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# BOOK OF ABSTRACTS

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21 OCT 2022

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# 2ND IBERIAN CONGRESS ON MEDICINAL BIOTECHNOLOGY

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# PROGRAMME

13:30 • Registration

14:00 • Opening Session

14:20 • Invited Speaker
Patrícia Poeta • UTAD

14:50 • Plenary Session
Meritxell Teixidó • Gate2Brain

15:50 • Oral Comunications I
Carmen Sieiro • Moderator

"Bringing together Spent Yeast biomass and Seaweeds to address the Iodine Deficiency Disease" • Elsa Vieira

"Evaluation of the probiotic potential of a strain of *Saccharomyces cerevisiae*" • Lara Areal-Hermida

16:20 • Coffe Break • Poster Session

16:50 • Plenary Session
Simão Soares • P-BIO & Silico Life

17:50 • Oral Comunications II
Cristina Prudêncio • Moderator

"Effects of tropomyosin analysis by a label-free electrochemical immunosensor" • Ricarda Torre

"Calcium-based cements on the regeneration of dental pulp: an *in vitro* study" • Gabriel Kato

18:20 • Closing Session • Awards

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# ORAL COMMUNICATIONS

# Bringing together Spent Yeast biomass and Seaweeds to address the Iodine Deficiency Disease

Elsa F. Vieira[1], Andreia D. M. Silva[1] Sónia A. Figueiredo[1] Tiago Brandão[2] & Cristina Delerue-Matos[1]

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Iodine deficiency disease (IDD) is a global public health problem, which affects both developed and developing countries. According to WHO, IDD is the world's most prevalent, yet easily preventable, cause of brain damage". Although universal salt iodization is an efficient way to treat IDD, it presents chemical disadvantages, which argues the developemrnt of efficient natural iodine-food-carriers. Brown seaweeds are naturally high iodine-enriched foods, but its direct consumption can lead to hyperthyroidism, and frequently there is consumption resistance to its taste, smell, and texture. Taking into in consideration, this work evaluated the potential of brewer´s spent yeast (BSY) – the second major by-product of brewing process, to adsorbe the iodine of three brown seaweeds species: *Himanthalia elongata, Fucus vesiculosus* and *Eisenia bicyclis*.

Seaweed extracts were prepared by Subcritical Water Extraction (90 °C, 100 bar, flow rate of 10 mL/min) and kinetic and equilibrium experiments were performed using non-living (lyophilized) BSY biomass, at pH 4, room temperature and at predefined initial concentrations of seaweed extract and mass of BSY. The quantification of iodine was made by the Sandell-Kolthoff method. The BSY, before and after iodine uptake, was characterized by the determination of the point of zero charge (pHPZC), Fourier transform infrared (FTIR) analysis, and scanning electron microscopy with energy dispersive spectroscopy (SEM/EDS).

Results showed that the Elovich's, pseudo-first-order's, and pseudo-second-order's models were fitted to the experimental kinetic results. The equilibrium was achieved in 5 min and the kinetic constant was 0.65 ± 0.04, 0.45 ± 0.08 and 0.43 ± 0.05 $g_{BSY}/\mu g_{iodine}$·min, respectively, for the *Himanthalia elongata, Fucus vesiculosus* and *Eisenia bicycli*-iodine extracts. The maximum biosorption capacity of *Himanthalia elongata, Fucus vesiculosus* and *Eisenia bicyclis*-iodine extracts by the BSY biomass were, respectively, 35 ± 6 $\mu g_{iodine}/g_{BSY}$, 26 ± 4 µgiodine/gBSY and 23 ± 3 $\mu g_{iodine}/g_{BSY}$.

Overall, these findings suggest the potential of the iodine-enriched BSY as an efficient iodine-carrier nutraceutical for the treatment/prevention of IDD. The mean ingestion of 5.0 g/day of iodine-enriched BSY could satisfy between 35-60% of iodine RDA for adults and around 22-35% of iodine RDA for pregnant women.

**Keywords**: Iodine deficiency disease, inutraceutical, brewer´s spent yeast, seaweeds.

**References:**

Lazarus, J.H. The importance of iodine in public health. Environ Geochem Health. 2015, 37, 605-618; DOI:10.1007/s10653-015-9681-4

WHO/UNICEF/ICCIDD. Assessment of iodine deficiency disorders and monitoring their elimination: A guide for program managers. 3. Geneva: World Health Organization. 2007, https://apps.who.int/iris/handle/10665/43781

02

ORAL COMMUNICATION

# Evaluation of the probiotic potential of a strain of *Saccharomyces cerevisiae*

Lara Areal-Hermida[1], Ángeles Pichardo-Gallardo[1], Pedro Coelho[2], Cristina Prudêncio[2], And Carmen Sieiro.[1]

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According to the World Health Organization (WHO) and the Food and Agriculture Organization of the United Nations (FAO), probiotics are living microorganisms that, when administered in appropriate quantities, can confer a beneficial effect on the health of the host. Probiotic potential has been demonstrated especially for many strains of different species of bacteria. However, there are fewer strains of yeast for which their probiotic properties have been studied, despite their advantages, which include the fact that they are not affected by antibacterial antibiotics or their inability to disperse antibiotic resistance genes. As a result, there is a growing interest in characterizing new yeasts as probiotics. The aim of this study was to evaluate in our laboratories the probiotic potential of a strain of *Saccharomyces cerevisiae* var. *boulardii* (the main species marketed as a probiotic so far), to compare it with that of other yeasts. Strain identification was carried out by analyzing the ITS and D1/D2 regions of the rDNA. Regarding the ability of the strain to survive at 37ºC and resist gastrointestinal conditions, the strain showed 100 % viability at 37ºC and between 95-100 % in synthetic gastric and duodenal juices (pH 2-7.5, 0.3-10 % bile salts, 0.3 % pepsin and 0.1 % pancreatin). In addition, the selfaggregation and hydrophobicity properties shown by the strain allowed inferring the ability to adhere to the intestine, which was confirmed in Caco-2 cells. Additionally, the strain presented other properties of interest for probiotics, including a high antioxidant capacity and pathogen coaggregation, as well as a variable cytotoxic activity against different tumor cell lines.

**Keywords**: Yeast, probiotics, *Saccharomyces*

**References:**

Sanders, M.E.; Merenstein, D.J.; Reid, G. Gibson, G.R.; Rastall, R.A. Probiotics and prebiotics in intestinal health and disease: from biology to the clinic. Nat Rev Gastroenterol Hepatol. 2019, 16, 605–616; https://doi.org/10.1038/s41575-019-0173-3

Staniszewski, A.; Kordowska-Wiater, M. Probiotic and Potentially Probiotic Yeasts-Characteristics and Food Application. Foods. 2021, 10, 1306. https://doi.org/10.3390/foods10061306

# Tropomyosin analysis by a label-free electrochemical immunosensor

Ricarda Torre[1], Maria Freitas[1], Estefanía Costa-Rama[2], Henri P.a. Nouws[1], Cristina Delerue-Matos[1]

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Crustaceans are one of the main food allergens. In many countries legal food labelling requirements obliges the indication of the presence of this type of seafood in commercial products. However, allergic individuals are at risk due to labelling errors caused by inaccurate detection and sometimes fraud. The major crustacean allergen is tropomyosin, a 34-39 kDa protein.

To provide a user friendly and expeditious method that could be useful for food producers, a label-free electrochemical immunosensor for the detection of this allergen was developed. The transducer used in this work was a screen-printed carbon electrode that was modified with a carbon-based material. After the immobilization of a monoclonal anti-tropomyosin antibody and the incubation with the allergen (standard/sample) a redox probe ($[Fe(CN)_6]^{3-/4-}$) was added to record the analytical signal by differential pulse voltammetry. With this approach the analysis time was about ~1h and the results were precise (RSD = 6.5 %). The sensor was selective towards the target allergen and no interferences from other food allergens and ingredients were observed. The linear relationship between the analytical signal and the tropomyosin concentration was found to be between 0.50 and 75 ng $mL^{-1}$ (limit of detection: 0.20 ng $mL^{-1}$). The accuracy of the results was confirmed through recovery assays (81.6 to 102.6 %) and comparison of the results of the analysis of commercial food samples with a conventional ELISA kit.

Based on these results and the fact that the sensing surface is stable for at least one month, the developed sensor is a promising tool for quality control in the food industry.

**Keywords**: Electrochemical immunosensor, Label-free assay, Tropomyosin, Seafood allergy

**Acknowledgements:**

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04

ORAL COMMUNICATION

# Calcium-based cements on the regeneration of dental pulp: an *in vitro* study

Gabriel Kato[1], Cláudia Rodrigues[1], Helena Fernandes[1,2] & Liliana Grenho[1,2]

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Calcium-based cements have been exploited to the management of deep carious lesions, in which a bioceramic cement is placed onto the remaining affected dentin, or directly onto the exposed pulpal tissue after pulpotomy or an accidental pulpal exposure[1,2,3]. Following application of calcium silicate-based cements, several cell types (osteoblasts, mesenchymal cells, and pulpal cells) are involved in the tissue regenerative/mineralizing activity[4,5,6]. The aim of the present study was to evaluate the effect of pulp capping cements in human dental pulp cells (hDPCs), by an indirect contact assay. DPCs (Poietics™ DPSC; Lonza Bioscience, Switzerland) were cultured in osteogenic medium (control) and in the presence of extracts from the cements - Dycal® (Dycal; Dentsply, USA); ProRoot® MTA (MTA; Dentsply, USA), Biodentine™ (Biodentine; Septodont, France), FKG TotalFill® BC RRM™ Fast Putty (FKG; FKG Dentaire SA, Switzerland) and Theracal LC® (Theracal; BISCO Inc., USA). Cells were exposed to the extract' cements for 4, 8 and 10 days and were characterized for cell viability (Resazurin assay) and Alkaline phosphatase (ALP) activity and histochemical staining. The null hypothesis was not having significant differences among exposure to the tested cements, whereas alternative hypothesis was assumed if significant differences were detected after ANOVA multiple comparisons with Tukey post-hoc test ($p \leq 0.05$). Compared to control hDPCs (not-exposed cells), viability of cells treated with the extracts was similar at day 4, but higher at days 8 and 10. Regarding ALP activity, values were significantly higher in the cell cultures exposed to the extract' cements, compared to control hDPCs. Histochemical staining of the cultures showed that cell density increased throughout the culture time, with the cell layer being organized in circular cellular arrangements, typical of osteogenic-differentiating cells, that stained positive for ALP. Therefore, all extracts permitted cell survival and induced ALP activity, an established osteogenic marker, confirming the bioactivity of calcium-based pulp capping cements.

**Keywords**: dental pulp capping, calcium silicate dental cements, dental pulp cells

**References:**

1. Cushley, S.; Duncan, H.F.; Lappin, M.J.; Chua, P.; Elamin, A.D.; Clarke, M.; El-Karim, I.A. Efficacy of direct pulp capping for management of cariously exposed pulps in permanent teeth: a systematic review and meta-analysis. Int. Endod. J. 2021, 54(4), 556-571; DOI:10.1111/iej.13449.

2. Kunert, M.; Lukomska-Szymanska, M. Bio-Inductive Materials in Direct and Indirect Pulp Capping—A Review Article. Materials. 2020, 13, 1204; DOI: 10.3390/ma13051204.

3. Bjørndal, L.; Simon, S.; Tomson, P.L.; Duncan, H.F. Management of deep caries and the exposed pulp. Int. Endod. J. 2019, 52, 949-973; DOI:10.1111/iej.13128

4. Camilleri, J.; Atmeh, A.; Li, X.; Meschi, N. Present status and future directions: Hydraulic materials for endodontic use. Int. Endod. J. 2022, 55, 710-777; DOI:10.1111/iej.13709.

5. Nie, E.; Yu, J.; Jiang, R.; Liu, X.; Li, X.; Islam, R.; Alam, M.K. Effectiveness of Direct Pulp Capping Bioactive Materials in Dentin Regeneration: A Systematic Review. Materials. 2021, 14, 6811; DOI:10.3390/ma14226811.

6. Park, S.-M.; Rhee, W.-R.; Park, K.-M.; Kim, Y.-J.; Ahn, J.; Knowles, J.C.; Kim, J.; Shin, J.; Jang, T.-S.; Jun, S.-K.; Lee, H.-H.; Lee, J.-H. Calcium Silicate-Based Biocompatible Light-Curable Dental Material for Dental Pulpal Complex. Nanomaterials. 2021, 11, 596; DOI:10.3390/nano11030596

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# POSTER COMMUNICATIONS

01

POSTER

# Quantification And Characterization Of Polyphenol Content In Apple Products

José Carlos Teixeira[1], Susana Soares[1], Rosa Pérez[1], Nuno Mateus[1], Victor De Freitas[1]

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Polyphenols result from plants' secondary metabolism and have gained great attention because of their widely proven bioactivities [1,2]. Due to their antiradical activity, can revert oxidative stress and promote defense against cancer, cardiovascular disease, diabetes mellitus, Alzheimer's, and many others[1–3].

Apple has been described with more than 60 polyphenol compounds [1]. Processing techniques would affect their profile in polyphenol compounds[3]. This study aims at understanding the effect of apple processing on the polyphenol profile depending on 1) centrifugation and 2) temperature. The samples were prepared at a national food industry company as follows: from the same raw apple puree, this sample was 1.1) centrifugated at 4400/10 rpm, and 1.2) centrifugated at 4400/35rpm giving turbid juices 1.1. and 1.2. Juice 1.2 was then clarified by filtration at 2.1) 60°C and 2.2) 40°C. To quantify and characterize the polyphenol profile, juices were then purified by Solid Phase Extraction (SPE) C18 Column. Turbid juices were centrifuged before the SPE procedure. To analyze the bounded polyphenols, the obtained precipitates were extracted followed by hydrolysis. The total polyphenol content was assessed by the colorimetric assay Folin-Ciocalteu and the polyphenol profile was characterized by high-performance chromatography coupled to UV-vis and tandem-mass spectrometry.

The juices 1.1 and 1.2, have the lowest content of total polyphenols, 0.470±0.025 mg/mL and 0.770±0.024 mg/mL, respectively. The lower content in polyphenols could be explained by their being trapped in the precipitates collected by centrifugation. So, the increase in the differential velocity of centrifugation seems to result in higher soluble polyphenol concentration. Regarding the clarified juices results show that the major content of polyphenols was for the clarified juice at 40°C with 1.081 ±0.130 mg/mL, while clarified juice at 60°C had 0.763 ±0.018 mg/mL (statistical different). This result seems to indicate that temperature leads to a decrease in polyphenols content. Ongoing analyses are exploring the polyphenols present in the precipitates. Regarding the polyphenols profile, the main polyphenols were chlorogenic acid and procyanidins.

In conclusion, filtration and centrifugations may affect the polyphenol content. The results suggested that apple fruit matrices can offer a high content of polyphenol compounds and can contribute to promoting health benefits.

**Keywords**: polyphenols compounds, apple, chlorogenic acid, procyanidins

**References:**

1. da Silva, L.C.; Viganó, J.; de Souza Mesquita, L.M.; Dias, A.L.B.; de Souza, M.C.; Sanches, V.L.; Chaves, J.O.; Pizani, R.S.; Contieri, L.S.; Rostagno, M.A. Recent Advances and Trends in Extraction Techniques to Recover Polyphenols Compounds from Apple By-Products. Food Chem. X 2021, 12, doi:10.1016/j.fochx.2021.100133.

2. Jakobek, L.; Buljeta, I.; Ištuk, J.; Barron, A.R. Polyphenols of Traditional Apple Varieties in Interaction with Barley a-Glucan: A Study of the Adsorption Process. Foods 2020, 9, doi:10.3390/foods9091278.

3. Starowicz, M.; Achrem-Achremowicz, B.; Piskuła, M.K.; Zielinski, H. Phenolic Compounds from Apples: Reviewing Their Occurrence, Absorption, Bioavailability, Processing, and Antioxidant Activity - A Review. Polish J. Food Nutr. Sci. 2020, 70, 321–336, doi:10.31883/pjfns/127635.

02

POSTER

# Insights into the development of *Actinidia arguta* leaves nutraceutical ingredient: Antioxidant activity and bioavailability study in rats

Ana Margarida Silva[1], Andreia Almeida[1], Bruno Sarmento[2,3,4], Paulo C. Costa[5,6], Cristina Delerue-Matos[1] & Francisca Rodrigues[1]

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*Actinidia arguta* is a perennial vine characterized by the presence of leaves extremely rich in antioxidant compounds, particularly phenolics (Silva et al., 2021). Recently, our research team applied the Response Surface Methodology (RSM) to determine the optimal extraction conditions of *A. arguta* leaves through Ultrasound-Assisted Extraction (UAE) (Silva et al., 2021). The optimal extract revealed to be rich in neochlorogenic and chlorogenic acids, caffeoylquinic acid, catechin, kaempferol-3-*O*-glucoside and isorhamnetin-3-*O*-rutinoside (Silva et al., 2022). These bioactive compounds may have an *in-vivo* pro-healthy impact, particularly antioxidant effects. Therefore, this study intends to evaluate the *in-vivo* antioxidant effects of UAE *A. arguta* leaves extract in rats, aiming to validate a new nutraceutical ingredient.

Briefly, the dried leaves of *A. arguta* were extracted by UAE using water as solvent and a solid: liquid ratio of 10 % (*w/v*) during 31.11 min, maintaining an ultrasonic intensity of 30 W/m2 (Silva et al., 2021). For the *in-vivo* assays, Wistar rats (*n* = 6 / group) were orally treated during 7 days with water (control; Group I), *A. arguta* leaves extracts (50 and 75 mg/kg bw/d, respectively, Group II and III) and vitamin C (positive control; Group IV). The antioxidant enzyme activities, namely superoxide dismutase, catalase, glutathione peroxidase and malondialdehyde levels were determined. The influence of the extract on the *in-vitro* erythrocyte hemolysis by AAPH induction was also assessed.

The results attested that the extract provided a strong inhibitory effect against the erythrocyte hemolysis, with an IC50 = 251.09 µg/mL. Regarding the biochemical and antioxidant parameters, the highest superoxide dismutase activity in kidneys and livers was found for Group III (75 mg/kg bw/d), respectively, 183.36 and 175.26 units/g protein. Similarly, both groups treated with extract achieved the best catalase results for livers. The rats administrated with 50 mg/kg bw/d of extract (Group II) significantly increased the glutathione peroxidase activity (kidneys = 205.35 units/g protein, livers = 133.60 units/g protein and serum = 64.57 units/mL protein). Regarding the malondialdehyde levels, groups II and III (treated with extracts) exhibited the lowest levels.

These results highlighted the *in-vivo* efficacy and safety of *A. arguta* leaves extracts to be used in nutraceutical field. Further studies should be performed to screen the possibility to counteract the progression of other diseases, such as prediabetes.

**Keywords**: *Actinidia arguta* leaves, *in-vivo* antioxidant effects, nutraceutical ingredient.

**References:**

1. Silva, A.M., Costa, P.C., Delerue-Matos, C., Latocha, P., Rodrigues, F. Extraordinary composition of *Actinidia arguta* by-products as skin ingredients: A new challenge for cosmetic and medical skincare industries. Trends Food Sci. Technol. 2022, 116, 842-853; DOI:10.1016/j.tifs.2021.08.031

2. Silva, A.M.; Pinto, D.; Moreira, M.M.; Costa, P.C.; Delerue-Matos, C.; Rodrigues, F. Valorization of Kiwiberry Leaves Recovered by Ultrasound-Assisted Extraction for Skin Application: A Response Surface Methodology Approach. *Antioxidants 2022*, 11, 763; DOI:10.3390/antiox11040763

# Honey from Natural Park of Montesinho: insights from DNA extraction methodology for botanical identification

Sónia Soares[1], Leandro Magalhães[1], Rui Barbosa[2] & Cristina Delerue-Matos[1]

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Honey is a sweet natural substance produced by bees from the nectar of plants and/or secretions of living parts of plants. It consists essentially of simple sugars (68%) and water (18%) and smaller quantities of other components such as vitamins, minerals, proteins, organic acids, phenolic compounds, among others [1]. Those are highly dependent of the botanical species visited by bees, conferring to each honey different and distinct organoleptic and biological properties. The botanical differences give rise to two types of honeys, monofloral, consisting essentially of the nectar of a single plant species, and multifloral, arising from several plant species [1]. This strict relation of botanical origin and its biological and organoleptic properties lead to a quality distinction of honey types. There are honeys with high market values due to its particular flavour, taste and specific biological properties, such as monofloral honey and honeys with the denomination of Protected Designation of Origin, such as honey produced in Natural Park of Montesinho. With this, honey is produced and marketed based on its colour, flavour, density and biological properties, being extremely important to assess its quality and authenticity. DNA-based methods are simple, fast and precise promising tools for species identification. However, honey is a complex matrix with high amounts of sugars and other compounds that inhibit the PCR reaction. Thus, it is important to find the best DNA extraction method to achieve good yields and purities. In this work, different pre-treatments were performed and results were compared. For the DNA extraction, a commercial DNA extraction kit was used. The quality of DNA extracts was assessed by spectrophotometry and electrophoresis. Results revealed that the use of ultrasounds in honey pre-treatments achieved the best yields and purity. Additionally, a PCR amplification targeting the gene 18S rRNA was performed to evaluate amplifiability of the extracted DNA. As expected, all extract samples revealed to have amplifiable DNA. Combining a simple and easy honey pre-treatment with an effective DNA extraction method, allows us to get desired good extracts. Thus, this methodology is the first step for further studies involving botanical identification of honey and honey quality studies.

