination are in development. The design of these genosensors consists on the ssDNA immobilization onto gold surfaces that act as the SNPs complementary probes. The hybridization reaction is performed in a sandwich format of the complementary ssDNA, using an enzymatic scheme to amplify the electrochemical signal.

Results and Conclusions

The study of the genetic variation indicated that the minor allele frequencies (MAF) for the *VKORC1* -1639G>A, *CYP2C9**2 and *3 SNPs were 33%, 10% and 5%, respectively.
The analytical features related to the genosensor development, such as DNA concentrations (DNA probe, DNA signaling and DNA target), temperature and time of the hybridization reactions, electrochemical parameters, are in study and optimization process.

Keywords: Polymorphisms; warfarin; genotyping, genosensors

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P41

Development of a new method for the detection and quantification of octopamine and 3-nitrotyrosine

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Introduction: Neurodegenerative diseases affect around 30 million people worldwide, thus new methods that can facilitate research of such diseases are essential. Neurotransmitters, characterised as critical regulators of several neurological disorders, have been the target for the development of such methods. Besides, oxidative/ nitrosative stress has been associated with several neurodegenerative diseases and is thought to play an important role in their pathogenesis. Therefore, the development of bioanalytical methods for quantification of neurotransmitters and oxidative stress-associated molecules seems to be an interesting approach. The aim of this study was to develop a simple, rapid, low-cost and sensitive octopamine (OCT) and 3-nitrotyrosine (3-NT) quantification method for use in biological models.

Materials and Methods: All experiments were performed on a Hitachi LaChrom Elite® HPLC system and detection was accomplished trough a diode array detector. Chromatographic separation was carried out using a Lichrocart® 250-4 Lichrospher 100 RP-18 (5µm) column. A solution of 0.5% CH_3COOH:MeOH:H_2O (15:15:70, v/v) was used as an isocratic mobile phase with a flow rate of 1 mL/min and at 25ºC. The method was developed and validated according to the International Conference on Harmonisation (ICH) guidelines.
Results: The protocol tested showed specificity for both biomolecules at 276 nm. Using this protocol, the calibration curve was linear (correlation coefficient = 1), the limit of detection (LOD) and the limit of quantification (LOQ) were in the order of ng/mL, and the time required for analysis did not exceed 15 minutes per sample.

Conclusion: The proposed method, which was successfully developed for OCT and 3-NT quantification, is simple, cheap and fast. In a near future, it would be interesting to assess whether this method is suitable for the quantification of OCT and 3-NT in several biological models.

Keywords: Octapamine, 3-Nitrotyrosine, HPLC, Neurodegenerative diseases.

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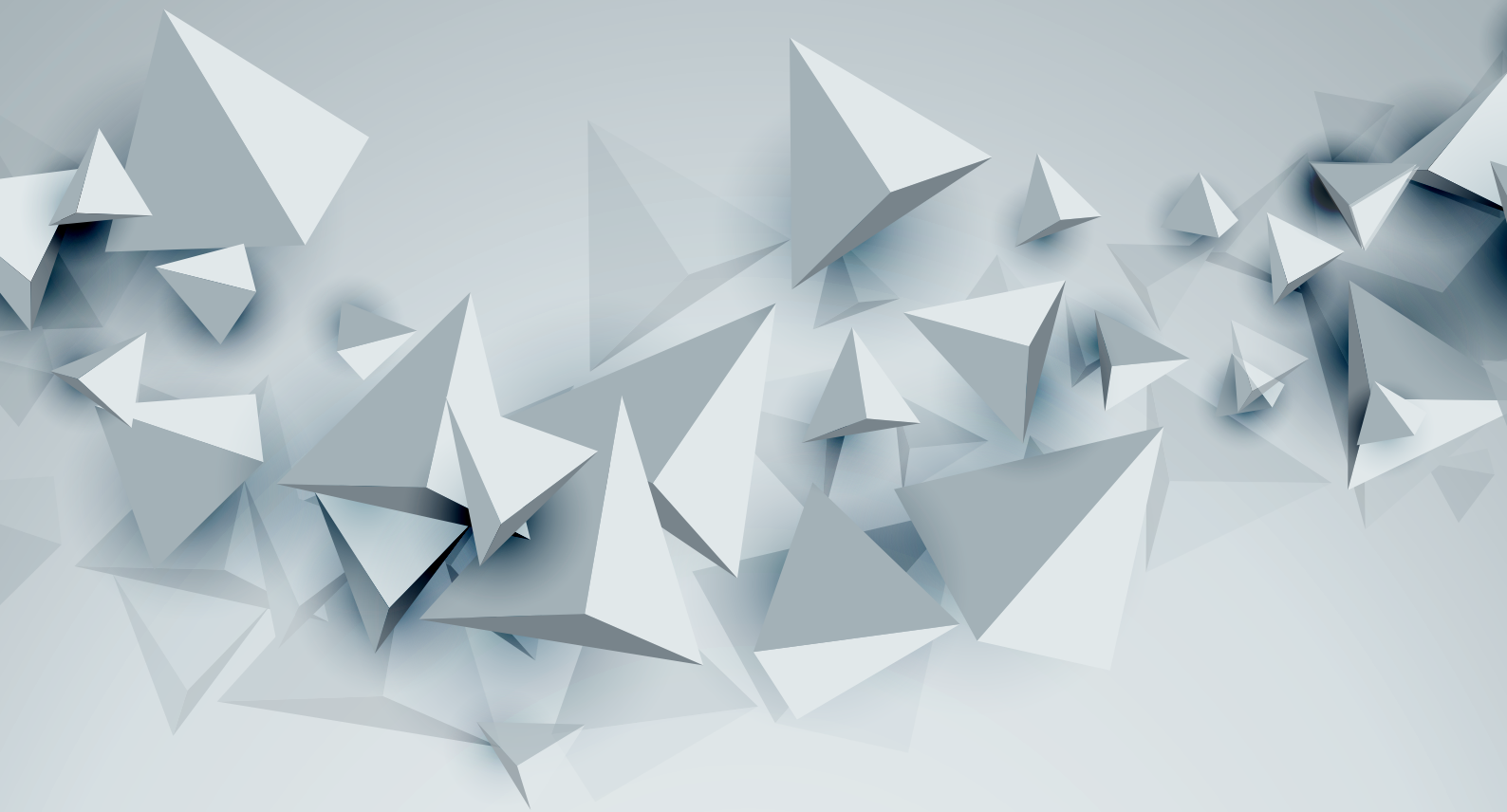
ÍNDICE
INDEX

ÍNDICE
INDEX



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