**Keywords**: Honey DNA, DNA extraction, PCR amplification, botanical identification.

**References:**

[1] Codex Alimentarius. STANDARD FOR HONEY, CXS 12-19811, Adopted in 1981. Revised in 1987, 2001. Amended in 2019, available at: https://www.fao.org/fao-who-codexalimentarius/codex-texts/list-standards/en/ (Accessed September 05, 2022.)

**Acknowledgements:**

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04

POSTER

# Sustainable valorization of phenolic compounds from *Castanea sativa* shells for pharmaceutical application

Ana Sofia Ferreira[1], Ana Margarida Silva[1], Diana Pinto[1], Manuela M. Moreira[1], Ricardo Ferraz[2], Jaroslava Švarc-Gajic[3], Paulo Costa[4], Cristina Delerue-Matos[1] & Francisca Rodrigues[1]

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Chestnut (*Castanea sativa*) fruits have an enormous global ecological and economic impact, despite the large generation of by-products during its industrialization process, namely shells, [1]. On the last years, the valorisation of chestnut shells for nutraceutical purposes arises as a challenge due to its richness in bioactive compounds, particularly phenolics. Subcritical Water Extraction (SWE) is an eco-friendly promising method to extract bioactive compounds using water as solvent, with fast processing time and high yield[2]. Therefore, the aim of this work was to produce a bioactive extract with antioxidant, antiradical scavenging power and antimicrobial activity, as well as low cytotoxic effect on buccal epithelial cell lines (HSC3 and TR146), by optimizing the extraction temperature (110 °C – 180 °C) of *C. sativa* shells through SWE. The optimal temperature of extraction was 110 ºC, revealing the highest phenolic and flavonoid contents (239.53 mg of gallic acid equivalents (GAE)/g dry weight (dw) and $IC_{50}$=148.68 µg/ml, respectively) as well as the highest antioxidant activity (4240.38 µmol of ferrous sulphate (FeS)/g). Additionally, the 110°C extract showed the higher antiradical activity ($IC_{50}$=426.88 g/ml for DPPH assay) and a good capacity to scavenge reactive oxygen species, namely HOCl and ROO· ($IC_{50}$=4.47 g/ml and 0.73 mol of Trolox equivalents/mg dw, respectively). Also, high concentrations of phenolic acids, such as gallic and protocatechuic acids and flavonoids (catechin, epicatechin and rutin), composed the phenolic profile. All extracts obtained demonstrated antimicrobial activity against different microorganisms present in the oral cavity during oral mucositis state, such as *Porphyromonas gingivalis*, *Streptococcus mutans*, *Staphylococcus aureus*, *Enterococcus faecalis* and *Escherichia coli*. The MTT assay revealed that the lowest $IC_{50}$ was achieved for the 110°C extract in the HSC3 and TR146 cell lines ($IC_{50}$=1325.03 and 468.15 µg/ml, respectively). This work demonstrated the potentialities of SWE to valorize *C. sativa* shells as a valuable source of compounds that may be suited for the potential application for oral mucositis treatment.

**Keywords**: bioactive compounds, chestnut shells, oral mucositis, sustainability.

**References:**

1. Barreira, J.C.M.; Ferreira, I.C.F.R.; Oliveira, M.B.P.P. Bioactive Compounds in Underutilized Fruits and Nuts. Murthy, H., Bapat, V.: New York, NY, USA, 2020, 303-313; https://doi.org/10.1007/978-3-030-30182-8_18

2. Zhang, J.; Wen, C.; Zhang, H.; Duan, Y.; Ma, H. Recent advances in the extraction of bioactive compounds with subcritical water: A review. Trends in Food Science & Technology. 2020, 95, 183-195; https://doi.org/10.1016/j.tifs.2019.11.018

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05

POSTER

# Human osteoclast derived exosomes

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Bone remodelling is a highly regulated process involving a balance between bone formation and resorption processes. Osteoclasts are resident bone cells responsible for bone resorption that also initiate the bone remodeling process. Osteoclast derived exosomes are nanometer sized vesicles loaded with bioactive molecules that play an essential role in regulation of bone remodeling. However, due to variations in osteoclast differentiation methodologies, the exosome isolation and characterization during the induction process are not well characterized. Further, the effect of exosome depleted serum on osteoclast induction efficiency has not been explored till now. This is crucial for future studies on osteoclast-derived exosomes. We have established a novel sequential method of osteoclast induction, wherein, a homogenous population of proliferative $CD14^+$ cells is generated from human peripheral blood monocytes. The cultures were subjected to M-CSF stimulation followed by differentiation into a mature/functional osteoclasts by co-stimulation with MCSF and RANKL. Exosomes were isolated from the supernatant of induced monocytes using a density gradient ultracentrifugation process. We compared osteoclast induction in regular and exosome depleted serum and characterized exosomes derived under those conditions. Our protocol resulted in generation of high efficiency and viability of $CD14^+$ cells which had higher proliferative potential than monocytes alone. Further, their induction into osteoclasts was found superior to that from monocytes alone at gene and protein level. We were further able to demonstrate generation of mature, functional osteoclasts. Finally, exosomes were successfully isolated and characterized at different stages of osteoclast induction.

**Keywords**: Bone resorption, osteoclasts, exosomes, CD14+ monocytes

**References:**

1. Steller, Daniel, Alexandra Scheibert, Tabea Sturmheit, and Samer G. Hakim. et al. Establishment and validation of an in vitro co-culture model for oral cell lines using human PBMC-derived osteoclasts, osteoblasts, fibroblasts and keratinocytes. Scientific Reports 10.1 (2020): 1-10. https://doi.org/10.1038/s41598-020-73941-0

2. Gebraad, Arjen, Roman Kornilov, Sippy Kaur, Susanna Miettinen, Suvi Haimi, Hilkka Peltoniemi, Bettina Mannerström, and Riitta Seppänen-Kaijansinkko et al. Monocyte-derived extracellular vesicles stimulate cytokine secretion and gene expression of matrix metalloproteinases by mesenchymal stem/stromal cells. The FEBS journal 285.12 (2018): 2337-2359. https://doi.org/10.1111/febs.14485

06

POSTER

# Cyanobacteria for skin care and cosmeceutical formulations

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Skin acts as a barrier from external stimulus such as pollutants and ultraviolet radiation. Disturbs in the skin are involved in skin aging, which mostly results on a thinner epidermis, dryness, wrinkles, and loss of elasticity. Synthetic ingredients in cosmetics are known to be more toxic and to cause negative impact on the environment. In a society increasingly worried with human and environment health, natural skin care products gain special attention and boosted the search for natural sources.

Due to the production of bioactive compounds, cyanobacteria emerged as an excellent source for cosmetic ingredients. Cyanobacteria strains of CIIMAR culture collection (LEGE-CC) have already proven to be interesting for cosmetic purposes [1,2]. In this project we aimed to evaluate the potential of four LEGE-CC cyanobacteria strains for skin care purposes. The cytotoxicity of acetonic and aqueous extracts was evaluated in keratinocytes (HaCAT), fibroblasts (3T3L1) and endothelial cells (hCMEC/D3) along with the total phenolic content and antioxidant properties.

Cytotoxic assays revealed toxicity of acetonic extracts to keratinocytes in the higher concentrations tested. Aqueous extracts revealed low or non-cytotoxicity. Mainly in low concentrations of extracts, cellular proliferation was registered. Aqueous extracts from strain JM/RS021A and acetone extracts from strain JM/RS035B revealed higher values for total phenolic compounds. The acetone extracts of strain JM/RS021A showed the greatest scavenging activity values on the DPPH assay.

**Keywords**: cyanobacteria, cosmetics, skin, phenols antioxidant

**References:**

1. Favas, R.; Morone, J.; Martins, R.; Vasconcelos, V.; Lopes, G. Cyanobacteria Secondary Metabolites as Biotechnological Ingredients in Natural Anti-Aging Cosmetics: Potential to Overcome Hyperpigmentation, Loss of Skin Density and UV Radiation-Deleterious Effects. Mar Drugs 2022, 20

2. Morone, J.; Lopes, G.; Preto, M.; Vasconcelos, V.; Martins, R. Exploitation of Filamentous and Picoplanktonic Cyanobacteria for Cosmetic Applications: Potential to Improve Skin Structure and Preserve Dermal Matrix Components. Marine Drugs 2020, 18, 486.

07

POSTER

# Development of Computational Methods for Aptamer Design for Biomedical Applications

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Aptamers are short single-stranded nucleic acids or peptide molecules that can bind with high affinity to specific protein targets. They have been employed as a precise tool for molecular recognition or to targeted drug delivery. Over the last decades, hundreds of aptamers have been selected to recognize different molecular targets, including small molecules, peptides, and specific proteins. Many have been patented for specific applications. Aptamer selection is normally accomplished by an "in vitro selection" process named SELEX, which stands for Systematic Evolution of Ligands by Exponential Enrichment. In this process, randomized libraries of different sequences are subject to iterative cycles of selection/separation/amplification, yielding round after round sequences with a higher affinity towards a specific target. More recently, in silico methods have been developed to help understand aptamer-target interaction and to rationally introduce modifications in selected aptamers to modulate their affinity, specificity, or ability to carry other molecules. In addition, in silico efforts to design aptamers with high affinity to specific targets, reducing the time and cost for developing rapid diagnostics tools and effective therapeutics, have been gaining momentum. Here, we present our efforts in developing methods for aptamer design, coupling molecular dynamics simulations, protein-DNA docking, and free energy calculations. This is illustrated with the identification and experimental confirmation of a novel aptamer for Cathepsin B, a predictive prostate cancer biomarker [1].

**Keywords**: Targeted-Drug Delivery; Computer-Aided Drug Discovery; In Silico Methods.

**References:**

[1] Pereira, AC et al. - Identification of novel aptamers targeting cathepsin B-overexpressing prostate cancer cells - Molecular Systems Design & Engineering (2022) DOI: 10.1039/D2ME00022A

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08

POSTER

# Antimicrobial Properties of Brown Algae: Solvent impact assessment

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Crude extracts and compounds isolated from brown algae have been demonstrated to present useful properties, namely antibacterial, antihypertensive, antiviral, anticancer, and antioxidant activity.

In this work, five different solvents (ethanol, chloroform, hexane, acetone, and ethyl acetate) were used to evaluate the antimicrobial activity of extracts obtained from nine species of brown macroalgae: *Ascophyllum nodosum*, *Himanthalia elongata*, *Undaria pinnatifida*, *Pelvetia canaliculate*, *Saccharina latissimi*, *Bifurcaria bifurcata*, *Laminaria ochroleuca*, *Sargassum muticum* and *Fucus spiralis*.

The microorganisms *Bacillus cereus*, *Escherichia coli*, *Salmonella enteritidis*, and *Pseudomona aeruginosa* were selected since they are known to be responsible for food spoilage and foodborne diseases. Additionally, *Staphylococcus aureus* and *Staphylococcus epidermidis*, two microorganisms responsible for opportunistic hospital infections were also included in the study.

The antimicrobial capacity of the extracts tested was variable in relation to the solvent choice, reinforcing the perception that this characteristic is solvent dependent and should be optimized for each individual case. *Saccharina latissimi* and *Bifurcaria bifurcata* were the most promising algae tested.

**Keywords**: extraction solvent, brown Algae, antimicrobial properties

**References:**

Silva, A.; Silva, S.A.; Lourenço-Lopes, C.; Jimenez-Lopez, C.; M. Carpena, M.; Gullón, P.; Fraga-Corral, M.; Domingues, V.F.; Fátima Barroso, M.; Simal-Gandara, J.; Prieto, M. A. Antibacterial Use of Macroalgae Compounds against Foodborne Pathogens. Antibiotics. 2020, 9, 712; DOI:10.3390/antibiotics9100712

**Acknowledgements:** This research was funded by Programa de Cooperación Interreg V-A España–Portugal (POCTEP) 2014–2020 (projects ref. 0181_NANOEATERS_01_E and ref. 0377_IBERPHENOL_6_E) and by MICINN supporting the Ramón&Cajal grant for M. A. Prieto (RYC-2017-22891); AlgaMar (www.algamar.com) that supports the pre-doctoral grant for C. Lourenço-Lopes. This work also received financial support from REQUIMTE/LAQV, National Funds (FCT, Fundação para a Ciência e Tecnologia) through project UID/QUI/50006/2019 and under project UIDB/50016/2020 and by FEDER–Fundo Europeu de Desenvolvimento Regional funds through the COMPETE 2020–Operational Programme for Competitiveness and Internationalisation (POCI), and by Portuguese funds through FCT–Fundação para a Ciência e a Tecnologia in the framework of the project POCI-01-0145-FEDER-030240–PTDC/OCE-ETA/30240/2017.

# Inverted Virtual Screening Approaches for the Identification of Protein Targets for Biologically Active Molecules

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Virtual Screening (VS) has become crucial in the drug discovery process because it can provide a list of potential hits in a more time effective and inexpensive manner, when compared to experimental methods. There are several success stories described in the literature and VS protocols continues to evolve by creating other approaches such as physics-based methods and machine learning. Inverted Virtual screening (IVS) consists in searching for targets or secondary targets for already known compounds. Identifying possible targets for a particular ligand is an appealing approach to prevent side effects but also, to identify targets and mechanisms of action for already known compounds. In this methodology, a molecular docking process is employed to screen a protein database for a query ligand.

In this work, we report the creation of a 6 step docking based IVS protocol: 1) Creation of Target Databases Associated with a Biological Activity; 2) Identification/Prediction of Associated Binding Pockets; 3) Validation of Target Specific Docking Protocols for the Different Targets; 4) Inverted Virtual Screening for Identification of the Most-likely targets; 5) MD simulations of the top predictions for validation and refinement; 6) MM-GBSA for characterization and prediction of the relative binding free energies. The subsequent IVS protocol can be applied to a several types of targets and molecules, when correctly adjusted [1,2].

**Keywords**: Inverted Virtual Screening, Molecular Docking, Target fishing, Target identification

**References:**

1. Pereira, R. B. et al. Amino Alcohols from Eugenol as Potential Semisynthetic Insecticides: Chemical, Biological, and Computational Insights. Molecules 2021, 26, 6616; DOI: 10.3390/molecules26216616

2. Natal, C. M. et al. New carvacrol and thymol derivatives as potential insecticides: synthesis, biological activity, computational studies and nanoencapsulation. RSC Adv. 2021, 11, 34024–34035; DOI: 10.1039/D1RA05616F

This work was supported by national funds from Fundacão para a Ciência e a Tecnologia UIDP/04378/2020 and UIDB/04378/2020, SFRH/BD/137844/2018, 2020.01423.CEECIND. Some of the calculations were produced with the support of INCD funded by FCT and FEDER under project 01/SAICT/2016 number 022153 and projects CPCA/A00/7140/2020 and CPCA/A00/7145/2020.

10

POSTER

# Computer-Guided Development of Novel Drugs Against SARS-CoV-2

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SARS-CoV-2 is a positive-strand RNA virus that causes severe respiratory syndrome in humans. This is the infectious agent behind the COVID-19 pandemic. Although multiple vaccines have been developed since the start of the pandemic, no effective drugs have been developed. The infection relies on the interaction between the viral spike S protein and the angiotensin-converting enzyme 2 (ACE2). This enzyme is expressed in the in human airway epithelia, lung parenchyma, vascular endothelia, kidney cells, and small intestine cells. The S/ACE2 interaction is the central entry pathway for SARS-CoV-2. By disrupting this interaction, the viral replication will be blocked.

In this work, *in silico* models were used to study the S/ACE2 complex and discover drugs that may target the S/ACE2 interface.

The interaction between spike and ACE2 was studied by performing molecular dynamics simulations with a length of 400 ns using the AMBER 21 software. The interfacial binding pocket was then defined using FPocket 2.0 [1]. Subsequently, a virtual screening protocol was employed. Using Autodock Vina and GOLD molecular docking software, 139,146 compounds were screened. These compounds belonged to different chemical libraries: the Chimiothèque Nationale, MuTaLig Virtual Chemotheca, and the Inhibitors of Protein-Protein Interactions Database.

From the results of the virtual screening experiment, 10 compounds were selected for experimental validation. These experimental studies tested the inhibition of infectivity, dose dependency, S/ACE2 binding inhibition, viral replication, and drug cytotoxicity.

From the experimental studies, two novel compounds were found which are capable of hindering S/ACE2 interaction and therefore inhibiting SARS-CoV-2 replication[2].

**Keywords**: SARS-CoV-2, Molecular Docking, MD Simulations

**References:**

1. Lapaillerie, D.; Charlier, C.; Fernandes, H.S.; Sousa, S.F.; Lesbats, P.; Weigel, P.; Favereaux, A.; Guyonnet-Duperat, V.; Parissi, V. In Silico, In Vitro and In Cellulo Models for Monitoring SARS-CoV-2 Spike/Human ACE2 Complex, Viral Entry and Cell Fusion. Viruses 2021, 13, 365, doi:10.3390/v13030365.

2. Lapaillerie, D.; Charlier, C.; Guyonnet-Dupérat, V.; Murigneux, E.; Fernandes, H.S.; Martins, F.G.; Magalhães, R.P.; Vieira, T.F.; Richetta, C.; Subra, F.; et al. Selection of Bis-Indolyl Pyridines and Triphenylamines as New Inhibitors of SARS-CoV-2 Cellular Entry by Modulating the Spike Protein/ACE2 Interfaces. Antimicrob Agents Chemother 2022, 66, e00083-22, doi:10.1128/aac.00083-22.

This work was supported by national funds from Fundacão para a Ciência e a Tecnologia UIDP/04378/2020 and UIDB/04378/2020, 2021.07128.BD, 2020.01423.CEECIND. Some of the calculations were produced with the support of INCD funded by FCT and FEDER under project 01/SAICT/2016 number 022153 and projects CPCA/A00/7140/2020 and CPCA/A00/7145/2020.

# Wild mushrooms for Alzheimer's disease

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Alzheimer's disease (AD) is among the most incident, debilitating, and widespread forms of dementia [1]. AD causes the decline of cognitive function, making overall day-to-day tasks difficult and even impossible [2]. Despite extensive research, its origin and progression are still unclear, and no efficient treatment is available to prevent disease progression or mitigate symptoms [3]. Due to the low efficiency in the available treatments, natural compounds have arisen as an alternative. These are gaining crescent relevance and have been explored, given their promising results as immunomodulatory, anti-inflammatory, or neuroprotective factors [4]–[7]. Mountain natural resources, specifically mushrooms, are among the most studied sources of bioactive molecules. The Montesinho Natural Park, located in northeast Portugal, has incredible mushroom biodiversity, which has been exploited to obtain health-beneficial compounds, namely with anti-inflammatory potential [8]–[11]. This work aimed to study different mushroom extracts and isolated compounds for the prevention and progression of AD. So far, our work aimed at bringing insight into the effect of extract administration in neuroblastoma and endothelial cell lines through the MTT viability assay, as well as studying their effects on acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE), enzymes known to be involved in AD pathogenesis through a modified Ellman's method: dry extracts were resuspended in tris-HCl 50mM + 10% DMSO to concentrations between 2 and 0.03125 mg/mL for enzymatic inhibition assays. To be considered promising, extracts must not cause cellular death of over 15-20% and cause enzymatic inhibition. So far, our results using nine mushroom extracts show low-to-no cytotoxicity in concentrations between 100 and 6.25 µg/mL in endothelial and neuroblastoma cell lines. High concentrations of *Lycoperdon umbrinum* extract caused cytotoxicity levels of approximately 35% in endothelial cells after 24h treatment; different extracts caused cell proliferation in both cell lines, the most evident being 128% in the lowest concentrations of *Russula delica* and *Boletus fragrans*. The most promising results were obtained for *Boletus aereus*, which, while not cytotoxic, inhibited both enzymes by 60%. Around 30% AChE inhibition was achieved for the two highest concentrations of *Agaricus silvicola* and *Boletus fragrans* making both extracts good candidates for further studies.

**Keywords**: Alzheimer's disease, Neurodegenerative disease, Natural products, Bioactive compounds

**References:**

1. H. Niu, I. Álvarez-Álvarez, F. Guillén-Grima, and I. Aguinaga-Ontoso, "Prevalence and incidence of Alzheimer's disease in Europe: A meta-analysis," 2017. [Online]. Available: www.elsevier.es/neurologia

2. M. Prince et al., "World Alzheimer Report 2014 Dementia and Risk Reduction AN ANALYSIS OF PROTECTIVE AND MODIFIABLE FACTORS SUPPORTED BY Dr Maëlenn Guerchet Dr Matthew Prina," 2014. [Online]. Available: www.daviddesigns.co.uk

3. J. M. Long and D. M. Holtzman, "Alzheimer Disease: An Update on Pathobiology and Treatment Strategies," Cell, vol. 179, no. 2. Cell Press, pp. 312–339, Oct. 03, 2019. doi: 10.1016/j.cell.2019.09.001.

4. W. Lee et al., "Role of Mushrooms in Neurodegenerative Diseases," in Medicinal Mushrooms, Springer Singapore, 2019, pp. 223–249. doi: 10.1007/978-981-13-6382-5_8.

5. G. Venturella, V. Ferraro, F. Cirlincione, and M. L. Gargano, "Medicinal mushrooms: Bioactive compounds, use, and clinical trials," Int J Mol Sci, vol. 22, no. 2, pp. 1–31, Jan. 2021, doi: 10.3390/ijms22020634.

6. N. Kushairi et al., "Modulation of neuroinflammatory pathways by medicinal mushrooms, with particular relevance to Alzheimer's disease," Trends in Food Science and Technology, vol. 104. Elsevier Ltd, pp. 153–162, Oct. 01, 2020. doi: 10.1016/j.tifs.2020.07.029.

7. S. K. Yadav et al., "A Mechanistic Review on Medicinal Mushrooms-Derived Bioactive Compounds: Potential Mycotherapy Candidates for Alleviating Neurological Disorders," Planta Med, vol. 86, no. 16, pp. 1161–1175, 2020, doi: 10.1055/a-1177-4834.

8. E. Pereira, L. Barros, A. Martins, and I. C. F. R. Ferreira, "Towards chemical and nutritional inventory of Portuguese wild edible mushrooms in different habitats," Food Chem, vol. 130, no. 2, pp. 394–403, 2012.

9. I. C. F. R. Ferreira, L. Barros, and R. M. v Abreu, "Antioxidants in Wild Mushrooms," Current Medicinal Chemistry , vol. 16, pp. 1543–1560, 2009.

10. S. A. Heleno, L. Barros, M. J. Sousa, A. Martins, and I. C. F. R. Ferreira, "Tocopherols Composition of Portuguese Wild Mushrooms with Antioxidant Capacity," Food Chem, vol. 119, pp. 1443–1450, 2010.

11. O. Taofiq et al., "The contribution of phenolic acids to the anti-inflammatory activity of mushrooms: Screening in phenolic extracts, individual parent molecules and synthesized glucuronated and methylated derivatives," Food Res Int, vol. 76, no. Pt 3, pp. 821–827, 2015, doi: 10.1016/j.foodres.2015.07.044.

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12

POSTER

# Manufacturing of mRNA nanomedicines using thermoreversible aqueous biphasic systems and ionic liquids

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The COVID-19 pandemic has unlocked the potential of messenger RNA (mRNA) vaccines as an effective tool to contain infectious disease outbreaks. Over conventional vaccines, mRNA vaccines show advantages, namely improved safety and efficacy, and the possibility of repeatedly administration [1]. However, mRNA nanomedicine production is still complex and expensive, requiring improved technologies to produce more stable and widely accessible products by meeting a timely and sufficient manufacturing capacity.

Ionic liquids (ILs) are molten salts comprising organic cations, with a remarkable structural diversity and with promising applications as solvents and catalysts. If properly engineered, ionic liquids (ILs) can improve the stability of RNA [2] and contribute to the achievement of highly selective purification processes when applied as components of aqueous biphasic systems (ABS) [3]. Accordingly, this work proposes the integration of production and clarification steps of mRNA manufacturing processes using thermoreversible ABS comprising ILs to simplify subsequent purification steps.

So far, we have accomplished the production of mRNA by in vitro transcription using a T7 polymerase-based cell free system and its purification using conventional methods, followed by the implementation of quality control methods to evaluate mRNA purity. Considering the most promising ILs able to maintain the stability and integrity of mRNA, ongoing work is focused on the selection of the best production-clarification platform, resorting to thermoreversible IL-based ABS.

In conclusion, the integrated production-clarification platform to be developed can be used to overcome the challenges of mRNA nanomedicine production, namely by lowering costs and environmental impact of current manufacturing processes while improving mRNA stability, yield, and speed of production.

**Keywords**: mRNA nanomedicines; ionic liquid; thermoreversible aqueous biphasic system; in vitro transcription.

**References:**

[1] Pardi, N.; Hogan, M.J.; Porter, F.W.; Weissman, D. mRNA vaccines — a new era in vaccinology. Nature Reviews Drug Discovery 2018, 17, 261-279, doi:10.1038/nrd.2017.243.

[2] Pedro, A.Q.; Pereira, P.; Quental, M.J.; Carvalho, A.P.; Santos, S.M.; Queiroz, J.A.; Sousa, F.; Freire, M.G. Cholinium-Based Good's Buffers Ionic Liquids as Remarkable Stabilizers and Recyclable Preservation Media for Recombinant Small RNAs. ACS Sustainable Chemistry & Engineering 2018, 6, 16645-16656, doi:10.1021/acssuschemeng.8b03900.

[3] Ventura, S.P.M.; e Silva, F.A.; Quental, M.V.; Mondal, D.; Freire, M.G.; Coutinho, J.A.P. Ionic-Liquid-Mediated Extraction and Separation Processes for Bioactive Compounds: Past, Present, and Future Trends. Chemical Reviews 2017, 117, 6984-7052, doi:10.1021/acs.chemrev.6b00550.

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# FTIR analysis of gingival crevicular fluid for the identification of oral mucosal diseases

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Oral Lichen Planus (OLP) is a chronic inflammatory disease of the oral mucosa caused by a dysfunctional basal epithelium. It mimics different pathologies being hard to obtain a good clinical diagnosis. The development of a rapid and accurate analytic diagnostic technique, with a wider comprehension of biochemical abnormalities would be of great clinical value. Mid-infrared (MIR) spectroscopy gathers all features abovementioned being considered a fingerprint technique. It allows the identification of biomolecular changes as it monitors molecules' vibrations on biological samples. Accordingly, this work aims to access the effectiveness of mid-infrared (MIR) on the identification of OLP upon gingival crevicular fluid (GCF) analysis.

GCF was collected from 16 patients with OLP diagnosis and 16 control-group patients. MIR spectroscopy was conducted, and the attained spectral regions were assessed by chemometric tools.

MIR spectroscopy was able to discriminate between OLP and controls' GCF samples, with 95.1% of correct predictions. The results of this trial further allowed the identification of the most important components of the spectra correlated with the biochemical alterations of this condition.

Whether further studies are needed to validate the attained data, GCF MIR analysis may be regarded as an innovative, fast, and low-cost technique to assist on early diagnosis and clinical follow-up of OLP.

**Keywords**: Oral Lichen Planus, Gingival Crevicular Fluid, Spectroscopy, FTIR-MIR

**References:**

Gong, C., S. Sun, B. Liu, J. Wang, and X. Chen. Identification of Potential Therapeutic Target Genes, Key Mirnas and Mechanisms in Oral Lichen Planus by Bioinformatics Analysis. Arch Oral Biol 78 (Jun 2017): 122-28.

Mishra, Shovna Shivani, and T. N. Uma Maheswari. Evaluation of Oxidative Stress in Oral Lichen Planus Using Malonaldehyde: A Systematic Review. J Dermatol Dermatol Surg .18, no. 1 (2014/01/01/ 2014): 2-7.

Naes, T. et al. Interpreting Pcr and Pls Solutions. A User-Friendly Guide to Multivariate Calibration and Classification. 2004.

Barker, M and Rayens, W. "Partial Least Squares for Discrimination." Journal of Chemometrics 17, no. 3 (2003): 166-73.

14

POSTER

# Subcutaneous and visceral adipose tissue-derived mesenchymal stromal cells: effect of retinoic acid

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Mesenchymal stromal cells (MSCs)-based regenerative orthopedic therapies have been used as a promising and innovative therapeutic approach to enhance the repair of bone defects, thus improving bone tissue regeneration and reconstruction [1-3]. Adipose-derived stromal cells (ADSCs) can be obtained from two main depots - subcutaneous and visceral - with further differences in structure, functional behavior, and cell content, according to the anatomical location [4,5]. However, the impact of these differences on osteogenic capability has not been thoroughly detailed, particularly with different osteogenic inductive programs. Thus, in the present study, we evaluated the functional characteristics and osteogenic capacity of two osteogenic strategies – Dex+β+AAM (dexamethasone, β-glycerophosphate and ascorbic acid) and RAM (retinoic acid), in cat-derived adipose tissue cell populations.

Adipose tissue from the visceral retroperitoneal (VAT) and subcutaneous abdominal region (SCAT) was collected from 5 healthy cats (*F. catus*). ADSCs were isolated through an enzymatic dissociation process and cultured in the presence of the two osteogenic induction media – Dex+β+AAM and RAM. The obtained cultures were characterized at different periods for proliferation, morphology, and osteogenic activity.

Our results demonstrated a distinct biological behavior of the cultured ADSCs, isolated from the two depots, namely in cell size, morphology, and functional activity. In the presence of the osteogenic inducers, cultures showed an increased expression of alkaline phosphatase (ALP) activity, compared to control. Additionally, VAT-derived ADSCs presented the highest osteogenic activity.

In conclusion, different characteristics were found in the two adipose tissue depots, which may reflect the differences found in the functionality of isolated ADSCs. However, VAT proved to be the tissue with the highest osteogenic potential, showing higher levels of ALP activity, particularly with RAM.

**Keywords**: adipose tissue, mesenchymal stromal cells, anatomic locations, osteogenic potential

**References:**

1. Kono, S.; Kazama, T.; Kano, K.; Harada, K.; Uechi, M.; Matsumoto, T. Phenotypic and functional properties of feline dedifferentiated fat cells and adipose-derived stem cells. The Veterinary Journal 2014, 199, 88-96, doi:10.1016/j.tvjl.2013.10.033.

2. Lee, B.-Y.; Li, Q.; Song, W.-J.; Chae, H.-K.; Kweon, K.; Ahn, J.-O.; Youn, H.-Y. Altered properties of feline adipose-derived mesenchymal stem cells during continuous in vitro cultivation. Journal of Veterinary Medical Science 2018, 80, 930–938, doi:10.1292/jvms.17-0563.

3. Voga, M.; Kovac, V.; Majdic, G. Comparison of Canine and Feline Adipose-Derived Mesenchymal Stem Cells/Medicinal Signaling Cells With Regard to Cell Surface Marker Expression, Viability, Proliferation, and Differentiation Potential. Frontiers in Veterinary Science 2021, 7, 610240, doi:10.3389/fvets.2020.610240.

4. Casteilla, L.; Planat-Benard, V.; Laharrague, P.; Cousin, B. Adipose-derived stromal cells: Their identity and uses in clinical trials, an update. World Journal of Stem Cells 2011, 3, 25-33 doi:10.4252/wjsc.v3.i4.25.

5. Cleal, L.; Aldea, T.; Chau, Y.-Y. Fifty shades of white: Understanding heterogeneity in white adipose stem cells. Adipocyte 2017, 6, 205–216, doi:10.1080/21623945.2017.1372871.

# Use of aqueous biphasic systems to improve the detection of pentraxin-3 in human serum

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Lung cancer is currently considered a major public health problem [1]. To improve the outcomes of patient care, the early detection of lung cancer is extremely important [1]. In this context, the quantification of biomarkers, such as pentraxin-3 (PTX-3) in human serum may play a key role [2]. Unfortunately, human serum is a complex sample, in which the presence of high abundance proteins, namely human serum albumin (HSA) and immunoglobulin G (IgG) is a barrier to accurate detection [3]. Thus, a sample pretreatment step is often required to separate the desired biomarker from the most abundant proteins, further improving detection [3]. To address this need, this study aims to design and evaluate three-phase partitioning (TPP) based on aqueous biphasic systems (ABS) as alternative approaches to perform human serum pretreatment. Systems composed of the homopolymers polypropylene glycol (PPG) and polyethylene glycol (PEG) or copolymers Pluronics and UCON combined with citrate buffered salt were investigated. Their ability to simultaneously deplete HSA and IgG at the interphase and extract PTX-3 in one of the aqueous phases was evaluated. When adequately designed, ABS-TPP present depletion efficiencies above 80% for high abundance proteins, while being able to extract PTX-3 to the polymer-rich phase in one step. A more accurate quantification of PTX-3 by ELISA was obtained in ABS-TPP-pretreated (relative error of 0.8%) than in non-pretreated serum samples (relative error of 28.1%). These results confirm the applicability of polymer-based ABS-TPP to improve the detection of lung cancer biomarkers in complex biological samples.

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**Keywords**: aqueous biphasic systems, pentraxin-3, lung cancer, extraction

**References:**

1. Sung, H.; Ferlay, J.; Siegel, R.L.; Laversanne, M.; Soerjomataram, I.; Jemal, A.; Bray, F. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. CA. Cancer J. Clin. 2021, 71, 209–249, doi:10.3322/caac.21660.

2. Diamandis, E.P.; Goodglick, L.; Planque, C.; Thornquist, M.D. Pentraxin-3 Is a Novel Biomarker of Lung Carcinoma. Clin. Cancer Res. 2011, 17, 2395–2399.

3. Lee, P.Y.; Osman, J.; Low, T.Y.; Jamal, R. Plasma/Serum Proteomics: Depletion Strategies for Reducing High-Abundance Proteins for Biomarker Discovery. Bioanalysis 2019, 11, 1799–1812, doi:10.4155/BIO-2019-0145.

16

POSTER

# A fast amperometric immunosensor for egg allergen analysis in foodstuff

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Food allergies are considered a pressing public health issue because it affects a considerable percentage of the population (over 10%). As this condition does not have a clinically approved treatment, assertive food labelling is required to prevent accidental allergen intake. European legislation establishes that commercial food products must declare the presence of major allergens, such as egg (Andrew T., et al. 2022). Ovotransferrin (Gal d 3) is an important egg white allergen that should be tracked in foodstuff since it causes adverse reactions in hypersensitive individuals. Therefore, this allergen was the target analyte of this study that consisted of the development of an amperometric immunosensor for food safety & quality control of commercial products. A set of experimental parameters of a sandwich-type assay was optimised, resulting in a 30-min assay with a remarkable analytical performance. The quantification range for Gal d 3 was established between 55 and 1000 ng/mL, with a limit of detection of 16 ng/mL. The coefficient of variation of the method (Vx0 = 5.5%) demonstrated the good precision of the developed biosensor. Gal d 3 was successfully applied to several samples: 25 foods were analysed, including products with labels that indicate "does not contain egg", "may contain traces of egg", "contains egg" and/or "contains egg white". Furthermore, the influence of food processing on the allergen amount was verified through the analysis of 7 raw and/or cooked egg and white egg samples.

**Keywords**: Food Allergy, Egg White, Ovotransferrin, Electrochemical Biosensor

**References:**

Andrew, T.; Fong, M.D.; Ahlstedt, S.; Golding, M.A.; Protudjer, J.L.P. The Economic Burden of Food Allergy: What We Know and What We Need to Learn. Curr Treat Options Allergy. 2022, 9, 169–186; DOI 10.1007/s40521-022-00306-5

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# Potential of *in vitro* models to address normal and diseased periodontium

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Periodontal disease (PD) is a multifactorial disease caused by plaque and inflammatory response that is a serious problem in dogs due to late diagnosis, poor oral hygiene, and the size of the oral cavity. PD usually leads to loss of quality of life and medical expenses in the treatment, maintenance, and subsequent prevention of the disease due to the different sequential phases and therapeutic needs. Currently, PD treatment involves complete surgical plaque removal, administration of systemic and local antibiotics, followed by guided tissue regeneration and bone grafting to restore periodontal tissues and bone. The use of biomaterials in humans has proven to be very relevant due to their properties, such as easy customisation in terms of composition and size, which allows reducing postoperative time and promoting patient well-being. As an alternative to more invasive materials, these biomaterials are being studied in dogs. These animals are used as pre-clinical models to study PD due to their biological attributes and the high prevalence of the disease.
The periodontal tissue is one of the most complex connective tissues due to the various types of cells that interact to achieve the maintenance of all the tissues that make up the periodontium. As such, most studies addressing this tissue are performed *in vivo* due to the complexity of the cellular and molecular microenvironment. As expected, ethical issues are being raised in the use of animals in medical research. The main objective of this study is to address the lack of *in vitro* models in order to reduce the use of animals in pre-clinical studies, which will demonstrate major advances in medical research. Biomaterials approaches are being studied such as the generation of a multifunctional periodontal system involving a multiphase scaffolding system and *in vitro* models such as a 3D periodontal pocket. These advances demonstrate the potential to study and understand more about periodontal tissue and disease and address current ethical issues using the 3R's principle in animal experimentation (replacement, reduction and refinement).

**Keywords**: periodontal disease, dog, biomaterials, in vitro models.

**References:**

Andreasen, J. O.; Andersson, L. Critical considerations when planning experimental in vivo studies in dental traumatology. Dent Traumatol. 2011, 27(4), 275-280; DOI:10.1111/j.1600-9657.2011.00983.x

Dannan, A.; Alkattan, F. Animal Models in Periodontal Research: A Mini-Review of the Literature. The Internet Journal of Veterinary Medicine, 2008, 5(1).

Donos, N.; Park, J.C.; Vajgel, A.; de Carvalho Farias, B.; Dereka, X. Description of the periodontal pocket in preclinical models: limitations and considerations. Periodontol 2000, 2018, 76(1), 16-34; DOI:10.1111/prd.12155

Harvey, C.E. Management of periodontal disease: understanding the options. Veterinary Clinics of North America: Small Animal Practice, 2005, 35(4), 819-836; DOI:10.1016/j.cvsm.2005.03.002

Koch, F.; Meyer, N.; Valdec, S.; Jung, R.E.; Mathes, S.H. Development and application of a 3D periodontal in vitro model for the evaluation of fibrillar biomaterials. BMC Oral Health, 2020, 20(1), 148; DOI:10.1186/s12903-020-01124-4

Matarese, G.; Ramaglia, L.; Fiorillo, L.; Cervino, G.; Lauritano, F.; Isola, G. Implantology and Periodontal Disease: The Panacea to Problem Solving? Open Dent J, 2017, 11, 460-465; DOI:10.2174/1874210601711010460

Muthanandam, S.; Muthu, J.; Mony, V.; Rl, P.; K, P. Animal models in dental research – A review. International Dental Journal of Student Research, 2020, 8, 44-47; DOI:10.18231/j.idjsr.2020.010

Park, C.H. Biomaterial-Based Approaches for Regeneration of Periodontal Ligament and Cementum Using 3D Platforms. International Journal of Molecular Sciences, 2019, 20(18), 4364; DOI:10.3390/ijms20184364

Sculean, A.; Chapple, I.L.C.; Giannobile, W.V. Wound models for periodontal and bone regeneration: the role of biologic research. Periodontology 2000, 2015, 68(1), 7-20; DOI:10.1111/prd.12091

Silvério, K.G.; Rodrigues, T.L.; Coletta, R.D.; Benevides, L.; Da Silva, J.S.; Casati, M.Z.; Sallum, E.A.; Nociti, F.H.Jr. Mesenchymal stem cell properties of periodontal ligament cells from deciduous and permanent teeth. J Periodontol, 2010, 81(8), 1207-1215; DOI:10.1902/jop.2010.090729

18

POSTER

# Magnetic electrochemical immunosensing to track a major celery allergen in foods

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Celery (*Apium graveolens*) found in leaves, stalks, and roots is often used in daily nutrition and can also be found in commercial meals and foodstuff. The seeds and/or seed power are largely used as a spice. However, the presence of celery, even in trace amounts, can trigger allergic reactions for sensitive individuals. Although the stalk is highlighted regarding its potential allergenicity, the root and celery oil have been described to cause anaphylactic reactions (Salehi B. et al. 2019). To track the presence of this plant-based allergen in food products, portable and easy-to-use biosensors can be a fit for purpose solution.

This work describes the development and application of the first nanomagnetic-based voltammetric immunosensor to track the major celery allergen Api g 1 in pre-packed food products from local supermarkets (Porto, Portugal). Besides this, the allergen was quantified in the distinct celery fractions (leaves, stalks, and roots). Using screen-printed electrodes as a disposable transducer, the developed methodology is sustainable and low-cost, allows product label content validation and quality control, in 2h30min, contributing to safe food intake, better health and wellbeing.

**Keywords**: Commercial food products, Api g 1, Trace analysis, hybrid magnetic nanomaterial, immunosensor

**References:**;

Salehi, B.; Venditti, A.; Frezza, C.; Yücetepe, A.; Altunta, U.; Uluata, S.; Butnariu, M.; Sarac, I.; Shaheen, S.; Petropoulos, S.A.; et al. Apium Plants: Beyond Simple Food and Phytopharmacological Applications. Appl. Sci. 2019, 9, 3547; DOI: 10.3390/app9173547

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# Fish allergen analysis in commercial products: electrochemical biosensor development

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Fish is an important constituent of the human diet. However, some species may cause severe allergic reactions, even in trace amounts, making fish allergies among the most common food allergies. The intake of food containing fish allergens can trigger immunoglobulin E (IgE)-mediated allergic reactions, such as urticaria, diarrhea, and dyspnea. Fish allergenicity is mainly related to parvalbumin. This ~12 kDa sarcoplasmic protein is believed to be responsible for 95% of allergic reactions in individuals affected by IgE-mediated fish hypersensitivity [1].
In the present work screen-printed carbon electrodes were used for the development of a portable electrochemical immunosensor for the analysis of beta-parvalbumin. In a single-step non-competitive (sandwich) assay (analysis time: 1 h), using an enzyme-labelled detection antibody and amperometry for signal acquisition, it was possible to quantify the target allergen in a set of foods that includes a variety of fish (e.g., salmon, codfish, mackerel, sardines, tuna, etc.), sushi, and others commercial products. The accuracy of the results was confirmed by comparison with a conventional enzyme-linked immunosorbent assay. The performance characteristics along with the simplicity of the analytical procedure makes this sensor a promising solution for both the food industry and regulatory agencies.

**Keywords**: Food safety, Food allergy, Amperometric immunosensor, Parvalbumin

**References:**

[1] D. Jiang, H. Jiang, J. Ji, X. Sun, H. Qian, G. Zhang, L. Tang, Mast-Cell-Based Fluorescence Biosensor for Rapid Detection of Major Fish Allergen Parvalbumin, J. Agric. Food Chem. 2014, 62, 6473−6480. doi.org/10.1021/jf501382t.

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20

POSTER

# Impact of Umbilical Cord Mesenchymal Stem Cells Secretome in Cancer Progression

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Ovarian cancer is a malignant neoplasm exclusive to females that develops in the ovaries, female sex organs and epithelial ovarian cancer [1]. It has a high mortality rate and is the seventh leading cause of death in women diagnosed with cancer because it develops silently and diagnostic tests have a low predictive value [1–3]. Thus, developing more accurate diagnostic tests combined with new anti-tumor therapies advance are needy measure to fight this disease. Mesenchymal stem cells (MSC) are multipotent cells, characterized by high proliferation rate and immunomodulatory, secretory, and paracrine properties. Theis cell type can be obtained from several sources in the human body such as marrow bone, adipose tissue and Umbilical cord (UC) [4]. UC stands out for being a source that is easily accessible and obtainable, non-invasive, and contains cells with high proliferation and differentiation rates [5]. It has been verified that these cells may be involved in tumor regulatory processes, exerting their effects on metabolic processes such as cell proliferation, survival, angiogenesis, and in tumor-associated inflammatory and immunosuppressive processes [6].
The present work aimed to evaluate how the secretome of human UC-derived MSC (hUC-MSC) influences the tumor capacity of the epithelial ovarian cancer cell line SKOV-3.
Conditioned medium (CM) was obtained from hUC-MSC culture and then tumor cells were incubated with different concentrations of conditioned medium, 100%, 50%, and 25%. Parameters such as viability, motility, and cell interactions were evaluated. From the results obtained it was found that CM decreases cell viability, proliferation, and cell-cell interactions, however it stimulated the motility of SKOV-3 cells. In summary, it is possible to conclude that MC from hUC-MSC presented an antitumor effect and can be used as a possible co-adjuvant therapy in the treatment of ovarian cancer.

**Keywords**: Mesenchymal stem cells; Acelluar Therapy; Conditioned medium; Ovarian cancer

**References:**

1. Jayson, G.C.; Kohn, E.C.; Kitchener, H.C.; Ledermann, J.A. Ovarian Cancer. Lancet 2014, 384, 1376–1388, doi:https://doi.org/10.1016/S0140-6736(13)62146-7.

2. Arora, T.; Mullangi, S.; Lekkala, M.R. Ovarian Cancer; 2022;

3. Momenimovahed, Z.; Tiznobaik, A.; Taheri, S.; Salehiniya, H. Ovarian Cancer in the World: Epidemiology and Risk Factors. Int. J. Womens. Health 2019, 11, 287–299, doi:10.2147/IJWH.S197604.

4. Driscoll, J.; Patel, T. The Mesenchymal Stem Cell Secretome as an Acellular Regenerative Therapy for Liver Disease. J. Gastroenterol. 2019, 54, 763–773, doi:10.1007/s00535-019-01599-1.

5. Nagamura-Inoue, T. Umbilical Cord-Derived Mesenchymal Stem Cells: Their Advantages and Potential Clinical Utility. World J. Stem Cells 2014, 6, 195, doi:10.4252/wjsc.v6.i2.195.

6. Liang, W.; Chen, X.; Zhang, S.; Fang, J.; Chen, M.; Xu, Y.; Chen, X. Mesenchymal Stem Cells as a Double-Edged Sword in Tumor Growth: Focusing on MSC-Derived Cytokines. Cell. Mol. Biol. Lett. 2021, 26.

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# Sweet bone – a new ex vivo model of the bone tissue development in hyperglycemic conditions

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Diabetes mellitus (DM) is a highly prevalent group of chronic metabolic conditions associated with high morbidity[1]. Its deleterious effects in different tissues and organs, including bone, are well described [1-3]. Hyperglycemia seems to be one of the most contributing etiological factors of bone-related alterations, altering metabolic functionality and inducing morphological adaptations [4-6]. Despite the established models for the assessment of bone functionality in hyperglycemic conditions, in vitro studies present limited representativeness given the restricted cell-cell and cell-matrix interactions, and three-dimensional spatial arrangement; while in vivo studies raise ethical issues and offer limited mechanistic characterization, given the modulatory influence of many systemic factors and/or regulatory systems [7,8].
This study aims to establish the influence of the hyperglycemic condition on bone tissue development, using the embryonic ex vivo chicken femur model. Thus, embryonic femurs of *Gallus domesticus* were obtained and cultured in organotypic air/liquid interface, for eleven days, in conditions that mimic hiperglycaemia. Upon that time, samples were further analyzed. Results show that, despite the enhanced collagen production under the presence of high levels of glucose, structural discrepancies were verified, possibly related to the increased oxidative stress. Also, the mineralization process is severely impaired with subsequent alteration of bone's three-dimensional structure. Furthermore, pro-inflammatory genes are overexpressed under hyperglycemic conditions. Present study findings are coherent with previous *in vitro* and *in vivo* studies, therefore the ex vivo embryonic chicken femur shows potential as a tool to further screen the effects of hyperglycemic conditions on bone tissue metabolism and development.

**Keywords**: Diabetes mellitus, bone tissue, embryonic chicken femur, ex vivo.

**References:**

Cole, J.B.; Florez, J.C. Genetics of diabetes mellitus and diabetes complications. Nat Rev Nephrol 2020, 16, 377-390, doi:10.1038/s41581-020-0278-5.

Olokoba, A.B.; Obateru, O.A.; Olokoba, L.B. Type 2 diabetes mellitus: a review of current trends. Oman Med J 2012, 27, 269-273, doi:10.5001/omj.2012.68.

Raisingani, M.; Preneet, B.; Kohn, B.; Yakar, S. Skeletal growth and bone mineral acquisition in type 1 diabetic children; abnormalities of the GH/IGF-1 axis. Growth Hormone & IGF Research 2017, 34, 13-21, doi:https://doi.org/10.1016/j.ghir.2017.04.003.

Filion, T.M.; Skelly, J.D.; Huang, H.; Greiner, D.L.; Ayers, D.C.; Song, J. Impaired osteogenesis of T1DM bone marrow-derived stromal cells and periosteum-derived cells and their differential in-vitro responses to growth factor rescue. Stem Cell Res Ther 2017, 8, 65, doi:10.1186/s13287-017-0521-6.

Kalaitzoglou, E.; Popescu, I.; Bunn, R.C.; Fowlkes, J.L.; Thrailkill, K.M. Effects of Type 1 Diabetes on Osteoblasts, Osteocytes, and Osteoclasts. Curr Osteoporos Rep 2016, 14, 310-319, doi:10.1007/s11914-016-0329-9.

Kanazawa, I.; Sugimoto, T. Diabetes Mellitus-induced Bone Fragility. Intern Med 2018, 57, 2773-2785, doi:10.2169/internalmedicine.0905-18.

Cramer, E.E.A.; Ito, K.; Hofmann, S. Ex vivo Bone Models and Their Potential in Preclinical Evaluation. Current Osteoporosis Reports 2021, 19, 75-87, doi:10.1007/s11914-020-00649-5.

Papadimitriou, K.; Kostopoulos, I.V.; Tsopanidou, A.; Orologas-Stavrou, N.; Kastritis, E.; Tsitsilonis, O.E.; Dimopoulos, M.A.; Terpos, E. Ex Vivo Models Simulating the Bone Marrow Environment and Predicting Response to Therapy in Multiple Myeloma. Cancers 2020, 12, 2006.

22

POSTER

# Biological evaluation of antimicrobial catheter surfaces functionalized by plasma-mediated bond of rhamnolipids

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Antimicrobial surfaces are an emergent need to overcome bacteria colonization on medical devices. The increasing usage of medical devices provoked an escalation of healthcare-associated infections (HAIs), resulting in higher morbidity and mortality. Among all HAIs, bloodstream catheter infections correspond to almost 50%, due to a surface prone to bacteria adherence and colonization[1]. To overcome this problem, a novel approach was devised by using biosurfactants in the surface's functionalization process. Rhamnolipids (RLs) are biosurfactant-molecules endowed with antimicrobial activities, biosynthesized by bacteria.

Previously, the surface of medical-grade polydimethylsiloxane (PDMS) was functionalized with a RLs mixture (RLs mix) and an isolated RL (di-RL)[2]. Further, antibiofilm properties of the materials were evaluated using representative bacteria of bloodstream catheter infections. Therefore, this study aimed to evaluate the *in vitro* biological response to the PDMS functionalized materials (RLs mix and di-RL).The biocompatibility of the functionalized materials was evaluated using human dermal fibroblasts (HDFs, AG22719). Metabolic activity was assessed by MTT and resazurin assays, while cell morphology was observed by fluorescent images, after staining cellular actin cytoskeleton and nucleus; vascular irritation potential was assessed through the Hen's egg-chorioallantoic membrane (HET-CAM) test; hemocompatibility was demonstrated via hemolysis rate and platelet adhesion using heparinized human blood. Assays were performed directly (cells seeded over the materials) and/or indirectly (cells seeded in the presence of materials' leachates).

HDF cells were unable to attach over functionalized surfaces, presenting a significantly lower metabolic activity, confirmed by fluorescent images, where cells were rarely identified and presented an irregular morphology. Oppositely, cells cultured with materials' leachates presented similar metabolic activity and morphology to control cultures. Moreover, materials were rated as non-irritating substrates, once no significant vascular alterations were observed during the HET-CAM test. Finally, developed materials were also considered hemocompatible, presenting a low hemolysis rate and limited platelet activation, with no identifiable dendritic or spread morphologic features.Overall, functionalized PDMS were found to be biocompatible and presented an adequate behavior for vascular catheters, avoiding cell adhesion over its surface, and, simultaneously, an adequate response to neighboring cells and tissues.

**Keywords**: Bio-functionalization; rhamnolipids; medical devices; antibacterial surfaces.

**References:**

1. Ricardo, S.I.C.; Anjos, I.I.L.; Monge, N.; Faustino, C.M.C.; Ribeiro, I.A.C. A Glance at Antimicrobial Strategies to Prevent Catheter-Associated Medical Infections. ACS Infect. Dis. 2020, 6, 3109–3130, doi:10.1021/acsinfecdis.0c00526.

2. Dardouri, M.; Bettencourt, A.; Martin, V.; Carvalho, F.A.; Santos, C.; Monge, N.; Santos, N.C.; Fernandes, M.H.; Gomes, P.S.; Ribeiro, I.A.C. Using plasma-mediated covalent functionalization of rhamnolipids on polydimethylsiloxane towards the antimicrobial improvement of catheter surfaces. Mater. Sci. Eng. C 2021, 134, 112563, doi:10.1016/j.msec.2021.112563.

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23

POSTER

# Evaluation of the Potential of Marine Cyanobacteria Towards Alzheimer's disease

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Alzheimer's disease (AD) is one of the most prevalent neurodegenerative diseases (NDs) and the major cause of dementia. Despite the constant efforts to achieve cure, no treatment is currently effective and new approaches are urgently needed[1].
Marine biotechnology has been at the frontline for the bioprospection of natural sources to treat ND[2]. Several marine compounds have already provided evidence for anti-AD drugs by acting in mechanisms such as the inhibition of acetylcholinesterase (AChE). Among marine organisms, cyanobacteria have been recognized as a major source of bioactive compounds and the potential towards AD has been highlighted, namely AChE inhibitors[3].
This project is included in a screening program that aims to evaluate the potential of cyanobacteria from the Culture Collection of CIIMAR -LEGE-CC against AD. In this work, 80 fractions of marine cyanobacteria were screened for the potential to inhibit AChE since this is a proven enzyme target to ameliorate the loss of cholinergic signal and to induce cytotoxicity in the neuroblastoma cell line SHY5Y, in the normal fibroblasts cell line 3T3L1, and in the endothelial cells from central nervous system capillaries hCMEC/D3. Results considering the enzymatic assays and cytotoxic assays revealed that the fractions are not toxic or with low toxicity. The strains *Phormidium sp;* an unidentified filamentous *Synechococcales* and *Nodosilinea nodulosa* revealed moderate toxicity towards AChE.

**Keywords**: Alzheimer disease; cyanobacteria; cytotoxicity; acetylcholinesterase

**References:**

1. Cummings, J.; Lee, G.; Zhong, K.; Fonseca, J.; Taghva, K. Alzheimer's Disease Drug Development Pipeline: 2021. Alzheimer's Dement. Transl. Res. Clin. Interv. 2021, 7, 1–24, doi:10.1002/trc2.12179.

2. Lakshmi, S.; Prakash, P.; Essa, M.M.; Qoronfleh, W.M.; Akbar, M.; Song, B.J.; Kumar, S.S.; Elumalai, P. Marine Derived Bioactive Compounds for Treatment of Alzheimer's Disease. Front. Biosci. - Elit. 2018, 10, 537–548, doi:10.2741/e840.

3. Castaneda, A.; Ferraz, R.; Vieira, M.; Cardoso, I.; Vasconcelos, V.; Martins, R. Bridging Cyanobacteria to Neurodegenerative Diseases: A New Potential Source of Bioactive Compounds against Alzheimer's Disease. Mar. Drugs 2021, 19, doi:10.3390/md19060343.

24

POSTER

# Actinobacteria as new probiotics for preventing fish diseases in aquaculture

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In order to meet the food needs of a constantly growing world population, aquaculture emerges as the fastest growing food production sector [1]. However, disease outbreaks due to opportunistic bacterial pathogens cause large losses [2, 3]. Antibacterial chemicals used indiscriminately have resulted in resistant bacteria and antibiotic resistance genes [4, 5]. Ecological solutions such as probiotics are needed [6]. Probiotics are microorganisms introduced into another organism that can have beneficial effects on the host as well as improve the external environment [7]. Actinobacteria are an important biotechnological resource, as they produce a great diversity of secondary metabolites with a high range of bioactivities, including antimicrobial [7]. The aim of this study was to explore the probiotic potential of some marine actinobacteria to prevent aquaculture fish diseases. For this, a screening of antimicrobial activity was performed using agar-based disk diffusion and Minimum Inhibitory Concentration (MIC) assays, against three Gram-negative bacterial fish pathogens: *Aeromonas hydrophila*, *Edwardsiella tarda* and *Pseudomonas anguilliseptica*. A total of 54 out of 103 actinobacterial extracts tested, presented bioactivity against these microorganisms, so that the growth of all three Gram-negative pathogens used was inhibited. The diameter of the inhibition halos varied between 8 and 20 mm. Tested extracts presented MIC values of 1000 µg/ml, except for *Isoptericola chiayiensis* (500 µg/ml). *Microbispora bryophytorum*, *Isoptericola chiayiensis* and *Nocardia nova* were some of the bacteria with the most promising results. Overall, actinobacteria associated with seaweed proved to be a potential source of probiotics to inhibit pathogens in aquaculture fish.

**Keywords**: Antimicrobial, aquaculture, marine actinobacteria, probiotics.

**References:**

1. Ahmed, N.; Thompson, S.; Glaser, M. Global Aquaculture Productivity, Environmental Sustainability, and Climate Change Adaptability. Environmental Management 2019, 63, 159-172; DOI:10.1007/s00267-018-1117-3

2. Bondad-Reantaso, M.G.; Subasinghe, R.P.; Arthur, J.R.; Ogawa, K.; Chinabut, S.; Adlard, R.; Tan, Z.; Shariff, M. Disease and health management in Asian aquaculture. Vet Parasitol 2005, 132, 249-272; DOI: 10.1016/j.vetpar.2005.07.005

3. Das, S.; Ward, L.R.; Burke, C. Prospects of using marine actinobacteria as probiotics in aquaculture. Appl Microbiol Biotechnol 2008, 81, 419-429; DOI: 10.1007/s00253-008-1731-8

4. Tan, L.T.; Chan, K.G.; Lee, L.H.; Goh, B.H. Streptomyces Bacteria as Potential Probiotics in Aquaculture. Front Microbiol 2016, 7, 79; DOI:10.3389/fmicb.2016.00079

5. Nhinh, D.T.; Le, D.V.; Van, K.V.; Giang, N.T.H.; Dang, L.T.; Hoai, T.D. Prevalence, Virulence Gene Distribution and Alarming the Multidrug Resistance of Aeromonas hydrophila Associated with Disease Outbreaks in Freshwater Aquaculture. Antibiotics-Basel 2021, 10; DOI:10.3390/antibiotics10050532

6. Wang, A.R.; Ran, C.; Wang, Y.B.; Zhang, Z.; Ding, Q.W.; Yang, Y.L.; Olsen, R.E.; Ringo, E.; Bindelle, J.; Zhou, Z.G. Use of probiotics in aquaculture of China-a review of the past decade. Fish & Shellfish Immunology 2019, 86, 734-755; DOI:10.1016/j.fsi.2018.12.026

7. Jagannathan, S.V.; Manemann, E.M.; Rowe, S.E.; Callender, M.C.; Soto, W. Marine Actinomycetes, New Sources of Biotechnological Products. Mar Drugs 2021, 19; DOI:10.3390/md19070365

# Development of a biosensor for detection of *Escherichia coli* in urine

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Urinary tract infections (UTI) are part of the range of infectious diseases and correspond to the abnormal presence of pathogens in some regions of the urinary tract. Although UTIs also affect men, the incidence rate in women is much higher (80% to 90%).(Haddad & Fernandes, 2019).The main causative agent of UTI (approximately 75% of cases) is *Escherichia coli* (*E.coli*).(Flores-Mireles *et al.*, 2015) Patients with symptomatic infections will have >$10^5$ bacteria/mL and inflammatory cells in their freshly excreted urine.(Chu & Lowder, 2018)

UTIs usually have their treatment summarized by taking antibiotics, therefore, in this project, a characterization of the antibiotic resistance of the different strains used are also made.In the present work, we aimed to develop a biosensor to facilitate the early diagnosis of UTIs. Polyclonal anti-*E.Coli* antibodies of capture (conjugated with Ulfa-Tag) and detection (conjugated with gold) were used to detect the presence of *E.Coli* through immunochromatography on a nitrocellulose membrane impregnated with anti-Ulfa-Tag antibodies based on the methodology of lateral flow tests. As a control, the gold-conjugated streptavidin-biotin complex was used.

Four distinct strains of *E.coli* were tested, *E.coli* S3R9, E.coli S3R22, *E.coli* ATCC 8739 and *E.coli* ATCC 25922, at concentrations from $10^3$ to $10^{10}$ bacteria/mL.Evaluating the results, it was possible to detect the presence of *E.Coli* at concentrations higher than 108 in samples with intact cells and in extracts. Although it is only possible to detect the presence of *E.Coli* at very high concentrations, the biosensor developed may, in the future, undergo protocol optimizations in order to detect lower concentrations.

In short, we can conclude that the development of a biosensor for the detection of UTIs caused by *E. Coli* (without the need for the previous culture), will be a decisive milestone in the early diagnosis of this type of infection with direct implications for treatment and prognosis, idealizing a better quality of life for society.

**Keywords**: Escherichia coli, infection, urinary, biosensor

**References:**

Chu, C. M., & Lowder, J. L. (2018). Diagnosis and treatment of urinary tract infections across age groups. American journal of obstetrics and gynecology, 219(1), 40-51.

Flores-Mireles, A. L., Walker, J. N., Caparon, M., & Hultgren, S. J. (2015). Urinary tract infections: epidemiology, mechanisms of infection and treatment options. Nature reviews microbiology, 13(5), 269-284.

Haddad, J. M., & Fernandes, D. A. O. (2019). Infecção do trato urinário. Femina, 47(4), 241-244.

26

POSTER

# Stabilization and purification of RNA using biocompatible ionic liquids

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The widespread use of RNA for the development of novel therapies has been hindered due to issues associated with its inherent chemical instability and fast degradation by ribonucleases[1]. In addition, the traditional extraction and purification techniques of this biomolecule are time-consuming and require highly toxic reagents, demanding for improved technologies able to provide RNA with high integrity, purity and biological activity.

To overcome the described bottlenecks, ionic-liquid-based aqueous biphasic systems (IL-based ABS), which are mainly composed of water[2], are herein investigated as more sustainable and efficient techniques for RNA purification. Due to the high affinity between amino-acids and RNA and the favourable nucleic acids-stabilization properties exhibited by amino-acid-based ILs (AA-ILs)[1], they were selected as components of ABS formulations. After identifying the most promising systems able to protect RNA from ribonuclease-mediated degradation, the ultimate goal of this work is to purify RNA from a complex recombinant lysate, envisaging the development of integrated purification-preservation platforms.

AA-ILs comprising L-arginine, L-tryptophan and L-lysine as cations combined with chloride or DL-aspartate ([Arg]Cl, [Trp]Cl, [Lys]Cl, [Arg][Asp], [Trp][Asp], and [Lys][Asp]) were synthesized and characterized, and their ability to form two phases with distinct salts and polymers investigated. With exception of [Trp]Cl and [Lys][Asp] that are unable to form ABS with the compounds in study, all the remaining AA-ILs form ABS with polypropylene glycol 400 (PPG 400). Initial studies on the stability and integrity of RNA have been performed in aqueous solutions of the studied ILs to assess its potential to act as preservation media. Several promising IL-based ABS were found and are currently under investigation as integrated purification-preservation platforms for RNA envisaging its use as biotherapeutics.

**Keywords**: RNA, Aqueous biphasic systems, Amino acid ionic liquid, Purification

**References:**

1. Quental, M. V.; Pedro, A. Q.; Pereira, P.; Sharma, M.; Queiroz, J. A.; Coutinho, J. A. P.; Sousa, F.; Freire, M. G. Integrated Extraction-Preservation Strategies for RNA Using Biobased Ionic Liquids. ACS Sustain. Chem. Eng. 2019, 7 (10), 9439–9448. https://doi.org/10.1021/acssuschemeng.9b00688.

2. Pereira, J. F. B.; Freire, M. G.; Coutinho, J. A. P. Aqueous Two-Phase Systems: Towards Novel and More Disruptive Applications. Fluid Phase Equilib. 2020, 505, 112341. https://doi.org/10.1016/j.fluid.2019.112341.

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27

POSTER

# Resistance of actinobacteria and rhizobia from wild Saharan plants to environmental stresses and their phosphate solubilization capacity

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Plant growth promoting bacteria (PGPB) enhance plant development through various mechanisms such as the fixation of atmospheric nitrogen, production of siderophores and phosphate solubilization [1]. This could be beneficial for the implementation of more sustainable agricultural practices. The objective of this study was to evaluate the effect of temperature and salinity variation as well as to evaluate the phosphate solubilization capacity of a collection of bacteria, originating from various sites in the Algerian Sahara Desert [2,3]. These sites are affected by abiotic stresses such as wide variations in temperature range and high salinity levels, so PGPB for inoculation in these soils need to be adaptable and withstand extreme conditions. To carry out this study, the bacteria were subjected to different temperatures and concentrations of NaCl [4]. The phosphate solubilization capacity was evaluated, in the National Botanical Research Institute's Phosphate Growth (NBRIP) medium. Overall, the tested bacteria did not solubilize phosphate, since no halos were observed around the bacterial colonies. The bacteria most tolerant to a wider range of temperatures and salinities and, therefore, most promising for inoculation in semi-arid and arid soils were *Afipia sp.* 61 and *Afipia massiliensis* 21, which showed significant growth in all studied conditions. In general, it was observed that most bacteria grow at temperatures and salinities, different from their common growth range.

**Keywords**: Abiotic stress; actinobacteria; N2 fixation; rhizobia-legume symbioses

**References:**

1. Purbani, D.C. Cyanobacteria from Sorghum Bicolor-Grown Fields of Ecopark at Cibinong Science Center-Botanic Gardens, Indonesia; 2019; ISBN 9789811367892.

2. Bhattacharyya, P.N.; Jha, D.K. Plant Growth-Promoting Rhizobacteria (PGPR): Emergence in Agriculture. World J. Microbiol. Biotechnol. 2012, 28, 1327–1350, doi:10.1007/s11274-011-0979-9.

3. Zahran, H.H. Rhizobium -Legume Symbiosis and Nitrogen Fixation under Severe Conditions and in an Arid Climate . Microbiol. Mol. Biol. Rev. 1999, 63, 968–989, doi:10.1128/mmbr.63.4.968-989.1999.

4. Backer, R.; Rokem, J.S.; Ilangumaran, G.; Lamont, J.; Praslickova, D.; Ricci, E.; Subramanian, S.; Smith, D.L. Plant Growth-Promoting Rhizobacteria: Context, Mechanisms of Action, and Roadmap to Commercialization of Biostimulants for Sustainable Agriculture. Front. Plant Sci. 2018, 871, 1–17, doi:10.3389/fpls.2018.01473.

28

POSTER

# Ionic Levothyroxine Formulations with Improved Bioavailability Properties

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Thyroid diseases affect a considerable portion of the population, with hypothyroidism being one of the most commonly reported thyroid diseases [1]. Levothyroxine (T4) is clinically used to treat hypothyroidism and to suppress thyroid stimulating hormone secretion in other thyroid diseases. The narrow therapeutic index of this drug, the need for a frequent administration and the influence of gastrointestinal diseases, foods and other drugs on its absorption are the shortcomings related with oral administration of T4 [2]. There are several approaches to enhancing the drug solubility and bioavailability, such as particle size reduction, nanosuspension, the use of surfactants, salt formation, and solid dispersion, among others [3]. In this work, an attempt to improve T4 solubility is made through the synthesis of T4 salts based on Ionic Liquids (ILs). ILs based on pharmaceutical drugs (API-ILs) are a class of low melting organic salts with promising therapeutic properties [4–5]. Herein, T4 was used as an anion in combination with choline and 1-ethanol-3-methylimidazolium [C2OHMIM] cations. All prepared compounds were characterized by 1H- and 13C-NMR, ATR-FTIR, elemental analysis to confirm their structures and purity levels. The water and serum solubilities of the prepared T4-ILs were compared with the original T4 drug, as was the thermal analysis, which was carried out through differential scanning calorimetry (DSC) studies. An improved adsorption capacity was determined through the permeability studies and, cytotoxicity assays revealed that cellular viability in Caco-2 cells was preserved. The ionic levothyroxine formulations were thus considered to present a good potential to be used as an alternative to levothyroxine commercial formulations with improved bioavailability.

**Keywords**: levothyroxine (T4); API-ILs; solubility studies

**References:**

1. Chiovato, L.; Magri, F.; Carlé, A. Hypothyroidism in context: Where we've been and where we're going. Adv. Ther. 2019, 36, 47–58. DOI: 10.1007/s12325-019-01080-8

2. Wartofsky, L. Levothyroxine: Therapeutic use and regulatory issues related to bioequivalence. Expert Opin. Pharmacother. 2002, 3, 727–32. DOI: 10.1517/14656566.3.6.727

3. Patel, V.R.; Agrawal, Y.K. Nanosuspension: An approach to enhance solubility of drugs Adv. Pharm. Technol. Res. 2011, 2, 81–87. DOI: 10.4103/2231-4040.82950

4. Marrucho, I.M.; Branco, L.C.; Rebelo, L.P.N. Annual. Reviews. Chem. Biomol. Eng. 2014, 527-546.

5. Santos, F.; Branco, L.C.; Duarte, A.R.C. Organic salts based on isoniazid drug: Synthesis, bioavailability and cytotoxicity studies. Pharmaceutics 2020, 12, 952-967. DOI: 10.3390/pharmaceutics12100952

# *Salicornia ramosissima*: A possible green alternative with valuable bioactive properties for cosmetics

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The cosmetic industry has been continuously growing worldwide, being one of the biggest and successful industries. In recent times, consumers have been more aware of the cosmetics origin and production processes as well as their ecological implications and safety. Since consumers correlate botanicals with safeness, these ingredients began to constitute desirable compounds over synthetic ones for cosmetic formulations. *Salicornia ramosissima* is a halophyte plant widely distributed. Despite its human consumption, halophytes remain under valorized regarding its bioactive composition, being rich in antioxidants and lipid components. This study aims to extract and validate a new potential cosmetic active ingredient from *S. ramosissima* through *in-vitro* assays, highlighting its potential use in skin formulations. The halophyte's extracts were obtained by subcritical-water extraction (SWE) at different temperatures (110, 120, 140, 160 and 180 °C) and the antioxidant and radical scavenging activities as well as the phenolic profile were screened. The sample extracted at 180 °C showed the highest phenolic content (1739.28 mg/100 g dw) evaluated by HPLC/DAD, along with phenolic acid predominance (1054.77 mg/100 g dw). Despite not being efficient in the sequestration of the $ABTS^{+\cdot}$ radical, this extract was the only that reasonably sequester the $DPPH^{\cdot}$ ($IC_{50}$ = 824.57 µg/mL). The scavenging capacity of superoxide ($O_2^{\cdot-}$) and hypochlorous acid (HOCl) were also considerable (respectively, $IC_{50}$=158.87 µg/mL and $IC_{50}$=5.80 µg/mL). The cell viability assays (assessed by MTT) on keratinocytes,and fibroblasts demonstrated that the keratinocytes viability was not affected after exposure to the extract (0.1 – 1000 µg/mL), while the fibroblasts viability slight decreased after exposure to the highest concentration. Enzymatic assays showed that 760 µg/mL of the halophyte's extract is capable to inhibit elastase by 5%. Contrastingly, a concentration of 64 µg/mL inhibit hyaluronidase by 10%. The results obtained support the bioactivity of *S. ramosissima* and its possible use as a cosmetic ingredient. Further studies, particularly *ex-vivo* permeation assays, should be performed to ensure this new application.

**Keywords**: *Salicornia ramosissima*, Subcritical-water extraction, Cosmetic ingredients, Eco-friendly

**References:**

Kazakova, I.; Lebedynets, V.; Kovalenko, S.; Kazakova, V. Research of the Activities of the Enterprises of the Perfume and Cosmetic Industry of Ukraine. EUREKA: Health Sciences 2021, 44–55, doi:10.21303/2504-5679.2021.001700.

Bilal, M.; Iqbal, H.M.N. New Insights on Unique Features and Role of Nanostructured Materials in Cosmetics. Cosmetics 2020, 7, doi:10.3390/cosmetics7020024.

Morais, T.; Cotas, J.; Pacheco, D.; Pereira, L. Seaweeds Compounds: An Ecosustainable Source of Cosmetic Ingredients? Cosmetics 2021, 8, doi:10.3390/cosmetics8010008.

Zhuang, D.; He, N.; Khoo, K.S.; Ng, E.-P.; Chew, K.W.; Ling, T.C. Application Progress of Bioactive Compounds in Microalgae on Pharmaceutical and Cosmetics. Chemosphere 2022, 291, 132932, doi:https://doi.org/10.1016/j.chemosphere.2021.132932.

Guzmán, E.; Lucia, A. Essential Oils and Their Individual Components in Cosmetic Products. Cosmetics 2021, 8, doi:10.3390/cosmetics8040114.

Borja-Martínez, M.; Lozano-Sánchez, J.; Borrás-Linares, I.; Pedreño, M.A.; Sabater-Jara, A.B. Revalorization of Broccoli By-Products for Cosmetic Uses Using Supercritical Fluid Extraction. Antioxidants 2020, 9, doi:10.3390/antiox9121195.

Kadereit, G.; Ball, P.; Beer, S.; Mucina, L.; Sokoloff, D.; Teege, P.; Yaprak, A.E.; Freitag, H. A Taxonomic Nightmare Comes True: Phylogeny and Biogeography of Glassworts (Salicornia L., Chenopodiaceae). Taxon 2007, 56, 1143–1170, doi:https://doi.org/10.2307/25065909.

Jang, H.-S.; Kim, K.-R.; Choi, S.-W.; Woo, M.-H.; Choi, J.-H. Antioxidant and Antithrombus Activities of Enzyme-Treated Salicornia Herbacea Extracts. Ann Nutr Metab 2007, 51, 119–125, doi:10.1159/000100826.

Isca, V.M.S.; Seca, A.M.L.; Pinto, D.C.G.A.; Silva, H.; Silva, A.M.S. Lipophilic Profile of the Edible Halophyte Salicornia Ramosissima. Food Chem 2014, 165, 330–336, doi:https://doi.org/10.1016/j.foodchem.2014.05.117.

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30

POSTER

# Valorization of fruit leftovers as substrate for inexpensive culture media

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Food waste is a serious problem in the current days [1]. Every year 670 and 630 million tons are wasted in developed and developing countries, respectively. Fruits, vegetables, roots and tubers are among the food items with highest wastage rate [2]. Most of this waste is still a valuable source of nutrients and bioactive compounds that can be a source for an array of microorganisms, not only for their growth and isolation but also to produce different industrial products[3,4]. Hence, the aim of this work is to validate fruit waste as a potential substrate for bacterial growth media. To achieve this, growth rates of several Gram-positive and Gram-negative bacteria, on fruit-based media, were assessed. For this, three different fruit waste media were prepared (A, B and C). Prior to the assays, these wastes were dried at 60°C for 24 hours and then crushed to obtain a powder substrate. For each substrate, 3 concentrations were prepared, 40g/L, 30g/L and 15g/L (dry weight) in distilled water and 1.5% of agar-agar was added to allow medium solidification. *P. aeruginosa*, *P. mirabilis*, *E. coli* and *S. aureus* were cultured in TSB at 37°C for 24 hours, followed by serial dilutions of each inoculation. Afterwards, 20µL of each bacteria culture was inoculated in mediums A, B and C in all distinct concentrations, as well as in TSA as positive control. Incubation carried out at 37°C for 24 hours. CFUs were counted and compared. Results demonstrated that *P. mirabilis* didn't grow in any media in any concentration and *S. aureus* had a slight growth in the highest concentration of B medium and in all concentrations of medium A. *P. aeruginosa* and *E. coli* grew in media A and B in all concentrations. The lack of growth of *P. mirabilis* and *S. aureus* could be due to low concentration of some key nutrients but also the lack of salts in the substrates used [5,6]. Hence, the media content needs further studies, in order to understand what substrates might help improve these strains' growth.

**Keywords**: Agroindustrial waste, Bacterial growth, Culture media.

**References:**

1. Kim, S.; Lee, Y.; Andrew Lin, K.-Y.; Hong, E.; Kwon, E.E.; Lee, J. The valorization of food waste via pyrolysis. Journal of Cleaner Production 2020, 259, doi:10.1016/j.jclepro.2020.120816.

2. UN environment. Worldwide food waste | ThinkEatSave. Available online: https://www.unep.org/thinkeatsave/get-informed/worldwide-food-waste (accessed on 16/09/2022).

3. Lin, Q.; Long, L.; Wu, L.; Zhang, F.; Wu, S.; Zhang, W.; Sun, X. Evaluation of different agricultural wastes for the production of fruiting bodies and bioactive compounds by medicinal mushroom Cordyceps militaris. J Sci Food Agric 2017, 97, 3476-3480, doi:10.1002/jsfa.8097.

4. Arias-Roblero, M.; Mora-Villalobos, V.; Velazquez-Carrillo, C. Evaluation of Fed-Batch Fermentation for Production of Polyhydroxybutyrate With a Banana Pulp Juice Substrate From an Agro Industrial By-Product. Frontiers in Sustainable Food Systems 2021, 5, doi:10.3389/fsufs.2021.681596.

5. Alteri, C.J.; Himpsl, S.D.; Mobley, H.L. Preferential use of central metabolism in vivo reveals a nutritional basis for polymicrobial infection. PLoS Pathog 2015, 11, e1004601, doi:10.1371/journal.ppat.1004601.

6. Becton Dickinson & Company. INSTRUCTIONS FOR USE - READY-TO-USE PLATED MEDIA BD Mannitol Salt Agar. 2013, 3.

31

POSTER

# *In situ* extraction of recombinant extracellular interferon alpha 2b using aqueous micellar two-phase systems

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In the past decades, the development of innovative medicines such as protein-based biopharmaceuticals has improved human lifespan and general health, providing novel avenues for the treatment of chronic disorders. Among them, the interferon alpha-2b (IFNα-2b) has been used in the treatment of chronic hepatitis C with a considerable impact on the global therapeutic proteins market [1]. One of the biggest hindrances to the widespread application of biopharmaceuticals, including IFNα-2b, is their high cost. It is usually associated with expensive chromatographic purification involving multiple steps as well as the low stability of some chromatographic supports. However, competitive, and more sustainable downstream processes can be designed by exploring the ability of aqueous biphasic systems to integrate different stages of bioprocesses [2]. From the exposed, this work investigated the use of poloxamers, polysorbates, and ionic liquids as phase-forming components of aqueous micellar two-phase systems (AMTPS) for the direct capture and extraction of recombinant extracellular IFNα-2b. The extracellular expression of IFNα-2b was achieved using *Pichia pastoris* X-33 harboring a recombinant pPICZα vector cloned with the IFNα-2b gene in frame with the secretion signal alpha mating factor, deemed essential to drive the protein toward the secretory pathway. The production conditions were optimized, being observed that the supplementation of the culture medium with 0.01 % (v/v) Tween-80 highly enhances the levels of correctly processed IFNα-2b in the culture medium. IFNα-2b was successfully extracted from the extracellular medium using AMTPS formed by poloxamers and ionic liquids and are currently being investigated for the *in situ* removal of IFN directly from the extracellular medium.
Overall, this work discloses the potential application of AMTPS formed by poloxamers copolymers to improve the *in situ* extraction of IFNα-2b from the extracellular medium.

**Keywords**: aqueous biphasic systems, ionic liquids, biopharmaceuticals, surfactants

**References:**

(1) Castro, L. S.; Lobo, G. S.; Pereira, P.; Freire, M. G.; Neves, M. C.; Pedro, A. Q. Interferon-Based Biopharmaceuticals: Overview on the Production, Purification, and Formulation. Vaccines 2021, 9 (4), 328. https://doi.org/10.3390/vaccines9040328.

(2) Magalhães, F. F.; Tavares, A. P. M.; Freire, M. G. Advances in Aqueous Biphasic Systems for Biotechnology Applications. Curr. Opin. Green Sustain. Chem. 2021, 27, 100417. https://doi.org/10.1016/j.cogsc.2020.100417.

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32

POSTER

# Development of integrated processes for the recovery of recombinant membrane proteins

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Recombinant protein production became an essential component of most modern small drug discovery programs [1], among of which membrane proteins (MP) are key players. Bioprocessing of recombinant proteins using aqueous biphasic systems (ABS) hold the potential to integrate several downstream units (*e.g.*, cell lysis and MP capture), contributing to reduce costs while ensuring that the stability of the extracted biomolecule is maintained throughout the process [2]. In this regard, it is herein investigated the ability of several surfactants and ionic liquids to solubilize the cell wall and plasma membrane of *Komagataella pastoris* for the recovery of the intracellular MP cyclooxygenase 2 (COX-2). The ultimate goal is to combine this step with the creation of an ABS able to extract and purify this recombinant protein in a single step. Initially, the solubility behaviour of N-acetyl-D-glucosamine (chitin monomers) and ergosterol, representative of structural components of *Komagataella pastoris* cell wall and plasma membrane, were evaluated *in silico* in a wide range of ionic liquids with the aid of conductor like screening model for real solvents (COSMO-RS), and the outcomes compared with experimental permeabilization assays. Moreover, the experimental results obtained so far indicate that intracellular COX-2 extraction is enhanced by a combination of 350 mM 1-decyl-3-methylimidazolium chloride and 162 mM 1-ethyl-3-methylimidazolium acetate using 1.4 x 10^9 cells/mL and during 60 min at 40 °C. The creation and recyclability of an ABS is currently under investigation, guaranteeing that the reproducibility and purity requirements of the original extraction stage are fulfilled. Overall, the approach reported here represents a step forward in the development of efficient processes to overcome the demand for high-quality membrane proteins for structural studies, while the integration of two downstream units may potentially reinforce the sustainability of the process and reduce its cost.

**Keywords**: Bioprocess integration; aqueous biphasic system; purification; ionic liquid; COSMO-RS.

**References:**

1. Pham, P.V. Chapter 19 - Medical Biotechnology: Techniques and Applications. In Omics Technologies and Bio-Engineering, Barh, D., Azevedo, V., Eds.; Academic Press: 2018; pp. 449-469.

2. Pereira, J.F.B.; Freire, M.G.; Coutinho, J.A.P. Aqueous two-phase systems: Towards novel and more disruptive applications. Fluid Phase Equilibria 2020, 505, 112341, doi: https://doi.org/10.1016/j.fluid.2019.112341.

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# Peptide-ionic liquid conjugates towards the treatment of skin infections

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The treatment of complicated skin infections, like diabetic foot ulcers and other chronic wounds, are often associated with persistent polymicrobial biofilms that delay and difficult the healing process.[1] The most severe cases culminate in inpatient hospital admission, where infections can be exacerbated by hospital-acquired pathogens, in particular, if caused by the so-called ESKAPE pathogens, for which few efficient antibiotics are available.[2] The current biomedical approaches to chronic wounds aim at providing both protection against multidrug-resistant (MDR) bacteria and a matrix scaffold, often collagen-based, to boost the reestablishment of healthy skin.[3] Therefore, new options and new antibiotics are urgently needed and having that in mind our strategy is to use: i) antimicrobial peptides (AMP) to prevent or treat infection in the open wound; ii) collagen-inducing peptides (CBP)[4] to induce fast healing; iii) and ionic liquids (IL) [5] with intrinsic antimicrobial chemical permeation enhancement properties for an improved skin permeation. Through different combinations of these three types of building blocks, we aim to find a new class of active pharmaceutical ingredients suitable for topical application in the treatment of complicated skin infections. All the different conjugates designed and tested in vitro thus far will be presented.[6-8] The most promising ones result from conjugation of CBP with IL, delivering a new type of conjugate with potent antibacterial, antifungal, and collagen-inducing effects on human dermal fibroblasts.[9] Hence, these peptide-ionic liquid conjugates are promising leads towards the development of a topical formulation for the treatment of complicated skin infections.

**Keywords**: skin infections, antimicrobial peptide, ionic liquid, wound healing

**References:**

1. Leong, H.N.; Kurup, A.; Tan, M.Y.; Kwa, A.L.H.; Liau, K.H.; Wilcox, M.H. Management of complicated skin and soft tissue infections with a special focus on the role of newer antibiotics. Infect Drug Resist 2018, 11, 1959-1974, doi:10.2147/IDR.S172366.

2. Mulani, M.S.; Kamble, E.E.; Kumkar, S.N.; Tawre, M.S.; Pardesi, K.R. Emerging Strategies to Combat ESKAPE Pathogens in the Era of Antimicrobial Resistance: A Review. Front Microbiol 2019, 10, 539-539, doi:10.3389/fmicb.2019.00539.

3. Holmes, C.; Wrobel, J.S.; Maceachern, M.P.; Boles, B.R. Collagen-based wound dressings for the treatment of diabetes-related foot ulcers: a systematic review. Diabetes, metabolic syndrome and obesity : targets and therapy 2013, 6, 17-29, doi:10.2147/dmso.s36024.

4. Gomes, A.; Teixeira, C.; Ferraz, R.; Prudencio, C.; Gomes, P. Wound-Healing Peptides for Treatment of Chronic Diabetic Foot Ulcer and Other Infected Skin Injuries. Molecules 2017, 22, doi:10.3390/molecules22101743.

5. Gomes, A.; Aguiar, L.; Ferraz, R.; Teixeira, C.; Gomes, P. The Emerging Role of Ionic Liquid-Based Approaches for Enhanced Skin Permeation of Bioactive Molecules: A Snapshot of the Past Couple of Years. International Journal of Molecular Sciences 2021, 22, doi:10.3390/ijms222111991.

6. Gomes, A.; Bessa, L.J.; Correia, P.; Fernandes, I.; Ferraz, R.; Gameiro, P.; Teixeira, C.; Gomes, P. "Clicking" an Ionic Liquid to a Potent Antimicrobial Peptide: On the Route towards Improved Stability. Int. J. Mol. Sci. 2020, 21, 1-11, doi: https://doi.org/10.3390/ijms21176174.

7. Gomes, A.; Bessa, L.J.; Fernandes, I.; Ferraz, R.; Mateus, N.; Gameiro, P.; Teixeira, C.; Gomes, P. Turning a Collagenesis-Inducing Peptide Into a Potent Antibacterial and Antibiofilm Agent Against Multidrug-Resistant Gram-Negative Bacteria. Front Microbiol 2019, 10, 1-11, doi:10.3389/fmicb.2019.01915.

8. Gomes, A.; Bessa, L.J.; Fernandes, I.; Ferraz, R.; Monteiro, C.; L. Martins, M.C.; Mateus, N.; Gameiro, P.; Teixeira, C.; Gomes, P. Disclosure of a Promising Lead to Tackle Complicated Skin and Skin Structure Infections: Antimicrobial and Antibiofilm Actions of Peptide PP4-3.1. Pharmaceutics 2021, 13, doi:10.3390/pharmaceutics13111962.

9. Gomes, A.; Bessa, L.J.; Fernandes, I.; Aguiar, L.; Ferraz, R.; Monteiro, C.; Martins, M.C.L.; Mateus, N.; Gameiro, P.; Teixeira, C.; et al. Boosting Cosmeceutical Peptides: Coupling Imidazolium-Based Ionic Liquids to Pentapeptide-4 Originates New Leads with Antimicrobial and Collagenesis-Inducing Activities. Microbiology spectrum 2022, 10, e0229121, doi:10.1128/spectrum.02291-21.

34

POSTER

# Diversity and antimicrobial activity of marine sponge associated Actinobacteria from Mozambique and Portugal

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The microbiome of marine sponges (Phylum Porifera) often comprises different species of Actinobacteria. These microorganisms are well-known producers of molecules with a wide range of bioactivities. We aimed to isolate and phylogenetically identify sponge associated Actinobacteria for the bioprospection of bioactive metabolites.

Nine marine sponges were collected in Mozambique and Portugal: six in the island of Inhaca, Mozambique, identified as *Haliclona* sp. (n=2), *Psammocinia* sp. (n=3) and *Callyspongia perforata* (n=1), and three were collected off-coast Ovar, Portugal, identified as *Cliona* cf. celata (n=1), *Axinella* sp. (n=1) and *Desmacidon fruticosum* (n=1). Samples of these sponges were inoculated in six selective media and incubated at room temperature for up to six months. The grown colonies were purified, cryopreserved, and phylogenetically identified using 16S rDNA sequencing. Over 160 strains of Actinobacteria, distributed by 21 genera, were isolated from the analysed sponges. The genera *Micromonospora* (57 isolates) and *Streptomyces* (11 isolates) were represented by the highest number of species. In addition, several rare and particularly unexplored actinobacterial genera were recovered, such as *Actinocoralia*, *Aeromicrobium*, *Agrococcus*, *Cellulosimicrobium*, *Dietzia*, *Nesteronkonia* and *Williamsia*, among others. Potential new species of *Streptomyces*, *Micromonospora* and *Microbacterium* were isolated as well.

All actinobacterial isolates are currently being grown in liquid culture for the preparation of organic extracts to be used in bioactivity assays. The antimicrobial activity of the crude extracts against a variety of reference bacteria and fungi will soon be tested using the disc diffusion assay.

In conclusion, a high number of isolates and a great variety of Actinobacteria were obtained from the marine sponges analysed. These isolates constitute a promising source for novel antimicrobial secondary metabolites.

**Keywords**: antimicrobial, Actinobacteria, bioprospection, bioactive metabolites

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35

POSTER

# Effect of arbuscular mycorrhizal fungi application in biodiesel producing plants

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Fossil fuels combustion is one of the major problems of the 21st century(1). Biodiesel could be one of the responses to this environmental problem. However, the yield and price of production come up as barriers to a global adoption. This study aimed to implement a strategy to improve the performance of biodiesel producing plants, through the application of arbuscular mycorrhizal fungi (AMF). Plants were inoculated with AMF and grown under controlled environmental conditions. Root staining showed the presence of AMF structures in inoculated plants and their absence in non-inoculated plants. No statistically significant seed biomass was observed between inoculated and control plants of *Nicotiana tabacum* (Tobacco) and *Linum usitatissimum* (Flaxseed). Chlorophylls assays showed higher chlorophyll content of non-inoculated *L. usitatissimum* plants in the first assay (before flowering stage) and no statistically significant results in the second assay (after flowering stage). In *N. tabacum*, no statistically significant results were obtained in both chlorophylls assays. Genetic evaluation in *N. tabacum* was performed to assess expression of genes involved in triacylglycerol synthesis (DGAT1, DGAT2 and PDGAT) before and during seed development. Results showed that during seed development inoculated plants overexpressed the studied genes compared to control plants. It was hypothesized that a defense mechanism to assure species survival in inoculated plants was strongly induced resulting in production of seeds with higher reserves, namely triacylglycerols(2).

**Keywords**: Arbuscular mycorrhizal fungi; Biodiesel; Genetic expression.

**References:**

1. Greenwell HC, Lloyd-evans M, Wenner C, Greenwell HC. Biofuels , science and society. 2013;2–5.

2. Konvalinková T, Jansa J. Lights off for arbuscular mycorrhiza: On its symbiotic functioning under light deprivation. Front Plant Sci. 2016;7(June2016):1–11.

36

POSTER

# The influence of phytochemicals on grade IV prostate adenocarcinoma

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Adenocarcinoma is a worldwide concern, being one of the main causes of death in men. (1) Bone metastases derived from this type of cancer, the fact that tumor cells are increasingly resistant to existing oncological drugs and the side effects caused by these are the main causes for the need to develop new therapies. (2) Thus, silymarin and cinnamic acid (CINN) are examples of two phytochemicals that have been studied for the treatment of this pathology, since they have a fundamental role in chemoprevention, that is, they prevent stages of initiation, promotion and progression, associated with carcinogenesis, reducing cancer morbidity and mortality. (3, 4)

The main objective of this project is to evaluate the effect of both phytochemicals on the PC3 cell line of grade IV prostatic adenocarcinoma. Thus, parameters such as viability (MTT) and cell motility (injury) were evaluated through the application of different concentrations of compounds in tumor cells. It was demonstrated, with the results obtained, that both silymarin and CINN have the ability to decrease the viability and motility of prostate cancer cells.

In short, it is possible to state that the phytochemicals in question have an antitumor effect, proving their potential as treatments against the PC3 cell line, through the reduction of cell proliferation and induction of apoptosis.

**Keywords**: Prostate cancer; phytochemicals; viability; motility

**References:**

1. Ward RD, Purysko AS. Magnetic Resonance Imaging of Prostate Adenocarcinoma: Detection and Staging. Top Magn Reson Imaging. 2020;29(1):17-30.

2. Li K, Fan J, Qin X, Wei Q. Novel therapeutic compounds for prostate adenocarcinoma treatment: An analysis using bioinformatic approaches and the CMap database. Medicine (Baltimore). 2020;99(51):e23768.

3. Deep G, Agarwal R. Chemopreventive efficacy of silymarin in skin and prostate cancer. Integr Cancer Ther. 2007;6(2):130-45.

4. Gopalakrishnan S, Ismail A. Aromatic monophenols from cinnamon bark act as proteasome inhibitors by upregulating ER stress, suppressing FoxM1 expression, and inducing apoptosis in prostate cancer cells. Phytother Res. 2021;35(10):5781-94.

# Biotechnology education and the SARS-CoV 2 pandemic: challenges, truths and takeaways.

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In early 2020, the COVID-19 pandemic forced the higher education institutions to adapt to a new form of teaching and learning, from presential to full remote, blended and hybrid environment. This challenge had a particular impact in the life sciences field where the courses have a high content of laboratorial classes.
The main issue was how to transition from hands-on practical classes to remote instruction, assuring that our students continued engaged and acquiring the necessary skills, in a short amount of time. To address this, we took advantage of digital learning tools and other online resources that had limited use to date. Digital platforms such as Moodle, Skype, Teams and ZOOM facilitated the interface with the students and online resources such as virtual labs, simulations and video demonstrations were explored to illustrate the contents and keep the students involved. Additionally, several classes and laboratorial experiments were recorded by the teachers in the school labs and the research labs where we develop our research activity and shared with the students. The creation of teachers' work groups to share experiences and tools was key in the success of this process.
The student's evaluation process constituted one of the major difficulties during the pandemic. Keeping a fair assessment of the knowledge acquired and prevent any kind of fraud was only possible with a deep understanding of Moodle, the virtual learning platform we preferentially used.
Despite all efforts from teachers and students, crucial elements of the high education experience, particularly for undergraduates, were disrupted. Namely the student-teacher and student-student contact and interaction and the integration in the academic setting, resulting in stress and feeling of isolation and overwhelm.
Even with the difficulties faced by all the academic community, we observed no major changes regarding the academic success reflected in the final grades, comparing with previous years. In our understanding the core skills proposed for these courses were acquired successfully and the digital tools used with exception for the student's evaluation, are now considered an added value and bring flexibility to the teaching-learning process.

**Keywords**: Biotechnology, Life Sciences, Science Education, SARS-CoV 2

38

POSTER

# Optimization of a phenotypic screening bioassay for antibiotic discovery against *Escherichia coli* and *Staphylococcus aureus* based on FTIR-Spectroscopy

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Antibiotic resistance by bacteria has become a growing threat to public health worldwide.
Current antibiotic discovery pipelines are focused on phenotypic screening due to the lack of success of target-oriented platforms. However, a limitation associated with phenotypic-based platforms is the prediction of Mechanism of Action (MOA). As a result, methods capable of elucidating the MOA in conjunction with high-throughput whole-cell screening are more important than ever, and Fourier-Transform Infrared Spectroscopy (FTIR) is a prominent metabolic fingerprinting technique that may allow us to achieve these goals. As a result, in the present work, a protocol based on high-throughput FTIR spectroscopy was optimized to detect metabolic changes induced by different classes of antibiotics against the bacterial models *Escherichia coli* and *Staphylococcus aureus*, as Gram-negative and Gram-positive bacteria, respectively. The following antibiotics were used to expose the bacteria to three different MOA: amoxicillin and ampicillin (β-lactams) as cell wall inhibitors; kanamycin and neomycin (aminoglycosides) as protein synthesis inhibitors; and sulfamethazine and sulfamethoxazole (sulfonamides) as folate synthesis inhibitors. Initially, pre-processing techniques were optimized for the distinction of bacterial strains. Next, Principal Component Analysis (PCA) and Hierarchical Cluster Analysis (HCA) were used to quantify the effect of variables such as incubation time, incubation medium composition, and cell washing step prior to spectra acquisition, and to optimize bioassay performance for each bacterium, namely its reproducibility and metabolic resolution. The resolution of the MOA improved when the cell washing step was not performed and in this way, it was possible to develop a high-throughput, sensitive, simple, faster, more economical, automatable and focused protocol with minimal manipulations. The biomarkers obtained in the spectral bands, highly significant ($p<0.001$), corresponded to the known MOA of the antibiotic.
As an overall conclusion, the phenotypic screening bioassay based on FTIR spectroscopy is a robust technique that provides valuable information about the MOA of antibiotics at the level of the main biosynthetic pathway. This suggests that it can be applied to a new generation of metabolic fingerprint screening bioassays for antibiotic discovery, increasing the likelihood of success for future commercialization.

**Keywords**: Fourier-Transform Infrared (FTIR) spectroscopy; Mechanism of action (MOA); High-throughput screening; Antibiotics

# CINtec PLUS cytology as a cervical cancer screening test at the IPO of Porto

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Cervical squamous cell carcinoma of the cervix is the fourth most common cancer between women and seventh globally. There are many women undergoing colposcopy without real need, so more effective screening tests are needed to decide who among HPV-positive women should receive additional diagnostic evaluation to avoid unnecessary colposcopies. So, this study is intended to evaluate CINtec PLUS cytology as a screening test for cervical cancer in the IPO-FG of the Port of women with hpv test positive other than 16/18, improving the referral for colposcopy.
CINtec PLUS cytology is an immunocytochemistry kit that simultaneously detects p16 and Ki-67 proteins. The presence of these proteins in the same cell indicates a deregulation of the cell cycle, and may be a marker of the persistence of HPV infections, their greater probability of progression and the severity of lesions secondary to the infection.
An experimental research was carried out, in which the immunostaining of 73 non-16/18 HPV cervico-vaginal hpv samples was performed from 73 women integrated in cervical cancer screening in the North region carried out at the IPO-FG of Porto. The results obtained in CINtec PLUS were compared with those of cytology.
2 samples present unsatisfactory cytology for evaluation, being excluded. Cintec Plus cytology would reduce by 17% the number of women sent for colposcopy (7 (about 10%) by Cintec Plus vs 19 (about 27%) by cytology). Of the 52 NILM samples, 3 (about 6%) were positive for Cintec Plus, so they should have been referred for colposcopy and were not. Of the 10 ASC-US samples 100% are negative for Cintec Plus, as well as 5 LSIL 4 (80%) are negative, of the 3 HSIL 2 (about 67%) are negative. So, 16 women (about 23%) made colposcopy unnecessarily.
In conclusion, Cintec Plus cytology would be an interesting tool as a second screening test in non-16/18 hpv cases in place of cytology, decreasing the number of women sent for colposcopy.

**Keywords**: HPV, Cintec Plus cytology, Colposcopy

**References:**

Gupta, N.; Srinivasan, R.; Rajwanshi, A. Functional biomarkers in cervical precancer: an overview. Diagnostic Cytopathology. 2010, 38, 8, 23-618. doi:10.1002/dc.21270.

Ferlay. J.; Colombet, M.; Soerjomataram, I.; Mathers, C.; Parkin, D.M., Piñeros, M.; et al. Estimating the global cancer incidence and mortality in 2018: GLOBOCAN sources and methods. International Journal of Cancer. 2019, 144, 53-1941. doi:10.1002/ijc.31937.

Koliopoulos, G.; Nyaga, V.N.; Santesso, N.; Bryant, A.; Martin-Hirsch, P.P.L.; Mustafa, R.A.; Schünemann, H.; Paraskevaidis, E.; Arbyn, M. Cytology versus HPV testing for cervical cancer screening in the general population. The Cochrane database of systematic reviews. 2017, 8. doi:10.1002/14651858.CD008587.pub2.

Roche. CINtec PLUS E-learning [Internet]. http://reagent-catalog.roche.com/static/CINtecPLUS-Cytology/ (accessed on 17 May 2022).

Yu, L.L.; Guo, H.Q.; Lei, X.Q.; Qin, Y.; Wu, Z.N.; Kang, L.N.; et al. p16/Ki-67 co-expression associates high risk human papillomavirus persistence and cervical histopathology: a 3-year cohort study in China. Oncotarget. 2016, 7, 9-64810. doi: 10.18632/oncotarget.11705.

Celewicz, A.; Celewicz, M.; Wezowska, M.; Chudecka-Glaz, A.; Menkiszak, J.; Urasinska, E. Clinical efficacy of p16/Ki-67 dual-stained cervical cytology in secondary prevention of cervical cancer. Polish journal of pathology : official journal of the Polish Society of Pathologists. 2018, 69, 42-7. doi:10.21873/anticanres.15738.

40

POSTER

# Neural control of the lower urinary tract during hydrocephalus

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Neurological impairments may affect the neural control of the Lower Urinary Tract (LUT), due to structural and functional changes. Hydrocephalic patients usually present a triad of symptoms, which include gait disturbances, dementia and urinary incontinence. The consequences of hydrocephalus in brain structures, namely those involved in neural control of micturition remain mostly unknown. We used a validated animal model of hydrocephalus (rats injected in the cisterna magna with kaolin) to study the neuronal control of the LUT in hydrocephalus, focusing on two circumventricular areas: the Locus Coeruleus (LC) and the Periaqueductal grey (PAG). Studying neuronal activation of the PAG, a decrease in the number of fos-immunoreactive IR cells in the group of hydrocephalic animals was observed. Analysing the different column of the PAG separately, it was observed that this reduction was statistically significant only in ventrolateral PAG (VLPAG).

During hydrocephalus the levels of tyrosine hydroxylase (TH, enzyme involved in noradrenaline biosynthesis) increase in the LC, which may affect surrounding areas, namely the Pontine Micturition Center (PMC). To analyse if hydrocephalic animals present changes in noradrenaline levels at the spinal cord which may affect micturition, the expression of dopamine beta-hydroxylase (DBH), another enzyme involved in noradrenaline biosynthesis, in L6 spinal cord sections, was evaluated by immunohistochemistry. Hydrocephalic animals showed a higher number of fibres immunoreactive to DBH. Cystometric analysis of hydrocephalic animals, 8 weeks after induction, was used to evaluate the bladder's function. It was found that hydrocephalic animals show an increase both in the number of bladder contractions and of minimum pressure.

These results suggest alterations in the brain-bladder control network leading to an exaggerated micturition reflex. Considering the significant role of PAG in the voiding reflex, its lower activation may lead to miscommunication with other areas involved in the network, namely the PMC or LC.

Noradrenergic projections from the LC are responsible for the coordination between bladder contractions and EUS relaxation during voiding. Our study shows an increase in the levels of DBH in the Onuf's nucleus. It is possible that the increased availability of the limiting enzyme in the synthesis of noradrenaline contributes to the increased number of bladder contractions during hydrocephalus.

**Keywords**: hydrocephalus, urinary incontinence, Periaqueductal Gray, Locus Coeruleus

# Detecting BDNF gene Polymorphisms using genosensors and molecular biology tools

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Major depressive disorder (MDD) is a complex and highly prevalent psychiatric disorder with a high impact on quality of life and negative effects on mood, behaviour, and cognition. Currently, the main medical treatment for MDD is antidepressant medication. The selective serotonin reuptake inhibitors (SSRIs), including fluoxetine, sertraline, fluvoxamine, paroxetine and citalopram, are the most commonly prescribed drugs. However, as with all antidepressant treatments, about 30–40% of MDD patients do not respond sufficiently to SSRIs. Several factors, including genetic factors, play important roles in antidepressant responses. BDNF is one of the most investigated genes regarding depression and antidepressant response. In fact, the rs6265 (Val66Met) non-synonymous polymorphism, has been demonstrated to decrease pro-BDNF processing, and consequently affect the dependent secretion of BDNF. Curiously, carriers of Met-allele have been described to have smaller hippocampal volume, either in healthy or depressed patients. So, it is likely they can contribute to the interindividual differences in patient´s responses to antidepressants. Therefore, it is crucial to develop methodologies to predict the individual antidepressant response.
In this work, two analytical approaches based in molecular biology and electrochemical genosensor techniques are under development to create a low-cost genotyping platform able to genotype BDNF SNPs related with antidepressants therapeutic response.

**Keywords**: Major depressive disorder, BDNF, genosensor, molecular biology

**References:**

Kishi, T.; Yoshimura, R.; Ikuta, T.; Iwata, N. Brain-Derived Neurotrophic Factor and Major Depressive Disorder: Evidence from Meta-Analyses. Front. Psychiatry. 2018, 8, 308; DOI:10.3389/fpsyt.2017.00308

Arosio, B.; Guerini F.R.; Voshaar, R.C.O.; Aprahamian, I. Blood Brain-Derived Neurotrophic Factor (BDNF) and Major Depression: Do We Have a Translational Perspective?. Front. Behav. Neurosci. 2021, 15; DOI:10.3389/fnbeh.2021.626906

Santos, M.; Carvalho, S.; Lima, L.; Mota-Pereira, J.; Pimentel, P.; Maia, D.; Correia, D.; Gomes, S.; Cruz, A.; Medeiros, R. The role of IL18-607C>A and IL18-137G>C promoter polymorphisms in antidepressant treatment phenotypes: A preliminary report. Neurosci Lett. 2016, 27, 622:107-12. DOI: 10.1016/j.neulet.2016.03.026

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42

POSTER

# Inhalable mPEG-PLGA nanoparticle formulation for the treatment of lung cancer

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Lung cancer has a high mortality rate among all common cancers [1]. Conventional therapies are usually administered intravenously with low selectivity for tumor cells, which requires high doses to achieve therapeutically effective drug concentrations, leading to serious side effects [2]. Therapeutic proteins such as antibodies are useful in treatment due to their higher specificity and bioactivity, and lower toxicity compared to small molecule drugs [3]. Antibody encapsulation into nanoparticles for inhalable delivery is a promising strategy, which combines targeted and controlled drug delivery with the ability to protect antibody structure and bioactivity [4]. Thus, the aim of this work was the development and optimization of mPEG-PLGA nanoparticles formulated into a dry powder by spray-drying aimed at lung cancer treatment. The formulation development followed a Design-of-Experiment (DoE) approach to target the desired nanoparticle features: small particle size and good colloidal stability. Between the evaluated surfactants, PVA and Tween®80, the latter showed better colloidal stability. The polymer mass and surfactant concentration were considered as variables and have a significant effect on nanoparticles properties, namely particle size. The optimized nanoparticles were produced with 150 mg mPEG-PLGA and 1% Tween® 80, presenting the lowest particle size of ≈ 300 nm, polydispersity index of 0.36, and zeta potential of -24 mV, considered suitable features for antibody encapsulation. The spray drying optimization has demonstrated that D-mannitol and L-leucine were the best performing matrix excipients to obtain microparticles. Indeed, their combination (at concentrations of 2% and 1% (w/v), respectively) allowed an increase in the yield, which reached 59%, and reduction in powder adhesion to the walls of the apparatus due to leucine ability as dispersibility enhancer [5,6]. L-leucine also seems to reduce of particle agglomeration after spray-drying due to its crystallization, surrounding the droplet with an outer shell [5,7]. On the other hand, the other tested excipients, lactose and D-trehalose resulted in hygroscopic powders with a sticky appearance. Further studies will be conducted to optimize the best process parameters to get a stable powder to allow the inclusion of antibodies, enabling lung cancer treatment by inhalable dry powders.

**Keywords**: Dry powder inhalation, lung cancer, therapeutic protein, spray-drying.

**References:**

1. Siegel, R.L.; Miller, K.D.; Fuchs, H.E.; Jemal, A. Cancer Statistics, 2021. CA: A Cancer Journal for Clinicians 2021, 71, doi:https://doi.org/10.3322/caac.21654.

2. Aslam, M.S.; Naveed, S.; Ahmed, A.; Abbas, Z.; Gull, I.; Athar, M.A. Side effects of chemotherapy in cancer patients and evaluation of patients opinion about starvation based differential chemotherapy Journal of Cancer Therapy 2014, 5, 8 doi:10.4236/jct.2014.58089.

3. Zahavi, D.; Weiner, L. Monoclonal antibodies in cancer therapy. Antibodies 2020, 9, 34, doi:10.3390/antib9030034.

4. Mangal, S.; Gao, W.; Li, T.; Zhou, Q. Pulmonary delivery of nanoparticle chemotherapy for the treatment of lung cancers: challenges and opportunities. Acta Pharmacologica Sinica 2017, 38, 6, doi:10.1038/aps.2017.34.

5. Ordoubadi, M.; Gregson, F.K.A.; Wang, H.; Nicholas, M.; Gracin, S.; Lechuga-Ballesteros, D.; Reid, J.P.; Finlay, W.H.; Vehring, R. On the particle formation of leucine in spray drying of inhalable microparticles. International Journal of Pharmaceutics 2021, 592, 120102, doi:10.1016/j.ijpharm.2020.120102.

6. Molina, C.; Kaialy, W.; Nokhodchi, A. The crucial role of leucine concentration on spray dried mannitol-leucine as a single carrier to enhance the aerosolization performance of Albuterol sulfate. Journal of Drug Delivery Science and Technology 2019, 49, doi:https://doi.org/10.1016/j.jddst.2018.11.007.

7. Alhajj, N.; O'Reilly, N.J.; Cathcart, H. Designing enhanced spray dried particles for inhalation: A review of the impact of excipients and processing parameters on particle properties. Powder Technology 2021, 384, doi:https://doi.org/10.1016/j.powtec.2021.02.031.

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43

POSTER

# Evaluation of valproic acid toxicity in duckweed, *Lemna minor*

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In the last years the contamination of water caused by drugs has increased significantly. The main cause is the increase of the number and quantities of drugs that is being used. Many of these compounds cannot be removed effectively by wastewater treatment plants. This problem affects the water cycle and, consequently, all the environment as well as the human health, so it is important to find new methods that can help the removal of drugs from the water.

This research is aimed to understand the influence of different concentrations of valproic acid on the growth of the duckweed *Lemna minor*. After 7 days of exposure it was analysed the number of fronds, the length of the root and the chlorophylls content. The chlorophylls of the *Lemna minor* samples were analysed by spectrophotometry.

The number of fronds was influenced by the concentration of valproic acid. The higher concentrations (0,1 mg/mL; 0,05 mg/mL; 0,025 mg/mL) showed an inhibitory effect while the lower concentrations (0,005 mg/mL; 0,0001 mg/mL) showed a stimulating effect. Relative to the control, the number of fronds was lower in the higher concentrations and higher in the lower concentrations. The root length was affected in all the concentrations. Comparative to the control the higher concentrations presented a diminution of the root's length while the higher ones had an increase. Like the other parameters the concentration of chlorophylls was affected in all the samples. In the higher concentrations (0,1 mg/mL; 0,05 mg/mL; 0,025 mg/mL) the quantity was lower, comparatively to the control, and in the lower ones (0,01 mg/mL; 0,005 mg/mL; 0,0001 mg/mL) it was higher.

In general, the valproic acid influenced all the parameters evaluated: number of fronds, length of the root and concentration of chlorophylls. In the higher concentrations it is notable an inhibitory effect while the lower concentrations have a stimulating one.

**Keywords**: *Lemna minor*, Duckweed, Valproic acid, Toxicity, Water contamination

44

POSTER

# Development of a microparticle platform for co-encapsulation of cells and growth factors by microfluidics

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Hydrogels are three dimensional networks of hydrophilic polymer chains that can retain large amounts of water and keep a moist environment for wound, absorbing extensive body fluids to assist wound healing [1]. The encapsulation of cells in microscale hydrogels can provide a mimic of a 3D microenvironment to support cell viability and functions, and to protect cells from the environmental stress. The versatility of microfluidics provides an excellent manner to control several processing parameters such as particle size, frequency, number of loaded cells and co encapsulation with bioactives [2]. The aim of this work was to develop a microparticle platform by microfluidics for delivery of mesenchymal stem cells and growth factors. Alginate microparticles have been successfully produced using a PDMS (polydimethylsiloxane) device, with a T-junction conformation fabricated by soft lithography [3], and two immiscible alginate solution of 1.2 % (m/v) and lipidic maisine CC. However, to avoid leakage, fluid adsoption, clogging and to improve the system throughput, a PMMA (poly(methyl methacrylate) device with T-junction conformation was fabricated by micromilling. Microparticles could be obtained, but further optimization is needed. A new PMMA device, which includes a microparticle precipitation and washing phase, is being developed to provide a continuous microparticle co-encapsulation of cells and growth factors. It is foreseen that such delivery system allows to maintain the stability of both the cells and growth factors and enhance wound healing.

**Keywords**: Mesenchymal stem cells; Microdroplet; Drug delivery; Wound healing.

**References:**

[1] Krishnamoorthy S.; Schwartz M. F.; Lisa Van den Broeck L.; Hunt A.; Horn T. J.; Sozzani R. Tissue Regeneration with Hydrogel Encapsulation: A Review of Developments in Plants and Animals. BioDesign Research. 2021, Volume 2021; DOI:10.34133/2021/9890319

[2] Lababidi N.; Sigal V.; Koenneke A.; Schwarzkopf K.; Manz A.; Schneider M. Microfluidics as tool to prepare size-tunable PLGA nanoparticles with high curcumin encapsulation for efficient mucus penetration. Beilstein Journal of Nanotechnology. 2019, 10, 2280-2293. DOI: 10.3762/bjnano.10.220

[3] Silverio V.; Cardoso S., Microfabrication Techniques for Microfluidic Devices (2017). DOI:10.1007/978-3-319-59593-1_2

**Acknowledgements:** This work was funded by Fundação para a Ciência e a Tecnologia (FCT) in the scope of the projects UIDB/04326/2020, UIDP/04326/2020 and LA/P/0101/2020 of the Research Unit Center for Marine Sciences–CCMAR; UIDB/04565/2020 and UIDP/04565/2020 of the Research Unit Institute for Bioengineering and Biosciences–iBB; UID/05367/2020 of the INESC Microsistemas e Nanotecnologias and LA/P/0140/2020 of the Associate Laboratory Institute for Health and Bioeconomy–i4HB.

# Optimization of PLGA-nanoparticle loaded hydrogels for wound healing

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Burns, acute and chronic wounds are health problems that affect people quality of life and have a significant economic impact in healthcare systems worldwide. Acute wounds heal in 12 weeks, whereas chronic wounds need months [1]. In this work, we propose the development of polyvinyl alcohol (PVA)/alginate hydrogels loaded with poly (lactic-co-glycolic acid) (PLGA) nanoparticles as carriers by Quality by Design for the delivery of growth factors to enhance wound healing. Nanoparticles were produced by a water-in-oil-in-water double emulsion technique [2]. Hydrogels were produced by freeze thawing. Sodium alginate concentration, glycerine concentration, cycle duration time, and number of cycles were studied. The freezing kept at -20ºC and thawing was performed at room temperature. Particle size and polydispersity index were assessed by Dynamic Light Scattering (DLS), and intermolecular interactions between nanoparticles and the hydrogel was evaluated by ATR-FTIR prior and after freeze-thawing. The hydrogels are formed upon freeze-thawing between the sodium alginate and PVA chains due to hydrogen bonds formed during thawing [2]. The freeze-thawing cycles of 12h and 24h did not affect the particle size achieving 266±39 nm and 269±34 nm, respectively with a PdI of 0.25. The addition of 5% glycerine and 10% glycerine had no effect in the particle size and it improved the flow properties of the hydrogel. The ATR-FTIR analysis showed a peak at 1700 $cm^{-1}$ characteristic of the PLGA, a band at 1450 $cm^{-1}$ and a broad peak between 1500-600 $cm^{-1}$ can also be identified in the hydrogels but not in the physical mixtures, showing interactions between the materials in the hydrogel. No differences in the spectra can be found between the freeze thawing times, indicating no new interactions between the materials were formed. The multivariate design allowed to understand the influence the formulation parameters on the overall characteristics of the PLGA-nanoparticles loaded hydrogel. The freeze thawing cycles did not affect nanoparticles features. The formulations will be further used to deliver growth factors and their *in vitro* and *in vivo* performance will be evaluated.

**Keywords**: Wound healing, nanoparticle, insulin, hydrogel.

**References:**

1. Boateng J.S.; Matthews K.H.; Stevens H.N.; Eccleston G.M., Wound Healing Dressings and Drug Delivery Systems: A Review. J. Pharm. Sci. 2008, 8, 2892-923, DOI: 10.1002/jps.21210

2. Fonte, P.; Soares, S.; Sousa, F.; Costa, A.; Seabra, V.; Reis, S.; Sarmento, B. Stability study perspective of the effect of freeze-drying using cryoprotectants on the structure of insulin loaded into PLGA nanoparticles. Biomacromolecules. 2014, 10, 3753-3765; DOI: 10.1021/bm5010383.

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46

POSTER

# New probiotics for novel functional foods

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A wide range of health problems have been associated with alterations of the human gut microbiota. At the same time, probiotics have demonstrated their ability to modulate and restore the gut microbiota. Conventional probiotics are often supplied in combination with dairy products that are not suitable or accepted by the general population. As a result, there is a growing interest and demand for probiotic strains able to survive and develop in other food matrices. In this study, the probiotic potential of two strains of lactic acid bacteria of plant origin was evaluated. For this purpose, after confirming their identification, different functional and technological properties of interest were studied. In addition, their stability in different plant-based matrices has been evaluated under standard storage conditions. The results showed that the two strains have probiotic potential and that they are stable in the matrices analyzed, so they could be proposed for the development of new functional foods.

**Keywords**: Probiotics, non-dairy functional foods

**References:**

Küçükgöz, K.; Trz askowska, M. Nondairy Probiotic Products: Functional Foods That Require More Attention. Nutrients 2022, 14, 753. https://doi.org/10.3390/nu14040753

Palanivelu, J.; Thanigaivel, S.; Vickram, S.; Dey, N.; Mihaylova, D.; Desseva, I. Probiotics in Functional Foods: Survival Assessment and Approaches for Improved Viability. Appl. Sci. 2022, 12, 455. https://doi.org/10.3390/app12010455

47

POSTER

# Organic micropollutants in edible algae: Determination of PAH by chromatography

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As humanity progresses towards industrial development, the marine environment has been suffering of increased levels of pollution due to the existence of persistent pollutants such as Polycyclic Aromatic Hydrocarbons (PAHs). This family of pollutants emerges in the ocean ecosystem by the natural decomposition of organic matter. However, its presence is mainly due to anthropogenic sources such as maritime trade, spillage of petroleum or petroleum products, atmospheric deposition, river runoff, and domestic and industrial emissions. The deterioration of the marine environment and consequent contamination of the marine ecosystem led to the necessity of evaluation and monitoring these priority pollutants in aquatic plants and animals.

Macroalgae are under scrutinous for the presence of PAHs because of their interest as a functional food and as a source of biomolecules with potential application in several important industries like pharmaceuticals or cosmetics. Nonetheless, the macroalgae habitat in the coastal areas, is subject to higher concentration of pollutants from anthropogenic origin, suffering further effects of bioaccumulation and bio amplification, which may be dangerous levels for human consumption.

Seaweeds are not included in the European legislation on PAHs present in foods, but the maximum levels for fresh marine foods such as bivalves are 5 µg/kg of fresh weight (FW) for benzo[a]pyrene (B(a)P) that is considered the marker of exposure to carcinogenic PAHs, and 30 µg/kg FW for the sum of B(a)P, chrysene (Chry), benzo[a]anthracene (B(a)A), and benzo[b]fluoranthene (B(b)Ft).

The objective of this work was to assess and quantify the PAHs in edible macroalgae by using High Performance Liquid Chromatography with a fluorescence detection methodology after microwave assisted extraction with acetonitrile. Six species of edible algae native to the north of the Iberian Peninsula: *Undaria pinnatifida*, *Ascophyllum nodosum*, *Himanthalia elongata*, *Fucus spiralis*, *Bifurcaria bifurcata* and *Sargassum muticum* were studied. Regarding the four PAHs considered in the legislation (B(a)P, Chry, B(a)A, and B(b)Ft), the quantities found in seaweeds were lower than the recommended limit of 5 µg/kg FW of B(a)P and 30 µg/kg FW for the sum of the 4 PAHs indicated. Although PAHs are present in seaweed, the values found do not present a human health concern.

**Keywords**: Macroalgae, Polycyclic aromatic hydrocarbons, High performance liquid chromatography, Safety

**References:**

Lidia Wolska, Agata Mechlińska, Justyna Rogowska & Jacek Namieśnik Sources and Fate of PAHs and PCBs in the Marine Environment, Critical Reviews in Environmental Science and Technology. 2012 42:11, 1172-1189, DOI: 10.1080/10643389.2011.556546

Qari HA, Hassan IA. Bioaccumulation of PAHs in Padina boryana Alga Collected from a Contaminated Site on the Red Sea, Saudi Arabia. Polish Journal of Environmental Studies. 2017 26(1), 435-439. doi:10.15244/pjoes/63937.

US EPA. Polycyclic Aromatic Hydrocarbons (PAHs)-EPA Fact Sheet; National Center for Environmental Assessment, Office of Research and Development: Washington, DC, USA, 2008.

EU Regulation (EU). No. 835/2011 of 19 August 2011 amending Regulation (EC) No. 1881/2006 as regards maximum levels for polycyclic aromatic hydrocarbons in foodstuffs. Off. J. Eur. Union 2011, L215, 4–8.

Soares, C.; Sousa, S.; Machado, S.; Vieira, E.; Carvalho, A.P.; Ramalhosa, M.J.; Morais, S.; Correia, M.; Oliva-Teles, T.; Domingues, V.F.; Delerue-Matos, C. Bioactive Lipids of Seaweeds from the Portuguese North Coast: Health Benefits versus Potential Contamination. Foods 2021, 10, 1366. https://doi.org/10.3390/foods10061366

48

POSTER

# Effect of Umbilical Cord Mesenchymal Stem Cells secretome in Melanoma.

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Melanoma of the skin is one of the most prominent and fastest growing malignancies. More than 300 thousand diagnosed cases and 57 thousand deaths in 2020 worldwide and roughly 517 thousand cases of were registered during the 2015-2020 period [1]. Melanoma is generally regarded as an aggressive and unpredictable cancer whose conventional therapies, such as local excision, chemotherapy and immunotherapy, have encountered difficulties to prevent larger scale-tumours and metastasis, as well as overcome recurrence and development of drug resistance [2]. Stem cell-based therapies have been studied as interesting therapeutical approaches for cancer whenever conventional therapy fails to impede its progression. That is owing to the anti-proliferative and immunomodulatory capacity of some SC, being one of the major examples, Mesenchymal Stem Cells. Due to its high abundance, well defined extraction and expansion protocols as well as documented anti-tumorigenic characteristics, Umbilical Cord derived Mesenchymal Stem Cells (UC-MSC) have been observed as a promising candidate for Melanoma treatment, specially through acellular therapy using its secretome [3,4]. MSC secretome is defined as the set of MSCs-derived bioactive factors available extracellularly and is responsible for the major therapeutic effects of MSCs, namely in oncological pathologies.

In this study we hypothesize the ability of UC-MSC's secretome to inhibit Melanoma growth in vitro. UC-MSC secretome, in the form of conditioned medium (CM), was obtained by extraction from selected umbilical cords and expansion of while murine melanoma cell line B16-F10 culture was established. After treating melanoma cells with different concentrations of CM (100%, 50% and 25%), common cancer hallmarks such as cell viability, motility, colony formation and cell interactions were assessed through MTT, Wound Healing and Colony formation and Hanging-Drop assays, respectively. General analysis of viability and motility showed no statistically significant difference between treated and control groups as well as no concentration-dependent effect whereas formation of cellular aggregates follows an inhibition trend on the treated groups.

These results put into perspective the effect of secretome of UC-MSCs. Moreover, further larger scale studies are needed for deeper understanding of MSC secretome mechanisms of action, therefore enabling their use in acellular therapies against melanoma in the future.

**Keywords**: Melanoma, Umbilical Cord Mesenchymal Stem Cells (UC-MSC), Secretome, Cancer

**References:**

1. Sung, H.; Ferlay, J.; Siegel, R.L.; Laversanne, M.; Soerjomataram, I.; Jemal, A.; Bray, F. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. CA Cancer J Clin 2021, 71, 209–249, doi:10.3322/caac.21660.

2. Ross, M.I.; Gershenwald, J.E. Evidence-Based Treatment of Early-Stage Melanoma. J Surg Oncol 2011, 104, 341–353.

3. Sun, B.; Wang, X.; Pan, Y.; Jiao, Y.; Qi, Y.; Gong, H.; Jiang, D. Antitumor Effects of Conditioned Media of Human Fetal Dermal Mesenchymal Stem Cells on Melanoma Cells. Onco Targets Ther 2019, 12, 4033–4046, doi:10.2147/OTT.S203910.

4. Otsu, K.; Das, S.; Houser, S.D.; Quadri, S.K.; Bhattacharya, S.; Bhattacharya, J. Concentration-Dependent Inhibition of Angiogenesis by Mesenchymal Stem Cells. Blood 2009, 113, 4197–4205, doi:10.1182/blood-2008-09-176198.

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# Construction of a collection of non-*Saccharomyces* yeast strains from different grape varieties

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Yeasts constitute a very broad and heterogeneous group of microorganisms that is increasingly attracting the attention of researchers and various industrial sectors. In addition to its essential role in the production of a wide range of fermented foods, its potential health-related applications are gaining increasing interest. These include, among others, their antioxidant activity, their ability to produce antibacterial and antifungal compounds, as well as their potential as new probiotics. All these properties depend not only on the species but also on the strain, hence the importance of having extensive collections of strains that can be subjected to screening. The aim of this study was to construct a collection of strains of non-*Saccharomyces* yeasts isolated from different grape varieties.
From samples of three grape varieties grown in Galicia (NW Spain) and during two consecutive years, the yeasts were isolated in WL medium, the different morphotypes were differentiated and several colonies of each of them were subjected to purification and conservation. Identification was carried out by analyzing the ITS and D1/D2 regions of the rDNA. A total of 9 genera (*Aureobasidium, Candida, Hanseniaspora, Metschnikowia, Papiliotrema, Pichia, Rhodotorula, Sporidiobolus* and *Zygosaccharomyces*) and 12 species (*Aureobasidium pullulas, Candida carpophila, Hanseniaspora opuntiae, Hanseniaspora uvarum, Metschnikowia sinensis, Papiliotrema laurentii, Pichia manshurica, Pichia terricola, Rhodotorula graminis, Sporidiobolus pararoseus, Zygosaccharomyces bailii* and *Zygosaccharomyces bisporus*) were identified, and differences were found between varieties and annuities. In addition, the study of several biochemical and physiological characteristics allowed to establish differences between strains.

**Keywords**: Grapes, yeasts diversity, identification, non-*Saccharomyces*.

**References:**

Cordero-Bueso, G.; Vigentini, I.; Foschino, R.; Maghradze, D.; Ruiz-Muñoz, M.; Benitez Trujillo, F.; Cantoral, J.M. Culturable Yeast Diversity of Grape Berries from Vitis vinifera ssp. sylvestris (Gmelin) Hegi. J. Fungi. 2022, 8, 410; DOI: 10.3390/jof8040410

Fakruddin, M.; Hossain, M.N.; Ahmed, M.M. Antimicrobial and antioxidant activities of Saccharomyces cerevisiae IFST062013, a potential probiotic. BMC Complement. Altern. Med. 2017, 17, 64; DOI: 10.1186/s12906-017-1591-9

50

POSTER

# Improving the performance of SLNs - combining lipids extracted from larvae biomass and choline-based ILs

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The search for bioinspired and sustainable materials for innovative nanotechnology-based formulations with pharmaceutical and cosmetic purposes is growing.
The biomass from the *Hermetia illucens* larvae is particularly relevant to provide emollient ingredients for skin applications, since its lipid fraction is mainly composed of saturated fatty acids, such as lauric acid. On the other hand, biobased ionic liquids (ILs) are versatile compounds and their incorporation in nanodelivery systems has been studied to improve the overall properties of nanoparticles.
Thus, this work aimed to compare the performance of the preparation of solid lipid nanoparticles (SLN) using commercial lipids with that using a bioinspired and sustainable approach combining insect oil and ILs.
For that, different solid lipid nanoparticles (SLN) were prepared from the lipid fraction of *H. illucens* larvae biomass or using commercial lipids. Moreover, the ILs were incorporated in both types of SLNs. During stability studies, these nanosystems were characterized in terms of size, polydispersity index, and zeta potential.
SLNs with good characteristics were obtained. The results showed that ILs stabilized the nanoparticles and contributed to improve the physicochemical properties towards a topical application. In conclusion, the production of innovative lipid nanocarriers from bioinspired and sustainable resources combined with ILs seems to open a new paradigm for skin delivery.

**Keywords**: SLNs, Ionic liquids, Larvae extract, Skin delivery.

**References:**

1. Júlio, A.; Costa, J.G.; Pereira-Leite, C.; de Almeida, T.S. TransfersomILs: From Ionic Liquids to a New Class of Nanovesicular Systems. Nanomaterials 2022, 12, doi:10.3390/nano12010007.
2. Almeida, C.; Filipe, P.; Rosado, C.; Pereira-Leite, C. Nanodelivery Strategies for Skin Diseases with Barrier Impairment: Focusing on Ceramides and Glucocorticoids. Nanomaterials 2022, 12, doi:10.3390/nano12020275.

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# Purification of antibodies using alternative materials

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Currently, the number of biopharmaceuticals available for clinical use has increased rapidly due to the growing interest of the pharmaceutical industry in these products. Antibodies are the best-selling class on the market, due to their specific action and reduced immunogenicity [1,2,3]. Immunoglobulin G (IgG) is the most widely used class of antibodies in a wide variety of scientific, medical, and therapeutic applications. Despite the advantages and therapeutic potential of antibodies, the extraction and purification of these biomolecules form their complex biological media with high quality and purity level is still based on multi-step approaches and extremely high cost [4,5,6]. In this way, downstream processing represents a frontier in clinical drug research, as it represents up to 80% of the total production costs and is also considered the bottleneck in manufacturing of antibodies [4,5,6]. Therefore, there is a high demand for new techniques of recovery and purification of antibodies that are cost-effective and with the possibility of scaling up [4,5]. To overcome these drawbacks, in this work supported ionic liquids (SIL) were investigated as an alternative purification method for antibodies. The use of the SIL, [Alg][Im]Cl, and its respective precursor, [IM-Alg], were evaluated in the purification of IgG from human serum. When comparing both materials, the [IM-Alg], in the form of a hydrogel, proved to be a good candidate for the purification of IgG.

This work was developed within the scope of the projects CICECO-Aveiro Institute of materials, UIDB/50011/2020, UIDP/50011/202 & LA/P/0006/2020, financed by national funds through the FCT/MEC (PIDDAC). Ana P. M. Tavares acknowledges FCT for the research contract CEECIND/2020/02599.

**Keywords**: antibodies, immunoglobulin G, purification, supported ionic liquids.

**References:**

Hong MS, Severson KA, Jiang M, Lu AE, Love JC, Braatz RD. Challenges and opportunities in biopharmaceutical manufacturing control. Comput Chem Eng. 2018; 110:106-114, doi: https://doi.org/10.1016/j.compchemeng.2017.12.007

Fortune Business Insights. Monoclonal Antibody Therapy Market Size. https://www.globenewswire.com/news-release/2021/08/30/2288101/0/en/Monoclonal-AntibodyTherapy-Market-Size-Is-Projected-to-Grow-USD-451-89-billion-in-2028-at-a-CAGR-of-14- 1.html. Published 2021. Accessed November 25, 2021.

Bjelošević M, Pobirk AZ, Planinšek O, Grabnar PA. Excipients in freeze-dried biopharmaceuticals: Contributions toward formulation stability and lyophilisation cycle optimisation. Int J Pharm. 2020;576:119029.

Capela E V, Aires-Barros MR, Freire MG, Azevedo AM. Monoclonal antibodies—addressing the challenges on the manufacturing processing of an advanced class of therapeutic agents. Front Clin Drug Res Anti-Infectives. 2017;4:142-203.

Shukla AA, Hubbard B, Tressel T, Guhan S, Low D. Downstream processing of monoclonal antibodies—Application of platform approaches. J Chromatogr B. 2007;848(1):28-39. doi:https://doi.org/10.1016/j.jchromb.2006.09.026

Chon JH, Zarbis-Papastoitsis G. Advances in the production and downstream processing of antibodies. N Biotechnol. 2011;28(5):458-463. doi:https://doi.org/10.1016/j.nbt.2011.03.015

52

POSTER

# A Portugal Perspective of Silicosis in a Poster Review

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Silicosis is a well-known fibrogenic lung disease which is probably one of the most ancient occupational illness. Silicosis is a prevalent disease currently without specific treatment. So, a preventive strategy is always the best option. An effective silicosis preventive strategy should be based on the primary and secondary prevention approaches.
An effective silicosis preventive strategy should be based on the primary and secondary prevention approaches.
Despite all efforts to prevent it, silicosis is widely spread in most parts of the world and millions of workers continue to be exposed to noxious dusts running a high risk of developing silicosis and other pneumoconioses. In some cases like Portugal, silicosis is present in particular regions of the country.
Silicosis is a prevalent disease currently without specific treatment.
Among the potential complications is tuberculosis, for which silicotic patients may have a risk up to 40 times higher than that of the general population.
This work will give a perspective of this disease from Portugal to the world.

**Keywords**: Epidemiology, pneumoconiosis, public health, silicosis.

# Optimisation of a molecular methodology for the detection of virulence factors of enterotoxigenic *Escherichia coli* for the diagnosis of swine colibacillosis

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The most common bacterial pathogen causing enteric infections in pigs is enterotoxigenic *Escherichia coli* (ETEC). Since pigs represent the largest livestock category in the European Union, ETEC-associated diseases, better known as swine colibacillosis leading to acute diarrhea and eventual death of the animal, result in significant costs to the pig industry. These diseases are traditionally prevented or treated with antibiotics, and this has had a huge impact on the emergence of resistant bacteria, correlating with the emergence of resistant infections in humans. Recognition of this problem has led the authorities to set ambitious goals for the reduction of this type of drug in animal husbandry, leading to the creation of a national project, APTAcoli, which aims to select aptamers (consisting of small single-stranded oligonucleotides capable of binding to target molecules with great affinity and specificity, due to the specific secondary and/or tertiary structures they can acquire) as an alternative in the treatment of colibacillosis. The present experimental study, which is on the APTAcoli agenda, focused on the optimization of a molecular methodology - Multiplex PCR - for the detection of the main virulence factors of ETEC to be used in an epidemiological study to characterize fecal samples from pigs in Portuguese farms. After using different optimization techniques, the results were two multiplex PCR amplification sets, one for amplification of the main toxigenic factors of ETEC (STa, STb, LT and STx2e) and another for amplification of the main adhesion factors (F4, F5, F6, F18 and F41).

**Keywords**: ETEC, Escherichia coli, Swine colibacillosis, Multiplex PCR

**References:**

Quinn, P.J.; Markey, B.K.; Leonard, F.C.; Hartigan, P.; Fanning, S.; Fitzpatrick, E.S. Veterinary Microbiology and Microbial Disease; P.J. Quinn, B.K. Markey, F.C. Leonard, E.S. FitzPatrick, S.F. and P.J.H., Ed.; 2nd ed.; Wiley-Blackwell, 2011; ISBN 9781405158237.

Eurostat Livestock Population in Numbers Available online: https://ec.europa.eu/eurostat/web/products-eurostat-news/-/ddn-20220517-2 (accessed on 24 June 2022).

Lei, X.J.; Park, J.W.; Baek, D.H.; Kim, J.K.; Kim, I.H. Feeding the Blend of Organic Acids and Medium Chain Fatty Acids Reduces the Diarrhea in Piglets Orally Challenged with Enterotoxigenic Escherichia Coli K88. Anim. Feed Sci. Technol. 2017, 224, 46–51, doi:10.1016/j.anifeedsci.2016.11.016.

Oliveira, R.; Pinho, E.; Sousa, A.L.; DeStefano, J.J.; Azevedo, N.F.; Almeida, C. Improving Aptamer Performance with Nucleic Acid Mimics: De Novo and Post-SELEX Approaches. Trends Biotechnol. 2021, doi:10.1016/j.tibtech.2021.09.011.

54

POSTER

# CytoPath®Easy: screening of cervical cancer

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Recently, CytoPath®Easy kit was created by DiaPath S.p.A. and started to be commercially available for the screening of cervical cancer. Using this methodology, epithelial cells are immersed in a preservative liquid, and a thin-layer of cells in the slide is obtained through gravity sedimentation and filtration.

The main objectives of this study are to evaluate the efficacy for the processing of cervical samples, for the detection of pre-neoplastic lesions and for the nucleic preservation and extraction by the kit. For this purpose, 215 cervical samples obtained by self-sampling were used: 174 were collected and processed by CytoPath® Easy and, as a control, 41 were collected and processed by the Thinprep® method. The samples were processed, stained by the Papanicolaou method, and independently evaluated microscopically for various morphological parameters; nucleic acids were isolated and evaluated for purity and integrity by spectrophotometry.

Results obtained showed that both methods have a good performance, allowing the morphological evaluation of the cervical epithelium. However, the statistical analysis reveals that the methods are different from each other, with overall lower results being obtained in the method under study ($p<0.001$). In turn, both methods allow the extraction of good quality and quantity of DNA.

Although some differences were found regarding morphology of the cells fixed and processed by the CytoPath®Easy method, this new methodology reveals efficient for the preservation of nucleic acids. Thus, its use in cervical cancer screening is recommended.

**Keywords**: pre-neoplastic lesion, CytoPath®Easy, Thinprep®, extraction of nucleic acids

**References:**

Buskwofie, A.; David-West, G.; Clare, C. A. Review of Cervical Cancer: Incidence and Disparities. J Natl Med Assoc. 2020, 112(2), 229;

Pista, A.; Costa, C.; Saldanha, C.; Moutinho, J.A.; Moutinho, J.; Arrobas, F., et al. Budget impact analysis of cervical cancer screening in Portugal: comparison of cytology and primary HPV screening strategies. BMC Public Health. 2019, 19, 2-3;

DiaPath S.p.A. CytoPath®Easy: Complete solutions for Liquid-Based Cytology. Available from: https://www.diapath.com/product/cytopath-easy--cp350-2609

Fernandes, S.; Martins, C.; Silva, R.A.; CytoPath®Easy processing method validation through cervical self-sampling. Eur J Public Health. 2021; 31(2).

Campaner, A.B.; Fernandes, G.M. Detection of transformation zone cells in liquid-based cytology and its comparison with conventional smears. Diagn Cytopathol. 2020

Nuttchote, P.; Oranratanaphan, S.; Termrungruanglert, W.; Triratanachat, S.; Chaiwongkot, A.; Baedyananda, F. et al. Comparison of detetion rate of high risk HPV infection between self-collected HPV testing and clinician-cellected HPV testing in cervical cancer screening. Taiwan J Obstet Gynecol , 2019, 58

55

POSTER

# Performance evaluation of IH-500 automatic equipment for immunohematological studies.

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New technologies and automated instrument platforms have been developed to improve the efficiency and safety of immunohematology tests in recent years. Recently, the automatic equipment for Immunohematology IH-500 was developed. By assessing its fully automated performance, the current study sought to validate the IH-500 equipment for the immunohematological analysis of blood donations.

Pre-determined blood donor samples were selected on the pre-existing Techno TwinStation. Subsequently, these same samples were submitted to the new IH-500 platform, where 92 samples were processed for ABO/RhD blood group determination, 31 for Rh/Kell phenotyping, and 102 samples for antibodies screening. For the three tests performed, an analysis of the agreement of the results obtained by both equipments was carried out.

The agreement rates obtained for the determination of the ABO/RhD blood group, the Rh/Kell phenotype, and the antibodies screening were all equal to 100%. This study demonstrated that the IH-500 provided reliable results compared to the pre-existing Techno TwinStation equipment. Thus, the IH-500 was validated and therefore implemented for the immunohematology study of donations in the department where the study was performed.

**Keywords**: Performance Evaluation; IH-500; Validation; Automation

**References:**

Park, S.H.; Kim, J., Lim, J.H.; Jeong, J,; Lee, S.H. Performance Evaluation of Automated Immunohematology Analyzer IH-500 for Blood Bank Testing. Indian J Hematol Blood Transfus. 2019, 35(4),731–5.

Shin, J.W.; Shin, W.Y.; Lee, D.L. Comparison of ABO Blood Group Typing between Automated Blood Bank Analyzer IH-500 and Manual Method. Korean J Blood Transfus. 2017, 28(2),126–33.

Park,Y.; Kim, S.Y.;Koo, S.H.; Lim, J.; Kim, J.M.; Lim, Y.A.; et al. Evaluation of the Automated Blood Bank Systems IH-500 and VISION Max for ABO-RhD Blood Typing and Unexpected Antibody Screening. Lab Med Online. 2017, 7(4),170–5.

# INDEX